

Interphase microtubule bundles use global cell shape to guide spindle alignment in fission yeast

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Summary

Correct spindle alignment requires a cell to detect and interpret its global geometry and to communicate this information to the mitotic spindle. In the fission yeast, *Schizosaccharomyces pombe*, the mitotic spindle is aligned with the longitudinal axis of the rod-shaped cell. Here, using wild-type and cell-shape mutants we investigate the mechanism of initial spindle alignment and show that attachment of interphase microtubules to the spindle pole bodies (SPB), the yeast equivalent of the centrosome, is required to align duplicated SPBs, and thus the mitotic spindle, with the long axis of the cell. In the absence of interphase microtubules or attachment between the microtubules and the SPB, newly formed spindles are randomly oriented. We show that the axis of the mitotic spindle correlates with the axis along

which the SPB, as a consequence of interphase microtubule dynamics, oscillates just before mitosis. We propose that cell geometry guides cytoplasmic microtubule alignment, which in turn, determines initial spindle alignment, and demonstrate that a failure of the spindle pre-alignment mechanism results in unequal chromosome segregation when spindle length is reduced.

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Introduction

An important question in cell morphogenesis is how cells detect and interpret their geometry to position organelles and structures within a cell. One example of such a mechanism is mitotic spindle positioning. Proper spindle alignment within a cell requires mechanisms to sense the cellular axis of polarity and to rotate the mitotic spindle with respect to that axis to ensure an effective mitosis (Gonczy, 2002; Kusch et al., 2003). In the budding yeast *Saccharomyces cerevisiae*, astral microtubules (MTs) are guided by the actin cytoskeleton towards the bud tip and align the mitotic spindle along the mother-daughter axis. Spindle positioning at the bud neck, a prerequisite for proper cell division, depends on local control of dynein motor activity (Pearson and Bloom, 2004; Shaw et al., 1997). In animal cells, depending on cell type, mitotic spindle positioning and orientation relies on polarity factors that control astral MT dynamics and on the extracellular matrix, which controls the actin cytoskeleton and cell geometry (Colombo et al., 2003; Fernandez-Minan et al., 2007; O'Connell and Wang, 2000; Strauss et al., 2006; Thery et al., 2005; Tsou et al., 2003). However, the mechanisms that allow cells to read and interpret their geometry are poorly understood. Here, using wild-type haploid and diploid cells, as well as various cell shape mutants, we have investigated how a fission yeast cell detects its global shape to align the mitotic spindle along its longitudinal axis.

The fission yeast is a rod-shaped organism that grows by tip extension and divides by fission at a position determined by the position of the early mitotic nucleus (Daga and Chang, 2005; Mitchison and Nurse, 1985; Tolic-Norrelykke et al., 2005).

Interphase MTs are organized in antiparallel bundles initiated from multiple sites on the nuclear envelope and extending towards the cell tips. From this perinuclear region interphase MTs probe the cellular space dynamically by continuous growth and shrinkage (Carazo-Salas and Nurse, 2006; Daga et al., 2006a; Drummond and Cross, 2000; Hoog et al., 2007; Sawin and Tran, 2006; Tran et al., 2001). These MTs are responsible for the vectorial transport of cell-end factors as well as for nuclear positioning. The mechanism by which fission yeast cells position the nucleus at the geometric center of the cell is based on pushing forces generated by MTs. In wild-type *S. pombe* cells, transition from MT growth to shrinkage ('catastrophe') is normally restricted to cell tips. Therefore, when the plus end of a growing MT bundle hits the cell cortex at a position other than the cell tip, it slides and becomes aligned with the cellular axis. At cell tips, MT bundles dwell for a period of around 60-90 seconds before undergoing catastrophe. During this time MTs continue polymerizing so that the minus end of the MT bundle and the nucleus, which is attached to it, are pushed back in the opposite direction. Since MTs extend from sites of perinuclear nucleation towards each cell tip with equal number and frequency, and dwelling time is similar at both cell tips, the net pushing forces generated by polymerization are similar at both cell ends and the nucleus is positioned in a medial position between the cell tips (Daga et al., 2006b; Tran et al., 2001).

Upon entry into mitosis, interphase microtubules disassemble, the SPBs are inserted into the nuclear envelope, and the intranuclear mitotic spindle forms, bringing about a closed mitosis (Ding et al., 1997). In *S. pombe* the position of the division plane is determined

in early mitosis, therefore the mitotic spindle must be oriented perpendicular to the actomyosin ring to segregate chromosomes equally to daughter cells after spindle elongation in late mitosis (Daga and Chang, 2005).

The mitotic spindle is formed by four different subclasses of MTs: (1) kinetochore microtubules, which are responsible for kinetochore capture and chromosome segregation; (2) interpolar MTs, which extend from pole to pole. A subset of interpolar MTs form an overlapping region at the central spindle later in mitosis and are responsible for pushing the poles and the nuclei apart during anaphase B; (3) intranuclear astral MTs or INA (INA-MTs) nucleated within the nucleus and organized in linear arrays extending from the two SPBs towards the nuclear periphery (Sagolla et al., 2003; Zimmerman and Chang, 2005); (4) cytoplasmic astral microtubules (CA-MTs), which are nucleated from the cytoplasmic sides of the SPBs upon onset of anaphase B. These MTs are responsible for spindle alignment late in mitosis during pole and nuclei separation by a mechanism analogous to nuclear positioning during interphase (Tolic-Norrelykke et al., 2004).

During mitosis, spindle behavior is described in three separate phases known as Phases I-III. In Phase I, the mitotic spindle is assembled. During interphase, centrosomes of the three *S. pombe* chromosomes are attached to the nuclear side of the SPB. Concomitantly with spindle formation at the beginning of mitosis, centromeres release from the SPBs and sister kinetochores are captured by the plus end of spindle MTs emanating from both poles, giving rise to proper bipolar attachment. During Phase I, the two poles separate at a rate of about 1 $\mu\text{m}/\text{minute}$. During Phase II, spindle length is constant, and chromosomes are pulled apart towards the two poles, becoming completely separated by the end of the phase (anaphase A or early anaphase B). In Phase III, the poles with a complete set of chromosomes are pushed apart by the elongating spindle into the two daughter nuclei at a rate of 0.8-1 $\mu\text{m}/\text{minute}$ (Hiraoka et al., 1984; Mallavarapu et al., 1999; Nabeshima et al., 1998).

It has been proposed that the CA-MTs have a role in aligning the mitotic spindle late in mitosis when the nucleus is extended, but not during earlier phases of mitosis before chromosome segregation. The spindle alignment mechanism during late anaphase is based on the same principle as nuclear positioning – balanced pushing forces exerted against the cell cortex by CA-MT polymerization keep the mitotic spindle away from the cell cortex during spindle elongation (Tolic-Norrelykke et al., 2004). However, the current model for spindle alignment during early mitosis proposes that cells use the actomyosin ring as a positional cue and use astral MTs to correct spindle alignment. The model also proposes the existence of a checkpoint signal that delays metaphase-to-anaphase transition when the mitotic spindle is misaligned. This model of spindle alignment has stimulated discussion because astral MTs during early mitosis have been reported to be intranuclear (Zimmerman et al., 2004) and therefore cannot directly contact the cell cortex to correct spindle alignment. Furthermore, it has been suggested that there is no checkpoint that solely senses the alignment of the mitotic spindle to delay progression through mitosis (Vogel et al., 2007).

Here, based on our analysis of wild-type and mutant cells of different morphologies, we propose a model in which pushing forces of MTs at interphase aligned with cellular geometry to set up the initial spindle alignment of early spindles and demonstrate that the initial spindle alignment is critical when spindle length is reduced. Our model is similar to that proposed recently by Vogel et al. (Vogel et al., 2007).

Results

Microtubules, proper MT dynamics and the attachment of interphase microtubules to SPBs are required for proper initial spindle alignment

We have investigated the alignment between the axis of SPB separation and the long axis of the cell during spindle formation. Asynchronously growing cells were imaged from late G2 until the initial separation of the SPBs in early mitosis using the SIN pathway component Sid2 fused to GFP and the SPB component cut12 fused to GFP as SPB markers (Bridge et al., 1998; Sparks et al., 1999). To investigate initial spindle alignment we examined only very short early spindles (length $<1 \mu\text{m}$) that had not yet begun to rotate. The angle between the axis of SPB separation and the long cell axis (spindle axis) was calculated from 2D maximal projections of images in multiple focal planes (Fig. 1A). Consistent with previous reports (Gachet et al., 2001; Gachet et al., 2004; Tournier et al., 2004; Vogel et al., 2007) essentially all wild-type cells initiated SPB separation within 30 degrees of the long axis of the cell (Fig. 1B and supplementary material Fig. S1 and Movie 1), suggesting that

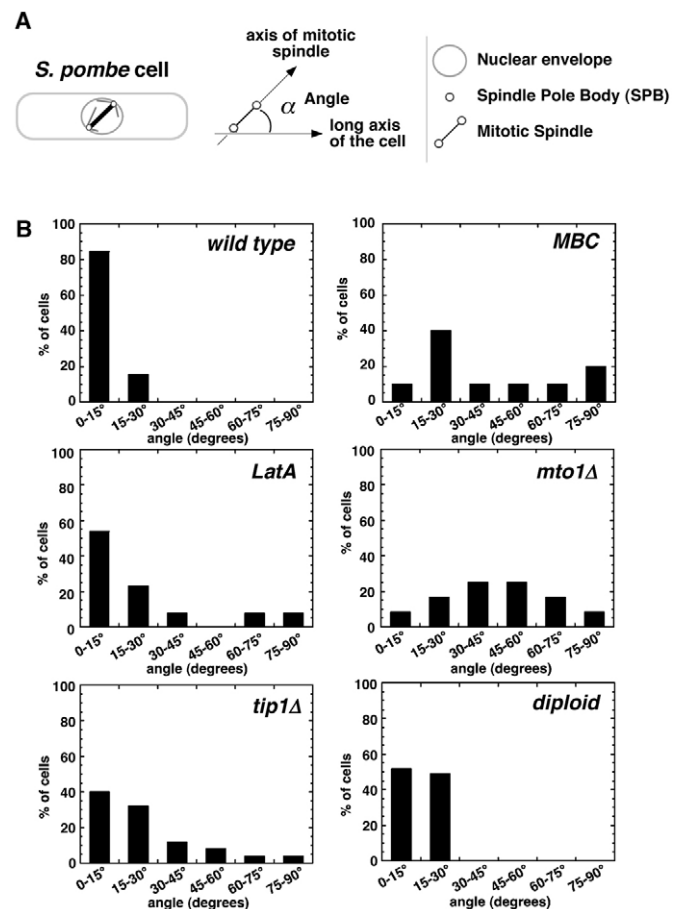


Fig. 1. Microtubule cytoskeleton is required for spindle alignment. (A) Scheme of a *S. pombe* cell with a misaligned spindle. The angle is that formed between the mitotic spindle axis and the long axis of the cell (both axes are indicated with arrows). (B) Percentage of wild-type haploid and diploid control cells, MBC (25 $\mu\text{g}/\text{ml}$) or Latrunculin A (50 μM) treated cells, and *mto1Δ* and *tip1Δ* cells, showing the angle of the mitotic spindle relative to the long axis of the cell. The range of mitotic spindle angles of *mto1Δ* cells MBC-treated cells and *tip1Δ* cells were significantly different to wild-type cells ($P < 0.001$) but no significant difference from the wild type was seen in Latrunculin-A-treated cells ($P < 0.1$) or diploid cells ($P < 0.2$).

fission yeast cells have a mechanism to align the spindle with the global axis of polarity.

We then tested whether the actin and microtubule cytoskeletons have any role during the initial steps of spindle alignment. Cells were treated with 50 μ M Latrunculin A to depolymerize the actin microfilaments or with 25 μ g/ml Carbendazim (MBC) to depolymerize interphase microtubules. Actin depolymerization inhibits entry into mitosis (Rupes et al., 2001), therefore initial SPB separation was followed only in those cells that had already become committed to enter mitosis when SPBs then separated within 2 minutes of drug addition. Although some differences in the angle of SPB separation were observed in cells treated with Latrunculin A compared with untreated wild-type cells, over 75% of the cells were within 30 degrees of the long axis (Fig. 1B). By contrast, MBC treatment at concentrations that depolymerize interphase MTs but not mitotic spindle MTs, generated an almost random spindle alignment relative to the long cell axis (Fig. 1B and supplementary material Fig. S1 and Movie 2). Thus, microtubules are much more significant than the actin cytoskeleton for proper initial spindle alignment.

Given that interphase MTs are required for proper initial spindle alignment, we checked whether attachment of the interphase MTs to the SPB was required for proper spindle alignment. We analyzed angles of SPB separation in the *mto1* deletion mutant. Mto1 is a component of the γ -tubulin complex, which is essential for proper cytoplasmic nucleation of MTs bundles. In the absence of Mto1, cells either have no interphase MTs, or MTs that are not attached to the SPB (Sawin et al., 2004; Venkatram et al., 2004; Zimmerman and Chang, 2005). We found that *mto1*-deleted cells were defective in mitotic spindle alignment relative to the long axis of the cell (Fig. 1B and supplementary material Fig. S1 and Movie 3). Since interphase microtubules can detect global cell geometry and align themselves parallel to the long cell axis (Carazo-Salas and Nurse,

2006; Daga et al., 2006a), we hypothesized that interphase MTs could somehow sense cell geometry and signal the position of the cellular axis to the SPBs. We therefore analyzed spindle orientation in a mutant with altered interphase MT organization. Deletion of the *S. pombe* CLIP170 homologue *tip1* results in cells with short MTs that often do not extend the entire length of the cell, and therefore are only partially aligned with the long axis of the cells (Brunner and Nurse, 2000; Carazo-Salas and Nurse, 2006; Daga et al., 2006b; Grallert et al., 2006). We observed in *tip1* Δ cells, which have short, poorly aligned MTs, that the frequency of spindle misalignment increased significantly, consistent with our hypothesis that interphase MTs aligned along the axis of the cell influence spindle orientation (Fig. 1B).

In wild-type cells, we found that the SPB oscillated around the cell center along a straight line parallel to the long axis (Fig. 2A-C). This oscillatory movement was not perturbed by Latrunculin A treatment, but was lost in cells treated with MBC or in *mto1* Δ cells, and was reduced in *tip1* Δ cells, the same three situations in which we have observed that the spindle is not properly aligned (Fig. 2B and data not shown) (Tran et al., 2001). During interphase, one of the MT bundles is always attached to the SPB (Ding et al., 1997; Hagan and Yanagida, 1995), therefore, as a consequence of MT dynamics, SPB movement during this phase is mainly caused by alternate pushing of MTs against either cell tip (supplementary material Fig. S2) (Tran et al., 2001). To better understand such a causal relationship between SPB oscillation during interphase and the initial alignment of the mitotic spindle, we utilized live-cell microscopy to analyze the angle of single SPB movements relative to the final axis of SPB separation in cells progressing from interphase to mitosis. In wild-type cells, the average angles of SPB movement relative to the cell axis during interphase was $13 \pm 13^\circ$ (range 0° to 45° , $n=124$, Fig. 3A,C), whereas the angle of SPB separation upon entry into mitosis was within $9 \pm 6^\circ$ of the angle of

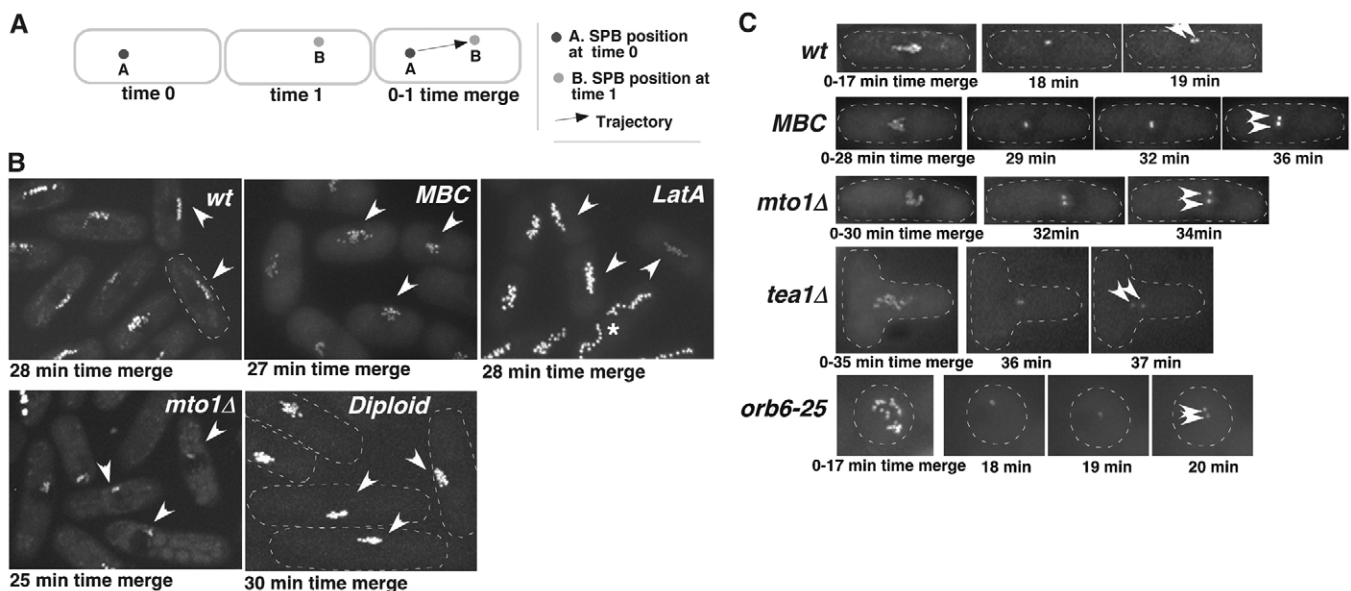
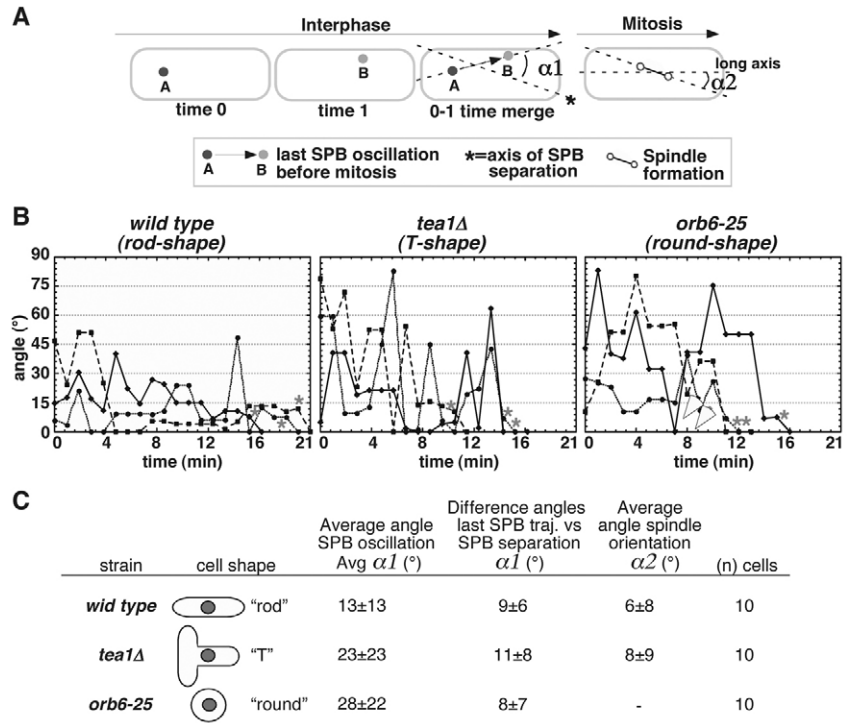


Fig. 2. Microtubules and microtubule-SPB attachment are required for proper spindle alignment. (A) Scheme showing the SPB trajectory from position A at time 0 to position B at time 1. (B) Overlaid images showing SPB position during a window of time ranging from 25 to 28 minutes in wild-type control cells, MBC (25 μ g/ml) or Latrunculin A (50 μ M) treated cells, and *mto1* Δ cells expressing the SPB marker Sid2-GFP. Arrowheads denote the overlaid SPB trajectory. The asterisk denotes a cell in mitosis. Dashed lines indicate the cell outline. (C) Overlaid images showing the SPB position during the indicated time interval followed by images taken one or two minutes before SPB separation in wild-type control cells, MBC-treated cells (25 μ g/ml), *mto1* Δ cells, and the cell morphology mutants *tea1* Δ and *orb6-25*, expressing *sid2-GFP*. Arrowheads indicate the initial SPB separation.

Fig. 3. Initial SPB separation at mitosis occurs along the same axis as the last SPB movement during interphase. (A) Scheme showing the angles measured. The angles of the SPBs trajectory with respect to the axis of SPB separation were measured between consecutive time points. (B) Graphs showing these angles in three representative cells of the indicated strains. The asterisks indicate the angle of the last SPB trajectory 1 minute before mitosis for every cell type. Note that the angle of SPB separation is the base comparison and so is zero. (C) Table of spindle orientation parameters in the wild type and the morphology mutants *orb6-25* and *tea1Δ*. Average angle of SPB oscillation, the difference between the angle of the last SPB trajectory and initial angle of SPB separation, and average angle of spindle alignment relative to the long axis of the cells are all shown in the indicated strains. n, number of cells analyzed.



the last trajectory of SPB oscillation just before interphase MT disassembly and spindle assembly (Fig. 2C and Fig. 3B,C). These data suggest that interphase MTs align the spindle, and that both proper MT-SPB interactions and MT dynamics are required to ensure that the initial spindle elongation is aligned with respect to the cellular axis.

Spindle alignment in cells with altered morphology

T-shaped cells or cells with two axes of polarity

To test the hypothesis that interphase MTs pre-align the axis of the mitotic spindle with the cellular axis, we used different cell-shape mutants to alter the axis along which interphase MTs became aligned and the SPBs oscillate, and then analyzed the angle of SPB separation in mitosis. We first analyzed spindle alignment in mutant cells with two axes of polarity. *Tea1* is required to position the sites of growth at the ends of the fission yeast cell. In *tea1*-deleted cells, an ectopic site of growth can be initiated generating 'T-shaped' cells with an extra axis of polarity (Mata and Nurse, 1997). In these cells, SPBs oscillated in both axes at different moments (Fig. 2C, data not shown) and the range of SPB trajectory angles during interphase was far greater than in wild-type cells (23 \pm 23 $^{\circ}$ vs 13 \pm 13 $^{\circ}$) (Fig. 2C and Fig. 3B,C). Importantly, the SPBs in T-shaped *tea1Δ* cells always separated along the axis in which the SPB was oscillating (17/17 cells). In addition, the SPB trajectory angle just before mitosis and the angle of SPB separation were very close: within 11 \pm 8 $^{\circ}$ of each other (Fig. 3B,C), as in wild-type cells. Similar results were obtained using another 'T' mutant, *tea4Δ/wsh3Δ; tea4/wsh3*, which encodes a cell-end factor essential for bipolar growth, and in its absence, cells form an extra axis of polarity (Martin et al., 2005; Tatebe et al., 2005). In *tea4Δ/wsh3Δ* cells, as in *tea1Δ* cells, the range of SPB trajectory angles during interphase was also greater than in the wild-type cells (26 \pm 20 $^{\circ}$ vs 13 \pm 13 $^{\circ}$, respectively); however, the last SPB trajectory angle and the angle of SPB separation were within 7 \pm 5 $^{\circ}$ of each other. *Tea1Δ* and *tea4Δ/wsh3Δ*

cells that were the normal rod shape were indistinguishable from wild-type cells with respect to the initial angles of spindle alignment (supplementary material Fig. S1) or any of the above measurements. These data suggest that the behavior observed with T-shaped *tea1* or *tea4*-deleted cells is unlikely to be an indirect consequence of the absence of any single proteins but rather reflects the different shape of the cells.

Spindle alignment in monopolar rod-shaped cells and nonpolarized round cells

The polarity mutant *pom1Δ* is rod shaped and monopolar (as are *tea1Δ* and *tea4Δ/wsh3Δ* cells) with the actin machinery assembled at only one cell tip instead of at both as in wild-type cells (Bahler and Pringle, 1998). *Pom1Δ* cells were also indistinguishable from wild-type cells in the initial angle of SPB separation. We found the same result in rod-shaped *tea1Δ* and *tea4Δ/wsh3Δ* cells (supplementary material Fig. S1). These data suggest that cells align their mitotic spindle with respect to cell geometry independent of cell polarity.

To confirm this suggestion we examined SPB oscillation in round cells using a temperature-sensitive allele of the gene encoding the protein kinase Orb6, which is essential for polarized growth (Verde et al., 1998). *Orb6-25* cells were grown at the restrictive temperature and then released to the permissive temperature for at least 1 hour before examination. MT bundles in these round cells are randomly oriented and frequently change their position (supplementary material Fig. S3). As expected, the range of SPB trajectory angles was much wider than in wild-type cells (Fig. 2C, Fig. 3B,C); however, the direction of the last SPB movement was still within 8 \pm 7 $^{\circ}$ of the axis of SPB separation (Fig. 3B,C and supplementary material Movie 4). Because round cells have no axis of polarity, the axes of SPB separation were set at zero degrees, and all the measurements were calculated relative to this position. Thus, SPB separation upon entry into mitosis occurs along an axis, which is

close to the last SPB movement during interphase, and this correlation is maintained regardless of cell shape.

Spindle alignment in wider rod-shaped diploid cells

Given that interphase MTs become aligned with the cellular axis by physical constraints (Carazo-Salas and Nurse, 2006; Daga et al., 2006a), we hypothesized that in wider cells, in which MTs can grow from perinuclear regions with higher angles relative to the long cellular axis, the initial angle of spindle separation would increase relative to narrower cells. To test this idea we used wild-type diploids cells. Diploid cells are on average around 15-20% wider than wild-type haploid cells ($3.5 \pm 0.1 \mu\text{m}$ vs $4.1 \pm 0.03 \mu\text{m}$ haploid vs diploid cellular diameter) (Mitchison and Nurse, 1985). Diploid cells expressing Sid2-GFP from one chromosomal locus ($h^+/h^- \text{sid2-GFP/sid2}^+$) were imaged every minute as they progressed from interphase into mitosis, and the initial angle of SPB separation relative to long cellular axis was measured. Consistent with the hypothesis that cellular width might affect initial spindle alignment, early mitotic spindles were found to be aligned with a somewhat higher range of angles in diploid than in haploids cells (Fig. 1B). The difference of initial spindle angles between haploid and diploids cells could also be potentially attributed to the impact of cell length on the proposed spindle pre-alignment mechanism, because diploid cells are also longer than haploids cells. The observation that in long diploid cells the SPB still oscillated around the cell center (Fig. 2B) suggests that cell length might not have a critical role on spindle pre-alignment (see Discussion).

Mitotic spindle formation and interphase MT disassembly

We then recorded wild-type cells coexpressing *GFP-atb2* and *sid2-TOMATO* (as microtubule and SPB markers, respectively) progressing from G2 to mitosis to analyze interphase MT depolymerization and the formation of the mitotic spindle; TOMATO is a variant of the monomeric red fluorescent protein (mRFP). We observed that in some cells interphase MTs and very short spindles coexisted for a brief period of time (1-3 minutes) (Fig. 4) (Sagolla et al., 2003; Vogel et al., 2007). We also observed this phenomenon by using immunofluorescence to detect the microtubule cytoskeleton (data not shown). In all cells ($n=15$) the SPBs were perfectly aligned with pre-existing or coexisting interphase bundles, when they separate to form the mitotic spindle (Fig. 4 and supplementary material Movie 5). We confirmed this observation in round or pear-shaped *orb6* mutant cells (supplementary material Fig. S4).

Biological relevance of spindle alignment in the fission yeast

We noticed that even highly misaligned spindles subsequently can separate the two nuclei into daughter cells with high efficiency in wild-type haploid or diploids cells (data not shown). What then could be the biological role of the pre-alignment mechanism described here? In wild-type cells the mitotic spindle elongates during Phase III, reaching the length of the whole cell (Hagan et al., 1990). We therefore speculated that the mechanism of spindle alignment might be more critical in cells in which the mitotic spindle length is reduced. To test this idea we treated wild-type cells labeled with the SPB marker *sid2-GFP* and the actomyosin ring marker *rlc1-GFP* with 25 $\mu\text{g/ml}$ MBC to depolymerize interphase microtubules (at this MBC concentration mitotic spindles still formed but were unable to elongate fully). Under these conditions, properly aligned spindles segregated DNA normally to two daughter cells. However, we also observed that those cells which initiated

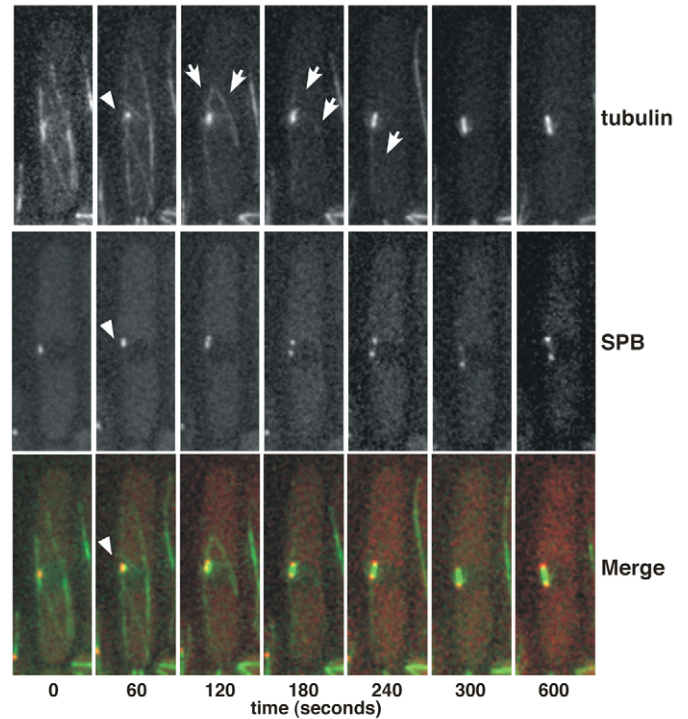


Fig. 4. Interphase MT disassembly and the formation of the mitotic spindle. Wild-type cells expressing *GFP-atb2* and *sid2-TOMATO* (tubulin and SPB markers, respectively) progressing from interphase to mitosis were recorded in multiple focal planes. Maximum projections of eight z-series are shown. Arrowheads indicate the incipient formation of the mitotic spindle. Arrows indicate interphase MTs that are being disassembled as the intranuclear mitotic spindle is formed.

spindle separation with misaligned spindles, underwent unequal segregation with both spindle poles remaining on one side of the actomyosin ring (Fig. 5A). The frequency of mis-segregation increased proportionally with the initial angle of spindle misalignment (Fig. 5B). We also observed that cells with misaligned spindles were able to properly segregate the poles to both sides of the actomyosin ring when the SPB was close to the cell center at the time of mitotic entry. Conversely, cells with the correct initial spindle alignment failed to separate their poles to both sides of the actomyosin ring when the SPB was displaced away from the cell center (data not shown). Thus, the position of the SPB is important for spindle alignment (Zimmerman and Chang, 2005) and becomes more critical in spindles of reduced length.

Discussion

We used cells with different morphologies to investigate the mechanism of initial spindle alignment in the fission yeast. We have shown here that interphase MT bundles attached to the SPBs are essential for proper spindle alignment in the fission yeast. A potential mechanism by which interphase microtubules might pre-align the mitotic spindle is suggested by the behavior of the SPB during G2 and mitosis. In fission yeast, the mother and daughter SPBs remain linked to each other by a bridge until the initiation of mitosis (Ding et al., 1997; Uzawa et al., 2004). Electron microscopic images show: (1) duplicated SPBs remain on the cytoplasmic site of the nuclear envelope until the initiation of mitosis; (2) the duplicated SPBs form an elongated structure with long and short axes of symmetry; and (3) interphase microtubules are bound to both mother and daughter

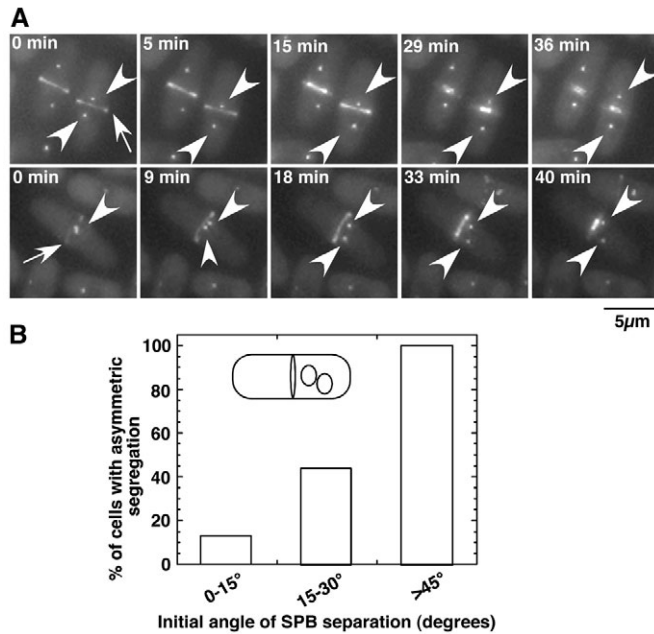


Fig. 5. The initial pre-alignment mechanism is critical for cell survival when the spindle length is compromised. (A) Wild-type cells expressing *sid2*-GFP and *rlc1*-GFP as SPB and actomyosin ring marker, respectively, were treated with 25 $\mu\text{g/ml}$ of the microtubule-depolymerizing drug Carbendazim (MBC) to inhibit the fully extension of the mitotic spindle. The upper panel shows a cell that initiates SPB separation at mitosis aligned with the cellular long axis. The lower panel shows a cell that initiated SPB separation misaligned with the cellular axis. Arrowhead indicates SPBs positions over time whereas the arrows indicate the position of the contractile actomyosin ring at the division site. (B) Quantification of the result shown in A. Graph showing the correlation between initial angle of SPB separation and the frequency of finding the two poles at one side of the division plane.

SPBs (Ding et al., 1997; Uzawa et al., 2004). Because the duplicated SPBs are elongated and both SPBs possess MT-binding activity, interphase microtubules will keep the duplicated SPBs aligned with the axis of the oscillating interphase MTs. Thus, the alignment of

MTs by physical constraint along the long axis of the cell will also align SPBs along the same axis (see Fig. 6). Consistent with this mechanism, in *mtol* mutant cells, which lack MT attachment to the SPBs, the long axis of duplicated SPB and the long axis of the cell are uncoupled resulting in a random spindle alignment.

Therefore, we propose that the attachment of duplicated SPBs to interphase MT bundles allows the spindle to sense the global geometry of the cell, to identify the long axis, and then to pre-align the two poles of the future spindle relative to that axis (Fig. 6). The mechanism by which interphase MTs sense global cell geometry is thought to act by pushing against cell cortex as a result of microtubule plus-end polymerization (Tran et al., 2001; Drummond and Cross, 2000; Daga et al., 2006b).

Thus, interphase MTs regulate spindle positioning by positioning the nucleus (Daga et al., 2006b; Hagan and Yanagida, 1997; Tolic-Norrelykke et al., 2005; Tran et al., 2001), and also regulate the initial steps of spindle alignment by aligning the SPBs with the cellular axis.

When the CLIP170 homologue Tip1 is absent, MTs undergo catastrophe more frequently than do wild-type cells, which results in shorter MTs that do not extend the whole length of the cell and therefore cannot detect the cell geometry as effectively as longer MTs. MT bundles in these cells are very often misaligned with respect to the long axis (Carazo-Salas and Nurse, 2006; Daga et al., 2006a) and are found to have similar misalignment of the mitotic spindle. Similar results were also found in cells lacking the *S. pombe* EB1 homologue Mal3, which also displays short MTs (Vogel et al., 2007).

Consistent with the proposed mechanism for initial spindle alignment, wider diploid cells show a higher range of initial angles of spindle formation that might simply reflect the wider range of angles that interphase MTs can adopt in these cells. Our results suggest that cell width might affect spindle alignment more strongly than cell length, because we observe that in long cells, interphase MTs still hit the cell tips. Furthermore, it might not be necessary that MT bundles make contact with the cell tips because long MT bundles will be aligned with the cellular axis by sliding on the cell cortex.

Our data also suggest that the actin cytoskeleton is not so critical for initial spindle pre-alignment; however, depolymerization of the actin cytoskeleton using drugs and mutants resulted in spindle misalignment later in mitosis (Phase II) (Gachet et al., 2001; Gachet et al., 2004; Tournier et al., 2004). Our unpublished observations are consistent with the idea that actin has a role in spindle alignment because cells treated with Latrunculin A rotate their spindle randomly immediately after the initial pre-alignment. This might explain why in the presence of Latrunculin A, a fraction of cells still misaligned their spindles (see Fig. 1). The molecular

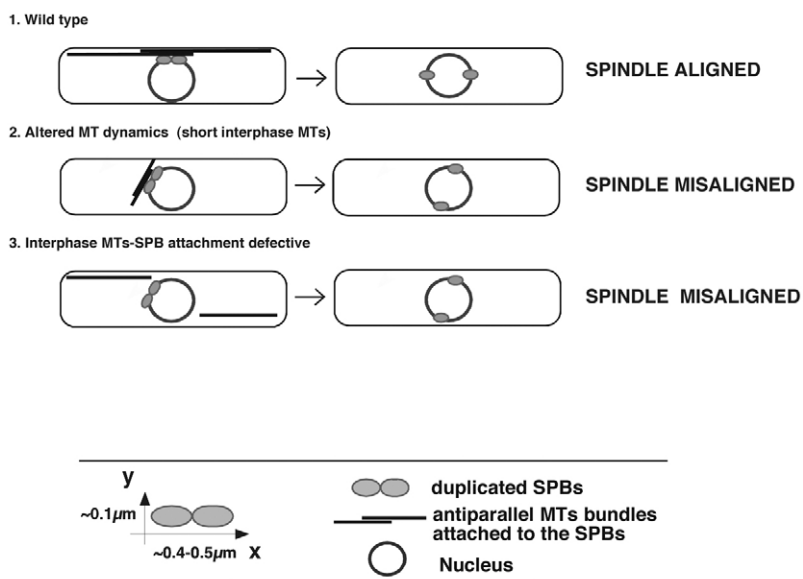


Fig. 6. Model of spindle alignment in *S. pombe*.

(1) Interphase MTs attached to the duplicated SPB align the axis of the duplicated SPBs with the axis of the cell. The drawing at the bottom left shows duplicated SPBs containing a long and a short axis. The distances shown are approximate. (2) Microtubule dynamics is important to align the interphase MTs with the cellular axis. When MTs are too short they are often misaligned with the cellular axis and consequently the mitotic spindle will be misaligned. (3) The attachment between the mitotic spindle and the interphase MTs is critical to transmit the spatial information obtained by dynamic MTs to the duplicated SPBs at the nuclear envelope.

mechanism by which a perturbation of the actin cytoskeleton results in spindle misalignment will require further investigation. We found no differences in the initial angle of spindle alignment between bipolar wild-type cells and different monopolar mutants, suggesting that fission yeast cells do not read cell polarity but read cell geometry.

We propose that spindle alignment in fission yeast occurs in three consecutive steps: in the first step the future axis of mitotic spindle is pre-aligned during interphase by the interaction of the SPB-associated MT bundle with duplicated SPBs at the nuclear envelope. The interaction between interphase MT bundles and the SPB allow alignment of the duplicated SPB with the cellular axis before interphase MT disassembly and SPB insertion into the nuclear envelope. Interphase MT disassembly is tightly coordinated with spindle formation in early mitosis (Sato and Toda, 2007) and so a delay in interphase MT disassembly might interfere with SPB insertion into the nuclear envelope contributing to spindle misalignment. Conversely, if interphase MTs depolymerize too early, the 'memory' of the SPB alignment might be lost before SPB insertion into the envelope and this would also contribute to spindle misalignment (as seen in MBC-treated cells). In the second step, the initial SPB alignment is maintained during Phase II. Microtubules and actin are required to maintain proper spindle alignment during this phase (our unpublished observations), but the mechanisms by which actin and MTs exert their functions to rotate or stabilize the spindle are not yet known. In the third step, which occurs after metaphase, pushing forces exerted by CA-MTs against the cell cortex align the elongating spindles with respect to cellular axis (Tolic-Norrelykke et al., 2004). The lack of any single mechanism is not critical for the cell, but the combined action of the three mechanisms is critical for proper chromosome segregation and cell survival when the length of the mitotic spindle is reduced, as shown in Fig. 5.

One model of spindle alignment, 'the compass model', suggests that in budding yeast, the asymmetry of factors that bind only one of two SPBs, such as Clb4/Cdc28 or Kar9, is required for proper spindle orientation (Kusch et al., 2003; Liakopoulos et al., 2003). Fission yeast cells might use the intrinsically asymmetrical shape of the duplicated SPBs with long and short axes, to align the spindle relative to the long axis of the cell. Defects in spindle positioning can cause genomic instability, defects in cell lineage specification, and alteration of the organ shape during development (Baena-Lopez et al., 2005; Betschinger and Knoblich, 2004; Le Borgne et al., 2002; Wodarz, 2005), and different organisms or cell types are likely to use different strategies to sense their axis of polarity and rotate their spindles. Our study of spindle alignment in the fission yeast has revealed a simple mechanism for interpreting global cellular geometry and for defining the long axis of the cell, which may be relevant to other organisms.

Materials and Methods

Yeast strains and methods

Strains are listed in the supplementary material Table S1. Standard *S. pombe* molecular genetic techniques, media and growth conditions were used as described in Moreno et al. (Moreno et al., 1990) and on the Forsburg Lab website (www.ref.usc.edu/~forsburg/). *Orb6-25* cells (Figs 2 and 3) were grown overnight at 25°C and then shifted to the restrictive temperature (36°C) for 4-6 hours. Cells were then shifted to the permissive temperature again once a significant population of them were completely round, and incubated for at least a further hour at this temperature before microscopic analysis. The swollen cells shown in Fig. 5 were generated as follows: *orb6-25* cells were grown overnight at 25°C and then shifted to 36°C for 4-6 hours. Cells were then shifted to the permissive temperature again to induce cell polarization. Images were taken after 30-90 minutes at 25°C. *tea1Δ* and *tea4Δ* cells were grown overnight to stationary phase in yeast extract medium (plus

supplements when required at 225 mg/l) and then inoculated in fresh medium for 4-5 hours before microscopic analysis. Strains carrying the *nmt*-based pDQ105 (Fig. 4F) were grown in Edinburgh minimal media (EMM) containing 5 μg/ml thiamine (Sigma).

Drug treatments

Exponentially growing *S. pombe* cells were treated with Methyl-2-benzimidazole-carbamate (MBC, Aldrich) used at a final concentration of 25 μg/ml from a 100× stock in DMSO (Sigma) and centrifuged for 2 minutes at 5000 rpm in a tabletop micro-centrifuge to concentrate the cells. Cell imaging started almost immediately after drug addition. Latrunculin A (Molecular Probes) was used at a final concentration of 50 μM from a 0.1 mM stock in DMSO (Sigma) and the same procedure described for the MBC treatment was followed.

Microscopy

Live-cell imaging was performed under Delta-vision wide-field microscope systems (Applied Precision, Issaquah, WA). Asynchronously growing cells placed in an EMM agar pad as described in Tran et al. (Tran et al., 2001). Every minute, 8-12 z-series of 0.5 μm focal-plane steps were acquired, with the exception of Fig. 4, where acquisition was every 2 minutes. The temperature during live-cell imaging was constant at around 24-25°C.

Image analysis

Angles of SPB trajectories, initial SPB separation and spindle alignment at mitosis were measured from maximum 2D projections of z-series. Image analyses and measurements were carried out using Image J (NIH, Bethesda, MD).

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References

- Baena-Lopez, L. A., Baonza, A. and Garcia-Bellido, A. (2005). The orientation of cell divisions determines the shape of Drosophila organs. *Curr. Biol.* **15**, 1640-1644.
- Bahler, J. and Pringle, J. R. (1998). Pom1p, a fission yeast protein kinase that provides positional information for both polarized growth and cytokinesis. *Genes Dev.* **12**, 1356-1370.
- Betschinger, J. and Knoblich, J. A. (2004). Dare to be different: asymmetric cell division in Drosophila, C. elegans and vertebrates. *Curr. Biol.* **14**, R674-R685.
- Bridge, A. J., Morphey, M., Bartlett, R. and Hagan, I. M. (1998). The fission yeast SPB component Cut12 links bipolar spindle formation to mitotic control. *Genes Dev.* **12**, 927-942.
- Brunner, D. and Nurse, P. (2000). CLIP170-like tip1p spatially organizes microtubular dynamics in fission yeast. *Cell* **102**, 695-704.
- Carazo-Salas, R. E. and Nurse, P. (2006). Self-organization of interphase microtubule arrays in fission yeast. *Nat. Cell Biol.* **8**, 1102-1107.
- Colombo, K., Grill, S. W., Kimble, R. J., Willard, F. S., Siderovski, D. P. and Gonczy, P. (2003). Translation of polarity cues into asymmetric spindle positioning in Caenorhabditis elegans embryos. *Science* **300**, 1957-1961.
- Daga, R. R. and Chang, F. (2005). Dynamic positioning of the fission yeast cell division plane. *Proc. Natl. Acad. Sci. USA* **102**, 8228-8232.
- Daga, R. R., Lee, K. G., Bratman, S., Salas-Pino, S. and Chang, F. (2006a). Self-organization of microtubule bundles in anucleate fission yeast cells. *Nat. Cell Biol.* **8**, 1108-1113.
- Daga, R. R., Yonetani, A. and Chang, F. (2006b). Asymmetric microtubule pushing forces in nuclear centering. *Curr. Biol.* **16**, 1544-1550.
- Ding, R., West, R. R., Morphey, D. M., Oakley, B. R. and McIntosh, J. R. (1997). The spindle pole body of Schizosaccharomyces pombe enters and leaves the nuclear envelope as the cell cycle proceeds. *Mol. Biol. Cell* **8**, 1461-1479.
- Drummond, D. R. and Cross, R. A. (2000). Dynamics of interphase microtubules in Schizosaccharomyces pombe. *Curr. Biol.* **10**, 766-775.
- Fernandez-Minan, A., Martin-Bermudo, M. D. and Gonzalez-Reyes, A. (2007). Integrin signaling regulates spindle orientation in Drosophila to preserve the follicular-epithelium monolayer. *Curr. Biol.* **17**, 683-688.
- Gachet, Y., Tournier, S., Millar, J. B. and Hyams, J. S. (2001). A MAP kinase-dependent actin checkpoint ensures proper spindle orientation in fission yeast. *Nature* **412**, 352-355.
- Gachet, Y., Tournier, S., Millar, J. B. and Hyams, J. S. (2004). Mechanism controlling perpendicular alignment of the spindle to the axis of cell division in fission yeast. *EMBO J.* **23**, 1289-1300.
- Gonczy, P. (2002). Mechanisms of spindle positioning: focus on flies and worms. *Trends Cell Biol.* **12**, 332-339.
- Grallert, A., Beuter, C., Craven, R. A., Bagley, S., Wilks, D., Fleig, U. and Hagan, I. M. (2006). S. pombe CLASP needs dynein, not EB1 or CLIP170, to induce microtubule

- instability and slows polymerization rates at cell tips in a dynein-dependent manner. *Genes Dev.* **20**, 2421-2436.
- Hagan, I. and Yanagida, M.** (1995). The product of the spindle formation gene *sad1+* associates with the fission yeast spindle pole body and is essential for viability. *J. Cell Biol.* **129**, 1033-1047.
- Hagan, I. and Yanagida, M.** (1997). Evidence for cell cycle-specific, spindle pole body-mediated, nuclear positioning in the fission yeast *Schizosaccharomyces pombe*. *J. Cell Sci.* **110**, 1851-1866.
- Hagan, I. M., Riddle, P. N. and Hyams, J. S.** (1990). Intramitotic controls in the fission yeast *Schizosaccharomyces pombe*: the effect of cell size on spindle length and the timing of mitotic events. *J. Cell Biol.* **110**, 1617-1621.
- Hiraoka, Y., Toda, T. and Yanagida, M.** (1984). The *NDA3* gene of fission yeast encodes beta-tubulin: a cold-sensitive *nda3* mutation reversibly blocks spindle formation and chromosome movement in mitosis. *Cell* **39**, 349-358.
- Hoog, J. L., Schwartz, C., Noon, A. T., O'Toole, E. T., Mastronarde, D. N., McIntosh, J. R. and Antony, C.** (2007). Organization of interphase microtubules in fission yeast analyzed by electron tomography. *Dev. Cell* **12**, 349-361.
- Kusch, J., Liakopoulos, D. and Barral, Y.** (2003). Spindle asymmetry: a compass for the cell. *Trends Cell Biol.* **13**, 562-569.
- Le Borgne, R., Bellaiche, Y. and Schweisguth, F.** (2002). *Drosophila* E-cadherin regulates the orientation of asymmetric cell division in the sensory organ lineage. *Curr. Biol.* **12**, 95-104.
- Liakopoulos, D., Kusch, J., Grava, S., Vogel, J. and Barral, Y.** (2003). Asymmetric loading of Kar9 onto spindle poles and microtubules ensures proper spindle alignment. *Cell* **112**, 561-574.
- Mallavarapu, A., Sawin, K. and Mitchison, T.** (1999). A switch in microtubule dynamics at the onset of anaphase B in the mitotic spindle of *Schizosaccharomyces pombe*. *Curr. Biol.* **9**, 1423-1426.
- Martin, S. G., McDonald, W. H., Yates, J. R., 3rd and Chang, F.** (2005). Tea4p links microtubule plus ends with the formin for3p in the establishment of cell polarity. *Dev. Cell* **8**, 479-491.
- Mata, J. and Nurse, P.** (1997). *tea1* and the microtubular cytoskeleton are important for generating global spatial order within the fission yeast cell. *Cell* **89**, 939-949.
- Mitchison, J. M. and Nurse, P.** (1985). Growth in cell length in the fission yeast *Schizosaccharomyces pombe*. *J. Cell Sci.* **75**, 357-376.
- Moreno, S., Klar, A. and Nurse, P.** (1991). Molecular genetic analysis of fission yeast *Schizosaccharomyces pombe*. *Methods Enzymol.* **194**, 795-823.
- Nabeshima, K., Nakagawa, T., Straight, A. F., Murray, A., Chikashige, Y., Yamashita, Y. M., Hiraoka, Y. and Yanagida, M.** (1998). Dynamics of centromeres during metaphase-anaphase transition in fission yeast: *Dis1* is implicated in force balance in metaphase bipolar spindle. *Mol. Biol. Cell* **9**, 3211-3225.
- O'Connell, C. B. and Wang, Y. L.** (2000). Mammalian spindle orientation and position respond to changes in cell shape in a dynein-dependent fashion. *Mol. Biol. Cell* **11**, 1765-1774.
- Pearson, C. G. and Bloom, K.** (2004). Dynamic microtubules lead the way for spindle positioning. *Nat. Rev. Mol. Cell Biol.* **5**, 481-492.
- Rupes, I., Webb, B. A., Mak, A. and Young, P. G.** (2001). G2/M arrest caused by actin disruption is a manifestation of the cell size checkpoint in fission yeast. *Mol. Biol. Cell* **12**, 3892-3903.
- Sagolla, M. J., Uzawa, S. and Cande, W. Z.** (2003). Individual microtubule dynamics contribute to the function of mitotic and cytoplasmic arrays in fission yeast. *J. Cell Sci.* **116**, 4891-4903.
- Sato, M. and Toda, T.** (2007). Alp7/TACC is a crucial target in Ran-GTPase-dependent spindle formation in fission yeast. *Nature* **447**, 334-337.
- Sawin, K. E. and Tran, P. T.** (2006). Cytoplasmic microtubule organization in fission yeast. *Yeast* **23**, 1001-1014.
- Sawin, K. E., Lourenco, P. C. and Snaith, H. A.** (2004). Microtubule nucleation at non-spindle pole body microtubule-organizing centers requires fission yeast centrosomin-related protein mod20p. *Curr. Biol.* **14**, 763-775.
- Shaw, S. L., Yeh, E., Maddox, P., Salmon, E. D. and Bloom, K.** (1997). Astral microtubule dynamics in yeast: a microtubule-based searching mechanism for spindle orientation and nuclear migration into the bud. *J. Cell Biol.* **139**, 985-994.
- Sparks, C. A., Morphew, M. and McCollum, D.** (1999). Sid2p, a spindle pole body kinase that regulates the onset of cytokinesis. *J. Cell Biol.* **146**, 777-790.
- Strauss, B., Adams, R. J. and Papalopulu, N.** (2006). A default mechanism of spindle orientation based on cell shape is sufficient to generate cell fate diversity in polarised *Xenopus* blastomeres. *Development* **133**, 3883-3893.
- Tatebe, H., Shimada, K., Uzawa, S., Morigasaki, S. and Shiozaki, K.** (2005). Wsh3/Tea4 is a novel cell-end factor essential for bipolar distribution of Tea1 and protects cell polarity under environmental stress in *S. pombe*. *Curr. Biol.* **15**, 1006-1015.
- Thery, M., Racine, V., Pepin, A., Piel, M., Chen, Y., Sibarita, J. B. and Bornens, M.** (2005). The extracellular matrix guides the orientation of the cell division axis. *Nat. Cell Biol.* **7**, 947-953.
- Tolic-Norrelykke, I. M., Sacconi, L., Thon, G. and Pavone, F. S.** (2004). Positioning and elongation of the fission yeast spindle by microtubule-based pushing. *Curr. Biol.* **14**, 1181-1186.
- Tolic-Norrelykke, I. M., Sacconi, L., Stringari, C., Raabe, I. and Pavone, F. S.** (2005). Nuclear and division-plane positioning revealed by optical micromanipulation. *Curr. Biol.* **15**, 1212-1216.
- Tournier, S., Gachet, Y., Buck, V., Hyams, J. S. and Millar, J. B.** (2004). Disruption of astral microtubule contact with the cell cortex activates a Bub1, Bub3, and Mad3-dependent checkpoint in fission yeast. *Mol. Biol. Cell* **15**, 3345-3356.
- Tran, P. T., Marsh, L., Doye, V., Inoue, S. and Chang, F.** (2001). A mechanism for nuclear positioning in fission yeast based on microtubule pushing. *J. Cell Biol.* **153**, 397-411.
- Tsou, M. F., Ku, W., Hayashi, A. and Rose, L. S.** (2003). PAR-dependent and geometry-dependent mechanisms of spindle positioning. *J. Cell Biol.* **160**, 845-855.
- Uzawa, S., Li, F., Jin, Y., McDonald, K. L., Braunfeld, M. B., Agard, D. A. and Cande, W. Z.** (2004). Spindle pole body duplication in fission yeast occurs at the G1/S boundary but maturation is blocked until exit from S by an event downstream of *cdc10+*. *Mol. Biol. Cell* **15**, 5219-5230.
- Venkatram, S., Tasto, J. J., Feoktistova, A., Jennings, J. L., Link, A. J. and Gould, K. L.** (2004). Identification and characterization of two novel proteins affecting fission yeast gamma-tubulin complex function. *Mol. Biol. Cell* **15**, 2287-2301.
- Verde, F., Wiley, D. J. and Nurse, P.** (1998). Fission yeast orb6, a ser/thr protein kinase related to mammalian rho kinase and myotonic dystrophy kinase, is required for maintenance of cell polarity and coordinates cell morphogenesis with the cell cycle. *Proc. Natl. Acad. Sci. USA* **95**, 7526-7531.
- Vogel, S. K., Raabe, I., Dereli, A., Maghelli, N. and Tolic-Norrelykke, I.** (2007). Interphase microtubules determine the initial alignment of the mitotic spindle. *Curr. Biol.* **17**, 438-444.
- Wodarz, A.** (2005). Molecular control of cell polarity and asymmetric cell division in *Drosophila* neuroblasts. *Curr. Opin. Cell Biol.* **17**, 475-481.
- Zimmerman, S. and Chang, F.** (2005). Effects of {gamma}-tubulin complex proteins on microtubule nucleation and catastrophe in fission yeast. *Mol. Biol. Cell* **16**, 2719-2733.
- Zimmerman, S., Daga, R. R. and Chang, F.** (2004). Intra-nuclear microtubules and a mitotic spindle orientation checkpoint. *Nat. Cell Biol.* **6**, 1245-1246.