

A switch in microtubule dynamics at the onset of anaphase B in the mitotic spindle of *Schizosaccharomyces pombe*

Aneil Mallavarapu*, Ken Sawin† and Tim Mitchison‡

Microtubule dynamics have key roles in mitotic spindle assembly and chromosome movement [1]. Fast turnover of spindle microtubules at metaphase and polewards flux of microtubules (polewards movement of the microtubule lattice with depolymerization at the poles) at both metaphase and anaphase have been observed in mammalian cells [2]. Imaging spindle dynamics in genetically tractable yeasts is now possible using green fluorescent protein (GFP)-tagging of tubulin and sites on chromosomes [3–8]. We used photobleaching of GFP-labeled tubulin to observe microtubule dynamics in the fission yeast *Schizosaccharomyces pombe*. Photobleaching did not perturb progress through mitosis. Bleached marks made on the spindle during metaphase recovered their fluorescence rapidly, indicating fast microtubule turnover. Recovery was spatially non-uniform, but we found no evidence for polewards flux. Marks made during anaphase B did not recover fluorescence, and were observed to slide away from each other at the same rate as spindle elongation. Fast microtubule turnover at metaphase and a switch to stable microtubules at anaphase suggest the existence of a cell-cycle-regulated molecular switch that controls microtubule dynamics and that may be conserved in evolution. Unlike the situation for vertebrate spindles, microtubule depolymerization at poles and polewards flux may not occur in *S. pombe* mitosis. We conclude that GFP-tubulin photobleaching in conjunction with mutant cells should aid research on molecular mechanisms causing and regulating dynamics.

Addresses: *Millennium Pharmaceuticals Inc., Cambridge, Massachusetts 02139, USA. †Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX, UK. ‡Department of Cell Biology, Harvard Medical School, 200 Longwood Avenue, Boston, Massachusetts 02115, USA.

Correspondence: Tim Mitchison
E-mail: timothy_mitchison@hms.harvard.edu

Received: 12 August 1999
Revised: 18 October 1999
Accepted: 18 October 1999

Published: 22 November 1999

Current Biology 1999, 9:1423–1426

0960-9822/99/\$ – see front matter
© 1999 Elsevier Science Ltd. All rights reserved.

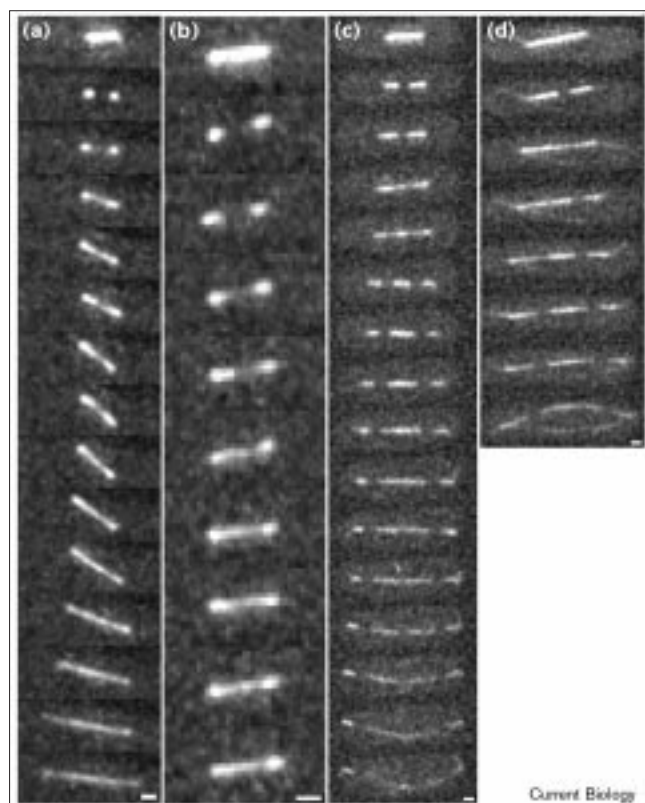
Results and discussion

We chose *S. pombe* to study mitotic spindle microtubule dynamics because its spindle is longer than that of

S. cerevisiae, its kinetochore structure may be more like vertebrates and its mitotic spindle structure has been reconstructed by serial section electron microscopy [11]. Each kinetochore of the 3 chromosomes is attached to 3–4 microtubules. An additional 12–16 non-kinetochore microtubules extend from the spindle poles, terminating with their plus ends distributed throughout the spindle. These overlapping microtubules increase in length and decrease in number during anaphase B. Electron microscopy and comparison with diatom mitosis [9] have led to a model for anaphase B in which spindle elongation is driven by the overlapping microtubules sliding apart and simultaneously elongating at their plus ends [10,11]. Live imaging of mitosis in *S. pombe* using GFP-tubulin and GFP targeted to sites on chromosomes has led to the identification of three phases of mitosis, corresponding to prometaphase, metaphase and anaphase B [5]. None of these studies measured the dynamic behavior of spindle microtubules or observed sliding directly, however. To probe spindle microtubule dynamics in *S. pombe*, we performed laser photobleaching of GFP-tubulin (see Supplementary material). We used similar strains and conditions to those used in previous work [5,6] and imaged with wide-field three-dimensional optical sectioning microscopy. The figures are flattened representations of the three-dimensional data with 0.16 μm pixels. In agreement with published data [5], we could divide mitosis into three phases (see Supplementary material): phase 1, spindle formation by rapid elongation ($\sim 1 \mu\text{m}/\text{min}$) to approximately 1.5 μm in length; phase 2 (metaphase and anaphase A), slow elongation ($\sim 0.09 \mu\text{m}/\text{min}$) from $\sim 1.5 \mu\text{m}$ to $\sim 3 \mu\text{m}$; phase 3 (anaphase B), rapid elongation ($\sim 0.8 \mu\text{m}/\text{min}$) from 2–3 μm to ~ 10 –12 μm , at which point the spindle disassembles. To test whether bleaching perturbed spindle function, we compared timing, length and velocity parameters for unbleached and bleached spindles and found no difference (see Supplementary material).

Bleach marks made on phase 2 spindles (Figure 1a,b) recovered fluorescence within 2–3 minutes, unless the cells went into phase 3. This indicates that bleached tubulin subunits exchanged with free (unbleached) subunits. To quantitate recovery, we plotted the amount of total fluorescence present in the whole spindle and in the bleached zone, and the ratio of these two values, as a function of time (Figure 2). Laser irradiation sufficient to bleach the center of the spindle to background levels diminished total spindle fluorescence to 30% of its initial value. Total spindle fluorescence recovered to 60% of its

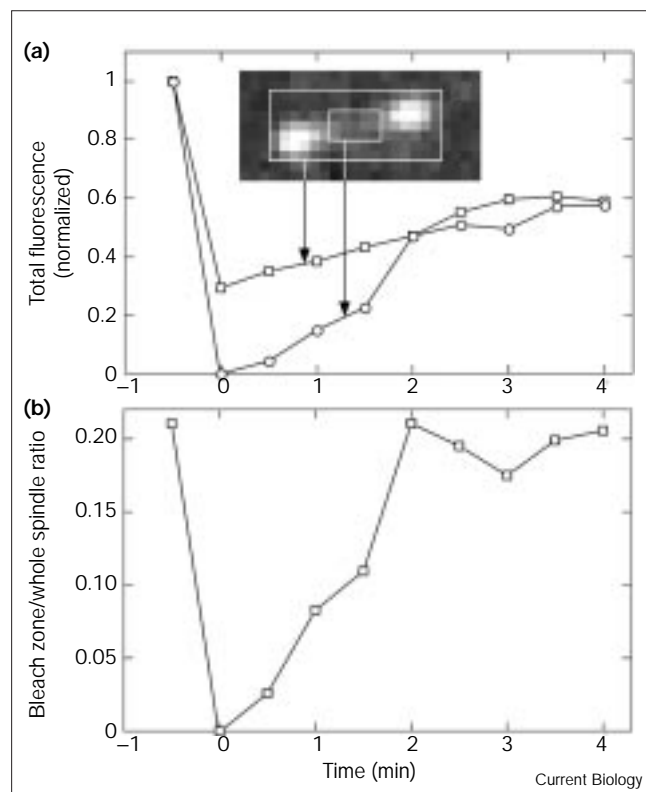
Figure 1



Photobleaching of GFP-tubulin in the *S. pombe* mitotic spindle. (a,b) Phase 2 and (c,d) phase 3 spindles are shown unbleached (top), a few seconds after bleaching (second to top) and (a,c,d) every 1 min or (b) every 30 sec thereafter. The scale bar represents 1.0 μm in all sequences. We collected seven focal planes at z-intervals of 0.5 μm and a final resolution of 0.12 μm . The images were deconvolved to generate a three-dimensional data set, and then projected to give a single flattened image (see Supplementary material). In (a,b), note the recovery of fluorescence in the bleached zone. Fluorescence intensity in the recovering region is often variable. In (c,d) note the lack of recovery, and sliding apart, of the bleached zone.

initial value in 2.5 minutes and then reached a plateau. The final level reflected the amount of unbleached GFP-tubulin remaining in the cell. Total fluorescence in the bleached zone was diminished to background immediately after the bleach, and it recovered to 60% of its initial value over a similar time period. The ratio of bleached-zone fluorescence to total fluorescence provides a measure of the anisotropy of fluorescence after bleaching. This ratio recovered to approximately its initial value in two minutes. From these data, we estimate a half-life for microtubule turnover in the range of 0.75–1.5 minutes for the *S. pombe* spindle in phase 2. Visual inspection of ten phase 2 bleaching experiments indicated that this estimated turnover rate is typical. We often observed small numbers of bright pixels appearing in the bleached zone in the spindle center during recovery (Figure 1a,b). These bright pixels sometimes appeared to move between

Figure 2

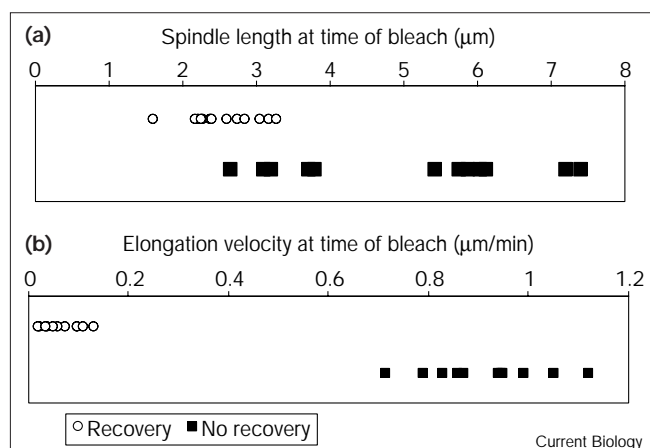


Quantitation of fluorescence recovery for the spindle shown in Figure 1b (in phase 2). (a) Fluorescence intensity data. Open squares, total spindle fluorescence (minus background) measured in a 7×17 pixel box around the spindle and normalized to the pre-bleach total; open circles, total bleached zone fluorescence (minus background) measured in a 3×5 pixel box in the spindle center and normalized to the pre-bleach total. The bleach was a few seconds before time zero. The inset shows the two measurement boxes superimposed on the spindle in mid recovery. (b) The ratio of total bleached zone fluorescence to total spindle fluorescence. This provides a measure of the rate at which spindle fluorescence becomes homogeneous during recovery.

frames, but we could not unambiguously distinguish movement from spatially inhomogeneous recovery at the spatial and temporal resolution of our data.

Bleach marks made on phase 3 spindles did not recover fluorescence at all. Instead, they moved outwards at the same rate as spindle elongation (Figure 1c,d). The switch from recovering to non-recovering appeared to be sharp. We grouped all our sequences into two categories: recovery (fluorescence in the bleached zone recovered to a value greater than the background fluorescence in the cell) and no recovery (fluorescence in the bleached zone remained not significantly different from background throughout the time-course). When spindle length (Figure 3a) or spindle elongation velocity at the time of bleaching (Figure 3b) of these groups were compared, a

Figure 3



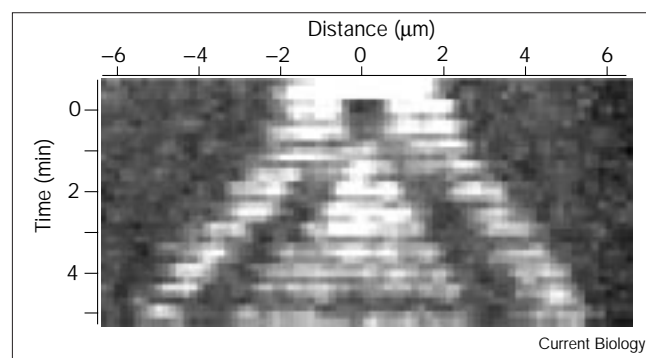
Switch in microtubule dynamics between phase 2 and 3. The 20 bleaching experiments were divided into two groups: those showing any fluorescence recovery significantly above background, and those showing no recovery. (a) The spindle length at the time of bleaching and (b) spindle elongation velocity at the time of bleaching were plotted for the two groups. Note that the recovering spindles were typically shorter and were always elongating less rapidly than the non-recovering spindles. These data indicate that the recovering spindles were in phase 2 at the time of bleaching, while the non-recovering spindles were in phase 3.

clear difference was apparent. The switch from recovering to non-recovering is sharp, and it occurs at the transition from phase 2 to 3, corresponding to the onset of anaphase B.

The non-recovery of marks made early in phase 3 allowed us to track microtubule sliding unambiguously for the rest of mitosis, either visually or by kymograph analysis (Figure 4). This analysis allowed us to trace the movement of the spindle poles and the two edges of the bleached zones. Lines drawn on these points in the composite were all parallel within experimental error, indicating that the poles and the two edges of the bleached zone moved outwards at the same rate. We also measured the length of the fluorescent zone between the pole and the polewards edge of the bleached zone as a function of time. We found that this distance remained constant within our measurement error (mean change $< 0.2 \mu\text{m}$, $n = 4$ cells, 8 marks). Both types of analysis suggest that no detectable microtubule polymerization or depolymerization occurs at the poles during phase 3 ($< 0.2 \mu\text{m}$ over phase 3).

With respect to the anaphase B mechanism, our data provide strong, direct confirmation of a model in which spindle elongation occurs with sliding apart of overlapping microtubules while they polymerize at their plus ends [9,11]. If any minus-end dynamics occur during anaphase B, they must be very minor. With respect to spindle dynamics, our data suggest that spindle microtubules turn

Figure 4



Kymograph analysis of a bleaching experiment in a phase 3 spindle. The image is a composite made from the phase 3 bleaching experiment shown in Figure 1c. Three lines of pixels containing most of the spindle fluorescence were cut from the data from each time point and pasted together in descending order. This presentation allows visual tracking of spindle elongation and outwards movement of the two bleached zones. Note that the poles, the poleward edge of the bleached zone, and the equatorial edge of the bleached zone all move outward at the same rate (approximately $0.45 \mu\text{m}/\text{min}$ in this sequence). The impression of slight widening of the right-hand bleach zones over time is probably due to decrease in signal intensity at the pole.

over rapidly at metaphase in *S. pombe*, as they do in metazoan spindles. Four known mechanisms might contribute to turnover: dynamic instability of free plus ends [12], polewards flux [13,14], polymerization and depolymerization of kinetochore microtubules at their plus ends coupled to directional instability of kinetochores [15] and release of microtubules from nucleating sites followed by depolymerization at minus ends [16]. Given the lack of depolymerization at poles in phase 3, the absence of evidence for flux in phase 2, the structure of the spindle pole [10] and data from *Saccharomyces cerevisiae* [7], we suspect that minus ends are not dynamic during mitosis in *S. pombe* and favor the first and third mechanisms [12,15] for turnover. Polewards flux may be a specialization for mitosis in larger cells in which longer distances of chromosome transport are required.

The abrupt switch from rapid fluorescence recovery in phase 2 to complete non-recovery in phase 3 could have two, non-exclusive interpretations: a change in the population of microtubules imaged (phase 2 spindles containing kinetochore and non-kinetochore microtubules and phase 3 spindles containing mostly non-kinetochore microtubules) or regulation of turnover rate within a population. In the center of the phase 2 spindle, where we made bleached zones, non-kinetochore microtubules account for at least 80% of the total [11]. Thus, we interpret our data as showing that the turnover rate of non-kinetochore microtubules decreases abruptly at the onset of anaphase B. Microtubules also become abruptly more stable at anaphase in vertebrate spindles [17], suggesting

that this switch may use a mechanism that is conserved in evolution. The change in dynamics in *S. pombe* correlates with an interesting change in the geometry with which overlapping, antiparallel microtubules interact: in phase 2 they interact by loose hexagonal packing, whereas in phase 3 they interact by tight square packing [11]. This change may indicate activation of an anaphase B microtubule cross-linking factor or motor that is also responsible for microtubule stabilization. By probing dynamics in mutant cells it should be possible to address the molecular mechanisms that regulate dynamics.

Supplementary material

Supplementary material including additional background material, figures showing the properties of bleached and unbleached GFP-tubulin and additional methodological details is available at <http://current-biology.com/supmat/supmatin.htm>.

Acknowledgements

We thank D-Q. Ding and Y. Hiraoka for providing the GFP-tubulin plasmid, and Paul Maddox, Ted Salmon and Jennifer Tirnauer for valuable discussions. This work was supported by grants (to T.M.) NIH GM39565 and GM48027.

References

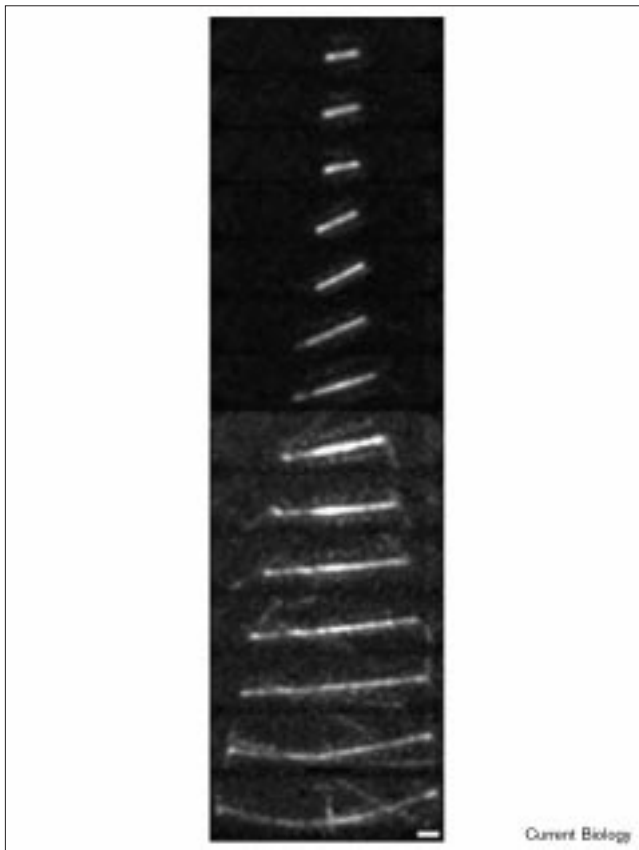
- Inoue S, Sato H: Cell motility by labile association of molecules. The nature of mitotic spindle fibers and their role in chromosome movement. *J Gen Physiol* 1967, 50 (suppl):259-292.
- Inoue S, Salmon ED: Force generation by microtubule assembly/disassembly in mitosis and related movements. *Mol Biol Cell* 1995, 6:1619-1640.
- Carminati JL, Stearns T: Microtubules orient the mitotic spindle in yeast through dynein-dependent interactions with the cell cortex. *J Cell Biol* 1997, 138:629-641.
- Straight AF, Marshall WF, Sedat JW, Murray AW: Mitosis in living budding yeast: anaphase A but no metaphase plate. *Science* 1997, 277:574-578.
- Nabeshima K, Nakagawa T, Straight AF, Murray A, Chikashige Y, Yamashita YM, et al.: Dynamics of centromeres during metaphase-anaphase transition in fission yeast: Dis1 is implicated in force balance in metaphase bipolar spindle. *Mol Biol Cell* 1998, 9:3211-3225.
- Ding DQ, Chikashige Y, Haraguchi T, Hiraoka Y: Oscillatory nuclear movement in fission yeast meiotic prophase is driven by astral microtubules, as revealed by continuous observation of chromosomes and microtubules in living cells. *J Cell Sci* 1998, 111:701-712.
- Maddox P, Chin E, Mallavarapu A, Yeh E, Salmon ED, Bloom K: Microtubule dynamics from mating through the first zygotic division in the budding yeast *Saccharomyces cerevisiae*. *J Cell Biol* 1999, 144:977-987.
- Tirnauer JS, O'Toole E, Berrueta L, Bierer BE, Pellman D: Yeast Bim1p promotes the G1-specific dynamics of microtubules. *J Cell Biol* 1999, 145:993-1007.
- Cande WZ, McDonald KL: In vitro reactivation of anaphase spindle elongation using isolated diatom spindles. *Nature* 1985, 316:168-170.
- Page BD, Snyder M: Chromosome segregation in yeast. *Annu Rev Microbiol* 1993, 47:231-261.
- Ding R, McDonald KL, McIntosh JR: Three-dimensional reconstruction and analysis of mitotic spindles from the yeast, *Schizosaccharomyces pombe*. *J Cell Biol* 1993, 120:141-151.
- Desai A, Mitchison TJ: Microtubule polymerization dynamics. *Annu Rev Cell Dev Biol* 1997, 13:83-117.
- Mitchison TJ: Polewards microtubule flux in the mitotic spindle: evidence from photoactivation of fluorescence. *J Cell Biol* 1989, 109:637-652.
- Sawin KE, Mitchison TJ: Poleward microtubule flux mitotic spindles assembled in vitro. *J Cell Biol* 1991, 112:941-954.
- Skibbens RV, Skeen VP, Salmon ED: Directional instability of kinetochore motility during chromosome congression and segregation in mitotic newt lung cells: a push-pull mechanism. *J Cell Biol* 1993, 122:859-875.
- Rodionov VI, Borisy GG: Microtubule treadmilling in vivo. *Science* 1997, 275:215-218.
- Zhai Y, Kronebusch PJ, Borisy GG: Kinetochore microtubule dynamics and the metaphase-anaphase transition. *J Cell Biol* 1995, 131:721-734.

A switch in microtubule dynamics at the onset of anaphase B in the mitotic spindle of *Schizosaccharomyces pombe*

Aneil Mallavarapu, Ken Sawin and Tim Mitchison

Current Biology 22 November 1999, 9:1423–1426

Figure S1

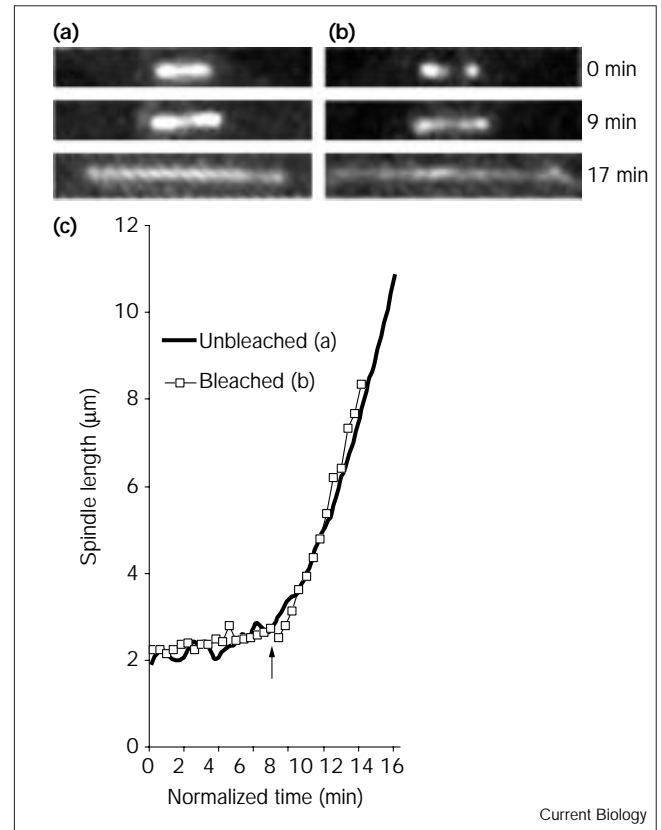


Mitosis in *S. pombe* imaged using GFP–tubulin without photobleaching. The images are 2 min apart; the scale bar represents 1 μm . In the original sequence the images were 1 min apart and some photobleaching occurred. The images in the second half of the sequence are presented at increased brightness to compensate for this. Our image sequences, and the elongation rates we measured from them, are similar to published ones [S8].

Supplementary background material

Photobleaching of fluorescein–tubulin shows that mitotic spindle microtubules exchange subunits with the soluble pool on a time scale of seconds to minutes in mammalian cells [S1] and sea urchin embryos [S2]. This rapid turnover is now thought to mainly reflect dynamic instability of spindle microtubules. Photoactivation of caged fluorescein–tubulin demonstrated a polewards flux of kinetochore [S3] and total [S4,S5] spindle microtubules. Polewards flux had previously been suspected from polewards movement of zones of reduced birefringence created by UV ablation of microtubules [S6].

Figure S2



Photobleaching does not perturb progress through mitosis.

(a,b) Selected time points from (a) an unbleached and (b) a bleached spindle. (c) Graph of spindle length as a function of time. The curves were superimposed so that the phase 2–3 transition (arrow) occurred at the same time in both. Note the very similar spindle behavior with and without photobleaching. Measurements of 10 unbleached and 20 bleached sequences confirmed this conclusion.

Supplementary materials and methods

Fission yeast (*S. pombe* strain PN43; *leu1-32 h⁻*) were transformed with the plasmid pDQ105 (gift of Da-Qiao Ding), which carries a GFP–atb2p fusion protein under the control of the *nmt1* promoter [S7]. Transformants were grown and imaged under conditions of low GFP–atb2p expression as described [S7]. For imaging experiments, 3.5 μl log-phase culture was mounted under a 25 mm² coverslip and sealed with wax. In this thin preparation, the cells are held in place but divide normally.

Cells were observed on an inverted microscope equipped with cooled CCD and ISIT (intensified video) cameras and a 488 nm laser to generate 0.2–0.8 sec photobleaching pulses. Cells early in mitosis were found and positioned using the ISIT image and dim epifluorescence illumination with a 100 \times objective. At each time point, a stack of 7 images at 0.5 μm z-step intervals were collected to the CCD using a

standard fluorescein filter set. The camera had 8 μm pixels that were binned 2×2 to give a pixel size in the image of 0.16 μm using a 100 \times objective. Image stacks were collected every 30 sec or 60 sec, and it took approximately 4 sec to collect one stack. Image stacks were deconvolved using software that comes with the Deltavision microscope (Applied Precision). For display and intensity measurement purposes, the resulting 3D image was flattened (projected in z) to generate a 2-dimensional image. For distance measurement we used the 3D data set. A more complete plan of the microscope and the image collection software will be placed on our web site (<http://iccbweb.med.harvard.edu/mitchisonlab/>).

Supplementary references

- S1. Saxton WM, Stemple DL, Leslie RJ, Salmon ED, Zavortink M, McIntosh JR: **Tubulin dynamics in cultured mammalian cells.** *J Cell Biol* 1984, **99**:2175-2186.
- S2. Salmon ED, Leslie RJ, Saxton WM, Karow ML, McIntosh JR: **Spindle microtubule dynamics in sea urchin embryos: analysis using a fluorescein-labeled tubulin and measurements of fluorescence redistribution after laser photobleaching.** *J Cell Biol* 1984, **99**:2165-2174.
- S3. Mitchison TJ: **Polewards microtubule flux in the mitotic spindle: evidence from photoactivation of fluorescence.** *J Cell Biol* 1989, **109**:637-652.
- S4. Sawin KE, Mitchison TJ: **Poleward microtubule flux mitotic spindles assembled in vitro.** *J Cell Biol* 1991, **112**:941-954.
- S5. Desai A, Maddox PS, Mitchison TJ, Salmon ED: **Anaphase A chromosome movement and poleward spindle microtubule flux occur at similar rates in *Xenopus* extract spindles.** *J Cell Biol* 1998, **141**:703-713.
- S6. Forer A: **Local reduction of spindle fibre birefringence in living *Nephrotoma suturalis* (Loed) spermatocytes induced by ultraviolet microbeam irradiation.** *J Cell Biol* 1965, **25**:95-117.
- S7. Ding DQ, Chikashige Y, Haraguchi T, Hiraoka Y: **Oscillatory nuclear movement in fission yeast meiotic prophase is driven by astral microtubules, as revealed by continuous observation of chromosomes and microtubules in living cells.** *J Cell Sci* 1998, **111**:701-712.
- S8. Nabeshima K, Nakagawa T, Straight AF, Murray A, Chikashige Y, Yamashita YM, *et al.*: **Dynamics of centromeres during metaphase-anaphase transition in fission yeast: Dis1 is implicated in force balance in metaphase bipolar spindle.** *Mol Biol Cell* 1998, **9**:3211-3225.