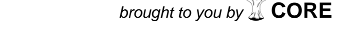
View metadata, citation and similar papers at core.ac.uk
brought to you by

provided by Elsevier - Publisher Connector

Current Biology 16, 1544–1550, August 8, 2006 ©2006 Elsevier Ltd All rights reserved DOI 10.1016/j.cub.2006.06.026

Report

Asymmetric Microtubule Pushing Forces in Nuclear Centering

Rafael R. Daga,[1,2] Ann Yonetani,[1] and Fred Chang[1,*]
[1] Department of Microbiology
Columbia University College of Physicians and Surgeon
New York, New York 10032

Summary

Dynamic properties of microtubules contribute to the establishment of spatial order within cells. In the fission yeast *Schizosaccharomyces pombe*, interphase cytoplasmic microtubules are organized into antiparallel bundles that attach to the nuclear envelope and are needed to position the nucleus at the geometric center of the cell [1, 2]. Here, we show that after the nucleus is displaced by cell centrifugation, these microtubule bundles efficiently push the nucleus back to the center. Asymmetry in microtubule number, length, and dynamics contributes to the generation of force responsible for this unidirectional movement. Notably, microtubules facing the distal cell tip are destabilized when the microtubules in the same bundle are pushing from the proximal cell tip. The CLIP-170-like protein tip1p [3] and the microtubule-bundling protein ase1p [4, 5] are required for this asymmetric regulation of microtubule dynamics, indicating contributions of factors both at microtubule plus ends and within the microtubule bundle. Mutants in these factors are defective in nuclear movement. Thus, cells possess an efficient microtubule-based engine that produces and senses forces for centering the nucleus. These studies may provide insights into mechanisms of asymmetric microtubule behaviors and force sensing in other processes such as chromosome segregation and cell polarization.

Results and Discussion

The process of nuclear centering involves sensing of the cell tips and measuring the medial point. Interphase *S. pombe* cells have longitudinal microtubule (MT) bundles arranged in an antiparallel manner, with the MT plus ends facing cell tips and MT minus ends in a medial overlapping region near the nucleus [1–3, 6]. Most MT bundles are attached to the nuclear envelope [1], with one associated laterally to the cytoplasmic face of the spindle pole body and others at other sites. The dynamics of MT plus ends are strictly regulated: MTs grow from the perinuclear region to the cell tips, contact the cell tip, and continue growing for about 80 s (dwell time), before shrinking back to the nucleus. Each time the MT plus ends contact and grow at cell tips, they can exert a small pushing force on the nucleus, causing small deformations and rocking of the nucleus. These types of observations, coupled with mathematical simulations, have suggested a model in which a balance of microtubule pushing forces by these bundles positions the nucleus at the center of the cell [1, 7]. However, because significant movements of the nucleus generally do not occur during interphase, it has been difficult to test this model in a quantitative manner. For instance, it has not been demonstrated whether these microtubules are capable of moving the nucleus over long distances with any efficacy, or whether they only function in maintaining nuclear position.

Here, we studied the mechanism of nuclear centering by using cell centrifugation to displace the nucleus many microns (avg. 3–4 μm) away from the center of the cell [8]. We observed that after centrifugation, nuclei moved back toward the cell center in an efficient and reproducible manner in 17 ± 6.7 min, depending on the length of the cell and extent of displacement (Figures 1A and 1B, Table 1, Movie S1 in the Supplemental Data available online; see also [7, 8]). Notably, nuclear movement was unidirectional until the nucleus reached the cell middle, at which point it oscillated, as in uncentrifuged cells. The average maximal velocity was 0.44 ± 0.18 μm/min (range 0.24–0.82 μm/min). Nuclear movement was dependent on microtubules and not actin, as shown by drug treatments (Figure 1C, Figure S1, Table S2). In the *mto1Δ* mutant (a microtubule-organizing-center protein, also known as *mbo1 or mod20*), cytoplasmic MTs are present, but not attached to the nucleus [9]. In this case, nuclear movement was greatly impaired (Figure 1C, Table S2, Figure S1, Movie S3). Thus, nuclear movement required not only MTs, but also the attachment of the MTs to the nuclear envelope.

Nuclear movement utilized a MT pushing mechanism generated from plus-end polymerization. The nuclear envelope developed large extensions that lead nuclear movement, suggesting considerable viscous drag opposing movement from the cytoplasm [10] (Figures 1 and 2). These membrane deformities occurred at multiple MT attachment sites on the nuclear envelope, although the most prominent deformities and movements were associated with the spindle pole body (SPB) (data not shown) [11]. The formation and movement of these extensions correlated with periods when one (or more) MT bundle in back of the nucleus contacted the proximal cell tip (Figures 1B and 2A). Many of these events were accompanied by the buckling of microtubules behind the nucleus, indicative of MT compressive forces. Imaging of tubulin-GFP speckles in the MT lattice showed that the movements are associated with MT plus-end polymerization at the proximal cell tip (the cell tip closer to the nucleus) (Figure S2). This movement was dynein independent (data not shown), and we detected no evidence for MT pulling from the distal cell tip (the cell tip farther away from the nucleus). In some longer cells, we observed nuclear movement even when no MTs contacted the distal cell tip (Figure 1B).

*Correspondence: fc99@columbia.edu
[2] Present address: CABD/Universidad Pablo de Olavide, Carretera de Utrera Km1 41013, Seville, Spain.

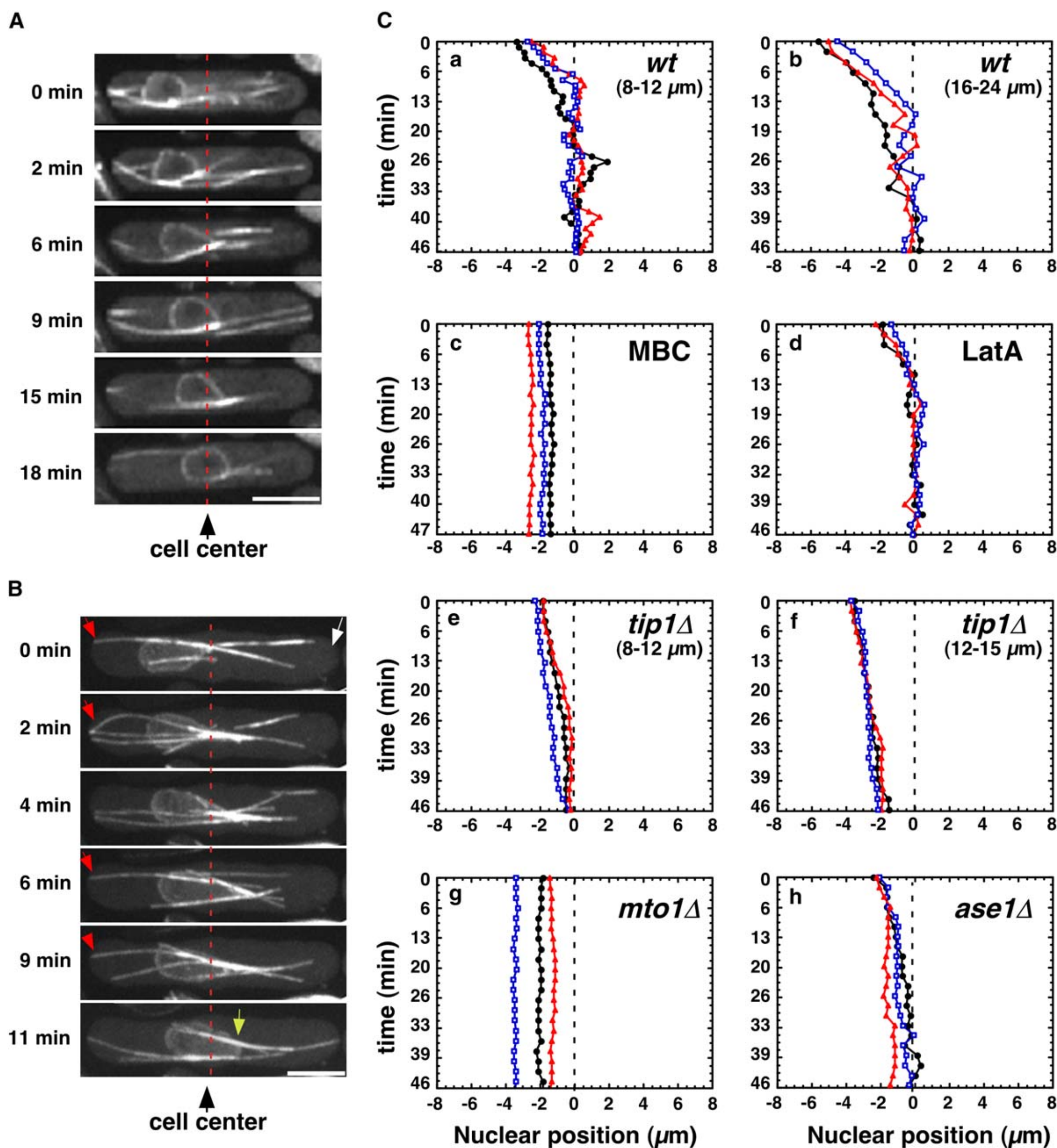


Figure 1. Nuclear Centering Is Dependent on Microtubules and Microtubule Regulatory Proteins

(A) Wild-type fission-yeast cells (RD92) expressing *cut11-GFP GFP-atb2* (GFP markers for the nuclear envelope and microtubules, respectively) were centrifuged to displace the nucleus and then immediately imaged by time-lapse 3D confocal microscopy. Representative images from a single Z section are shown.

(B) Similar to (A), except *cdc25-22* cells, which were grown at 36°C for 3 hr and imaged at 25°C, were used to increase cell length. Maximum-projection images are shown. Note that the nucleus moves when proximal MTs (in back of the nucleus) contact and buckle (red arrows), even when there is no MT contacting the distal cell tip (white arrow). Also, the nucleus becomes less stretched when the proximal MT shrinks (yellow arrow).

(C) Plots of nuclear position (centroid) over the time after centrifugation, each showing behavior in three representative cells. (C_a) Wild-type cells with indicated lengths. (C_b) Wild-type cells that were elongated by treatment with 11 mM hydroxyurea for 4 hr. (C_c and C_d) Wild-type cells treated with 25 μg/ml methyl-2-benzimidazole-carbamate (MBC) or 100 μM Latrunculin A (LatA), respectively. (C_e and C_f) *tip1*Δ (CLIP170) cells (RD90) of different lengths. (C_g) *mto1*Δ cells (RD122). (C_h) *ase1*Δ cells (RD232). On the x axis, 0 represents the cell center.
The scale bars represent 5 μm.

Thus, MT pushing forces can provide the sufficient force for moving the nucleus over many microns.

The directed, unidirectional movement of the nucleus indicates that there must be a net MT-based force toward the center of the cell. As predicted [1], the average number of MTs contacting the proximal tip at any one time was higher than at the distal tip (Table 1). This is due, in part, to the shorter time and distance the MTs need to grow to reach the proximal tip, as compared to reaching distal tip. A second source of asymmetry is

Table 1. Asymmetric Microtubule Behavior

Genotype	Nuclear Position	MT Location	Dwell Time (s)	Avg. # MT Contact	Cell Length (μm)	MT (n)	Cell (n)
wild-type	e e	either	83.7 ± 46.6	1.5 ± 0.4	11.8 ± 1.3	165	17
wild-type		proximal	91.9 ± 53.4	1.73 ± 0.3	12.4 ± 1.6	154	27
wild-type	p d	distal	45.6 ± 36.3	0.75 ± 0.4	12.4 ± 1.6	126	27
tip1Δ		either	41.4 ± 25.1	0.91 ± 0.3	11.4 ± 1.6	83	8
tip1Δ		proximal	43.8 ± 26.7	1.31 ± 0.3	11.7 ± 1.6	143	19
tip1Δ		distal	33.6 ± 27.2	0.47 ± 0.1	11.7 ± 1.6	57	19
ase1Δ		either	101 ± 52.8	1.8 ± 0.5	11.1 ± 3.2	83	8
ase1Δ		proximal	114 ± 70	2 ± 0.3	13.2 ± 1.2	96	17
ase1Δ		distal	112 ± 74.3	1.6 ± 0.3	13.2 ± 1.2	77	17
tip1Δ ase1Δ		either	43.5 ± 24.4	0.72 ± 0.5	11.3 ± 1.6	52	12
tip1Δ ase1Δ		proximal	41.1 ± 22.2	0.95 ± 0.3	11.9 ± 1.6	56	12
tip1Δ ase1Δ		distal	26.8 ± 16.9	0.4 ± 0.5	11.9 ± 1.6	28	12

The dynamics of MTs in cells with a symmetric or asymmetric nucleus were observed by time-lapse 3D confocal microscopy. "Proximal MTs" refers to those on the side of the nucleus closest to a cell tip, and "distal MTs" are those on the opposite side of the nucleus. "Dwell times" refer to the period that a MT contacts a cell end before shrinking. "Average number of MTs contact" refers to the number of MTs that contact the cell end at any one time. n is the total number assayed for these measurements.

predicted to be MT stiffness: In vitro experiments show that short MTs are stiffer and thus should be able to push more efficiently than longer ones [12, 13].

Unexpectedly, we discovered a third asymmetric parameter, MT dynamics. Previous in vitro studies show that compressive forces during MT pushing promote MT catastrophe [13], predicting that the pushing proximal MTs would be less stable than the distal MTs. Our in vivo measurements showed the opposite: MTs facing the proximal cell tip (the pushing ones) showed, on average, normal dwell times (91.9 ± 53.4 s versus 83.7 ± 46.6 s in cells with a medial nucleus), although some stayed at cell tips for much longer. In contrast, MT plus ends at the distal cell tip were less stable than those facing the proximal cell tip. Of MTs that reached the cell tip, dwell times at the distal tip were significantly less (45.6 ± 36.3 s; $p < 0.001$) than the dwell times at the proximal cell tip and less than those seen in cells with a symmetrically positioned nucleus (Table 1; Movie S2). Other abnormal events were also observed specifically in these distal MTs, including catastrophe even before hitting the cell tip, and pause and rescue events, which occur only rarely in normal cells. As a result of these abnormal behaviors, the distal MTs rarely contacted the distal cell tip and did not appear to generate pushing forces that would counteract the forces from the proximal MTs. These effects were spatially specific and transient; they disappeared when the nucleus re-centered.

Compressive forces within in the bundle may regulate asymmetric MT catastrophe. Catastrophe frequency of the distal MTs was significantly higher when the corresponding proximal MT in the same bundle was pushing, as compared to those when the proximal MT was not contacting the cell tip ($p < 0.005$) or when another proximal MT in a different bundle was contacting the cell tip ($p < 0.005$; Figure 2C). This suggested that pushing from a proximal MT in the bundle activates destabilization of a distal MT in the same bundle.

Asymmetry in MT dynamics was not due to the cell-centrifugation protocol. Similar effects were observed when the nucleus was displaced by using another method, in *cdc10* mutants, which grow asymmetrically (Figure S2). Also, MTs were not destabilized simply because they were too long. Abnormally long cells (*cdc25* cells without centrifugation) have much longer MTs, which exhibited largely normal dynamics (Movie S4). In addition, many of the distal MTs in centrifuged cells exhibited abnormal behaviors even when they were shorter than the proximal ones.

Because the dynamic properties of these microtubules differ from pure microtubules in the in vitro systems [13], we predicted that MT-regulatory proteins are operating for this mechanism in vivo. In screening for genes that affect nuclear centering, we identified two gene products required for asymmetric MT dynamics: the CLIP170-like protein tip1p and the PRC homolog ase1p.

Ase1p is a conserved MT-bundling protein that localizes to regions of antiparallel MT overlap and stabilizes interphase and spindle midzone MT bundles [4, 5, 14]. In uncentrifuged *ase1Δ* cells, interphase MT dynamics were not affected (Table S1, see also [4]), and antiparallel bundles were still present. After centrifugation, proximal and distal MTs were equally stable at both cell tips, with dwell times similar to those in noncentrifuged wild-type ones, (114 ± 70 s proximal tip versus 122 ± 74.3 s distal tip, Table 1, Figure 3, Movie S5). Notably, buckling of both proximal and distal MTs was apparent, suggesting that both sets of MT exert pushing forces (Figure 3), which did not occur in wild-type cells. Thus, ase1p is needed for the destabilization of the distal MTs and may play a role in the transmission of signals or forces that are propagated through the medial MT overlap region.

Nuclear centering in *ase1Δ* cells was very inefficient in our assay (Table S2), similar to previous observations [4, 5]. Our observations suggested multiple causes of this

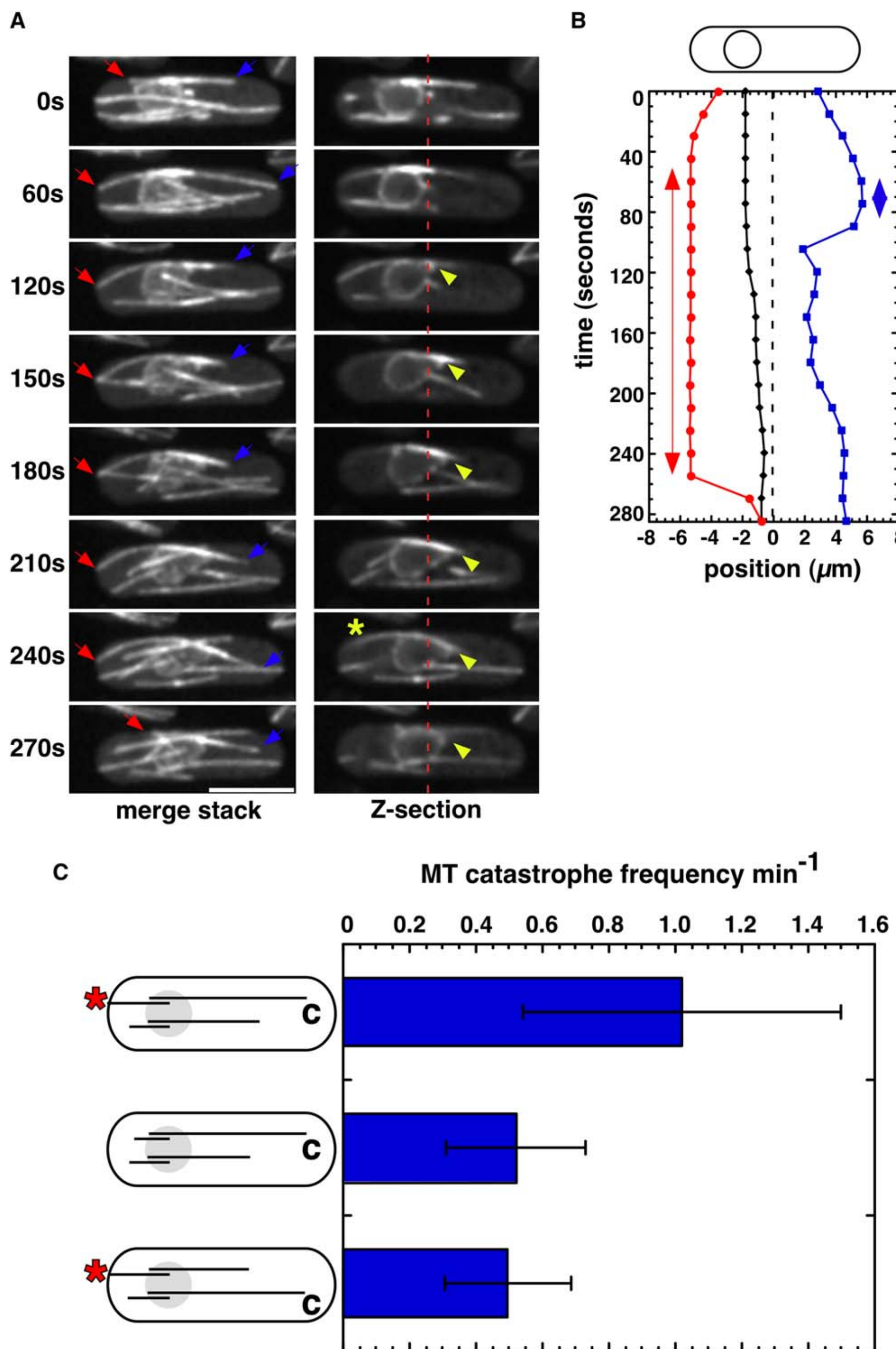


Figure 2. Asymmetric Microtubule Dynamics in Cells with a Displaced Nucleus

Wild-type cells expressing *cut11-GFP GFP-atb2* (RD92) were centrifuged and then imaged.

(A) Maximum-projection images (left) or single medial Z sections (right) of representative frames are shown. Proximal (red) and distal (blue) MT plus ends in the same bundle are highlighted. Yellow arrowheads highlight nuclear-envelope deformation, which ceases when the proximal MT shrinks (270 s). The scale bar represents 5 μm.

(B) Positions of the proximal (red) and distal (blue) MT plus ends in the bundle during nuclear movement (black). Vertical arrows indicate the time period that MT plus end contacts the cell end. The cell center is represented by 0 μm.

(C) Catastrophe rates of a distal MT (marked by "c") as a function of a when a proximal MT is contacting (*) or not contacting the cell end, in the same or different MT bundle (total times assayed = 20 min, 13 min, 71 min). Values are presented as the mean ± standard deviation.

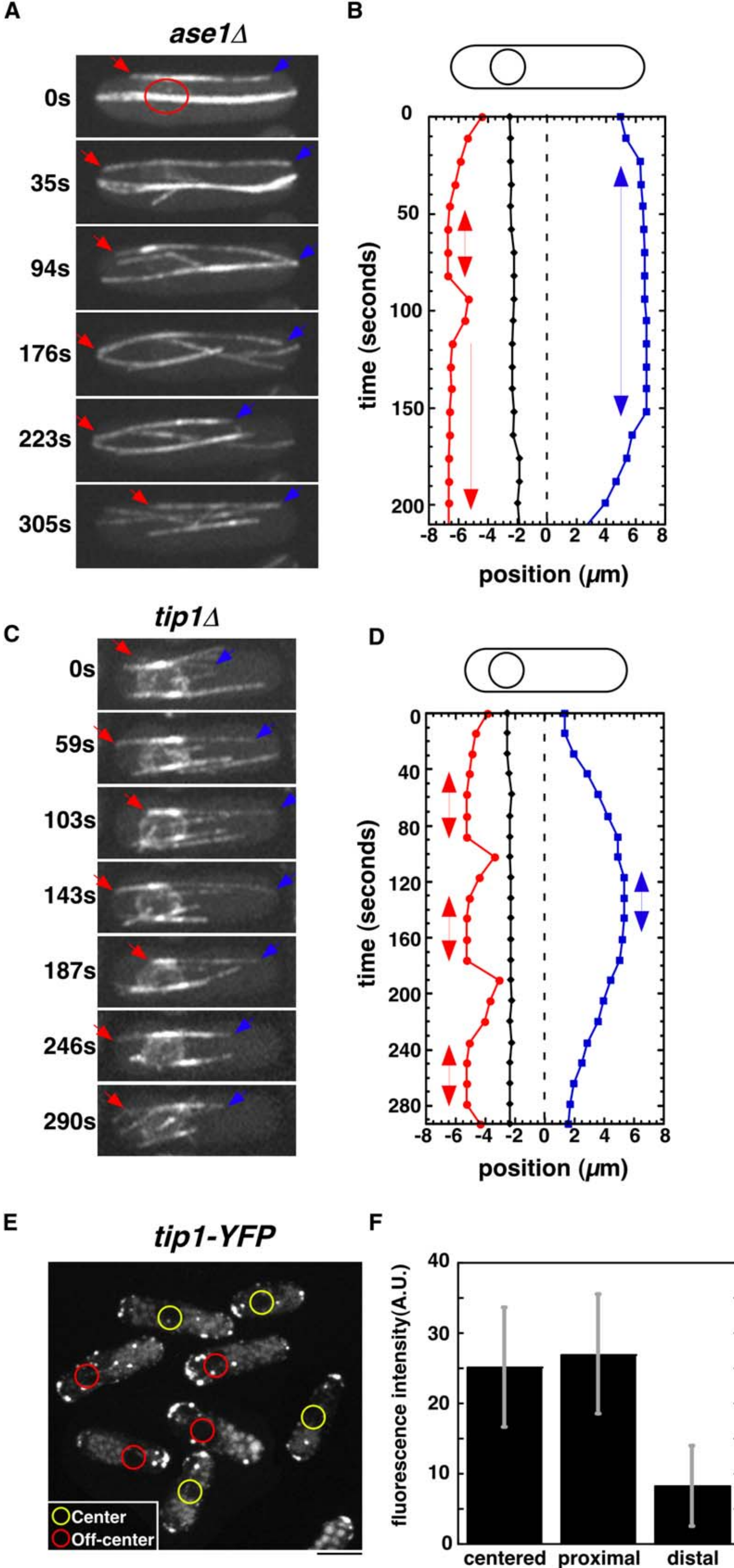


Figure 3. The MT-Bundling Factor Ase1p and CLIP170-like Tip1p Are Required for Asymmetric MT Dynamics

(A) *ase1Δ cut11-GFP GFP-atb2* cells (RD232) were centrifuged to displace the nucleus and then imaged. Maximum projections of representative frames are shown. Arrows indicate the proximal (red) and distal (blue) MT plus ends. Note that distal MTs (blue) stay at the cell tip much longer than in wild-type cells, and can buckle. Cut11-GFP was detectable but not readily visible because of photobleaching in the time frames shown.

(B) Plot of positions over time of the proximal (red) and distal (blue) MT plus ends and the nucleus (black) of the cell shown in (A). Vertical arrows indicate the time period that the MT plus end contacts the cell end. The cell center is represented by 0 μm.

(C) *tip1Δ cut11-GFP GFP-atb2* cells (RD90) were centrifuged and then imaged. Maximum projections of representative frames are shown.

(D) Positions of the proximal (red) and distal (blue) MT plus ends in the bundle during nuclear movement (black) in *tip1Δ* cell in (C).

(E) Wild-type cells expressing tip1-YFP were imaged 2 min after centrifugation and MBC washout. Circles denote the approximate positions of the nuclei. Note the asymmetric localization of tip1-YFP at cell tips in cells with displaced nuclei (red).

(F) Quantification of fluorescence intensities of tip1p-YFP at cell tips. “Centered” denotes either cell tip in cells with a centered nucleus. “Proximal” and “Distal” denote cell tips in cells with a displaced nucleus. Values are presented as the mean ± standard deviation.

defect in nuclear movement. MTs appeared to be pushing from both cell tips, as suggested by the buckling events on both sides of the nucleus and by occasional observations of nuclear-envelope deformations toward the proximal cell tip (Figure 3, Movies S5 and S6). Also, the nucleus often detached from the MTs during pushing events, suggesting that ase1p affects nuclear-MT attachment.

In wild-type cells, imaging of ase1p-GFP showed that the MT overlap zones were moved toward the distal side of the nucleus (Movie S7). MT sliding in these bundles, as visualized by ase1p-GFP or GFP-tubulin, was an occasional, but not prominent, feature and did not correlate with catastrophe events. Further, deletion of *klp2⁺*, a putative minus-end kinesin (KAR3/ncd-like) responsible for MT sliding within these interphase bundles [15], did not affect the asymmetry of MT dynamics (data not shown).

The CLIP170-like protein tip1p, a +TIP protein that regulates MT plus-end catastrophe [3], was also needed to generate asymmetric MT behavior. In *tip1Δ* mutants, MT dwell times are decreased about 2-fold, to 41 s [3] (Table 1; Movie S8). In *tip1Δ* cells with a displaced nucleus, MTs still reached both tips, but dwell times were decreased and nearly equal between the two sets of MTs (44 s at the proximal tip and 33 s at the distal tip; Table S1, Figure 3). Because these MTs shrank back so quickly, they were very inefficient at moving the nucleus (Movie S8, Table S2). This is consistent with subtle nuclear-positioning defects seen in noncentrifuged *tip1Δ* cells. Thus, proper MT plus-end dynamics are needed for nuclear movement.

Intriguingly, displacement of the nucleus led to a transient asymmetric distribution of tip1-YFP in wild-type cells. Tip1p normally cycles rapidly on growing MT plus ends and accumulates equally at both cell tips in cortical dots, which may be deposited by MT plus-end contact [3, 16]. Upon nuclear displacement, tip1p-GFP still localized to growing MT plus ends, but accumulated asymmetrically in cortical dots at the proximal end (Figure 3, Movie S9). This asymmetry developed in the first 5 min after centrifugation and appeared to be due to the increased number of MTs contacting the proximal versus distal cell tips (Figure S3). Interestingly, a tip1p-associated protein, tea1p, remained symmetric during this treatment (Figure 3).

The behavior of tip1p on distal MT plus ends was also not normal. Tip1p dots started moving away from the nucleus normally, but then often disappeared before reaching the distal cell tip (Figure S5, Movie S9). The dots often lost intensity, paused, and/or moved backward for a short distance before disappearing. These events most likely correlated with premature MT catastrophe events. Similar behaviors were seen with mal3-GFP (the EB1 homolog; data not shown) [17]. Imaging of tea2p (kinesin) particles, which normally deliver tip1p from the MT overlap zone to the MT plus end [18], showed that this delivery system still operates (Movie S10). Given that distal MTs have similar behavior to that of *in tip1Δ* mutants, these observations suggest that there may be a defect in maintaining these +TIP proteins on the plus ends of the distal microtubules. However, the causal relationship between tip1p behavior and catastrophe has not yet been established. The observed asymmetry in tip1p distribution suggests a model in which a cytoplasmic tip1p gradient stabilizes the proximal MT plus ends and destabilizes the distal ones, possibly by affecting the off-rates of tip1p from the MT end.

To test the genetic relationship between ase1p and tip1p, we analyzed an *ase1Δ tip1Δ* double mutant. This double mutant had a severe defect in nuclear positioning and exhibited decreased, symmetric MT dwell times, resembling those of a *tip1Δ* single mutant (Table 1). These results are consistent with a proposal that ase1p affects a process that is ultimately regulated by tip1p at the MT plus ends.

In summary, we show here that fission-yeast cells possess an efficient mechanism for centering the nucleus. After displacement, the nucleus, using a MT pushing mechanism, moves in a reproducible and unidirectional manner back to the cell center. This mechanism suggests that MT polymerization is able to produce significant forces to move the nucleus through the viscous cytoplasm [10]. Asymmetries in MT number, length, and dynamics between the MTs on either side of the nucleus are responsible for generation of asymmetric MT pushing forces. The asymmetric behaviors may prevent the distal microtubules from pushing back the nucleus, which would impede movement to the center. Notably, these asymmetrical behaviors are transient, induced simply by displacement of the nucleus, and cease when the nucleus reaches the center.

Although pure microtubule asters can find the center of dishes in vitro [19], it is clear that many additional proteins contribute to efficient centering in vivo. In particular, current in vitro models do not predict the destabilization of the distal MT. We identify two protein factors required for the asymmetry in MT dynamics, the CLIP170 tip1p [3] and the MT-bundling protein ase1p [4]. These suggest that mechanisms contributing to asymmetric MT dynamics are functioning at both ends of the MT.

A key question in a centering mechanism is how do the MT bundles sense the positions of the cell tips and the nucleus? We propose that the overlap zone of antiparallel MTs is used as a force sensor. Compressive force from the proximal MTs produces changes in the overlap region that ultimately affect MT plus-end dynamics in the antiparallel distal MTs. Ase1p, which binds to the overlap zone, appears to be required for this sensing process. In addition, we observed a potential cytoplasmic gradient of tip1p, which may also contribute to asymmetric plus-end regulation. Additional understanding into the complex relationships between tip1p, MT catastrophe, and compressive forces will be needed to test this proposal further. Studies in this relatively simple system will likely be relevant to understanding asymmetric MT dynamics in more complex processes such as chromosome congression during mitosis [20–22], cell migration [23], and cytokinesis [24].

Supplemental Data

Supplemental Data include Experimental Procedures, six figures, two tables, and ten movies and are available with this article online at: http://www.current-biology.com/cgi/content/full/16/15/1544/DC1/.

Acknowledgments

We thank P. Nurse for support of R.D. during a portion of this work, P. Tran and R. Carazo-Salas for strains, and F. Nedelec, R.Carazo-Salas, J. Peterson, I. Zhurinsky, and S. Bratman for discussions. This work was supported by National Institutes of Health GM069670 to F.C.

Received: May 5, 2006
Revised: June 5, 2006
Accepted: June 6, 2006
Published: August 7, 2006

References

1. Tran, P.T., Marsh, L., Doye, V., Inoue, S., and Chang, F. (2001). A mechanism for nuclear positioning in fission yeast based upon microtubule pushing. J. Cell Biol. *153*, 397–411.
2. Drummond, D.R., and Cross, R.A. (2000). Dynamics of interphase microtubules in *Schizosaccharomyces pombe*. Curr. Biol. *10*, 766–775.
3. Brunner, D., and Nurse, P. (2000). CLIP170-like tip1p spatially organizes microtubular dynamics in fission yeast. Cell *102*, 695–704.
4. Loiodice, I., Staub, J., Setty, T.G., Nguyen, N.P., Paoletti, A., and Tran, P.T. (2005). Ase1p organizes antiparallel microtubule arrays during interphase and mitosis in fission yeast. Mol. Biol. Cell *16*, 1756–1768.
5. Yamashita, A., Sato, M., Fujita, A., Yamamoto, M., and Toda, T. (2005). The roles of fission yeast ase1 in mitotic cell division, meiotic nuclear oscillation, and cytokinesis checkpoint signaling. Mol. Biol. Cell *16*, 1378–1395.
6. 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.
7. 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.
8. Daga, R.R., and Chang, F. (2005). Dynamic positioning of the fission yeast cell division plane. Proc. Natl. Acad. Sci. USA *102*, 8228–8232.
9. 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.
10. Sacconi, L., Tolic-Norrelykke, I.M., Stringari, C., Antolini, R., and Pavone, F.S. (2005). Optical micromanipulations inside yeast cells. Appl. Opt. *44*, 2001–2007.
11. 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.
12. Janson, M.E., and Dogterom, M. (2004). A bending mode analysis for growing microtubules: Evidence for a velocity-dependent rigidity. Biophys. J. *87*, 2723–2736.
13. Janson, M.E., de Dood, M.E., and Dogterom, M. (2003). Dynamic instability of microtubules is regulated by force. J. Cell Biol. *161*, 1029–1034.
14. Schuyler, S.C., Liu, J.Y., and Pellman, D. (2003). The molecular function of Ase1p: Evidence for a MAP-dependent midzone-specific spindle matrix. Microtubule-associated proteins. J. Cell Biol. *160*, 517–528.
15. Carazo-Salas, R.E., Antony, C., and Nurse, P. (2005). The kinesin Klp2 mediates polarization of interphase microtubules in fission yeast. Science *309*, 297–300.
16. Feierbach, B., Verde, F., and Chang, F. (2004). Regulation of a formin complex by the microtubule plus end protein tea1p. J. Cell Biol. *165*, 697–707.
17. Busch, K.E., and Brunner, D. (2004). The microtubule plus end-tracking proteins mal3p and tip1p cooperate for cell-end targeting of interphase microtubules. Curr. Biol. *14*, 548–559.
18. Busch, K.E., Hayles, J., Nurse, P., and Brunner, D. (2004). Tea2p kinesin is involved in spatial microtubule organization by transporting tip1p on microtubules. Dev. Cell *6*, 831–843.
19. Holy, T.E., Dogterom, M., Yurke, B., and Leibler, S. (1997). Assembly and positioning of microtubule asters in microfabricated chambers. Proc. Natl. Acad. Sci. USA *94*, 6228–6231.
20. Rieder, C.L., and Salmon, E.D. (1998). The vertebrate cell kinetochore and its roles during mitosis. Trends Cell Biol. *8*, 310–318.
21. Sprague, B.L., Pearson, C.G., Maddox, P.S., Bloom, K.S., Salmon, E.D., and Odde, D.J. (2003). Mechanisms of microtubule-based kinetochore positioning in the yeast metaphase spindle. Biophys. J. *84*, 3529–3546.
22. 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.
23. Gundersen, G.G. (2002). Evolutionary conservation of microtubule-capture mechanisms. Nat. Rev. Mol. Cell Biol. *3*, 296–304.
24. Canman, J.C., Cameron, L.A., Maddox, P.S., Straight, A., Tirnauer, J.S., Mitchison, T.J., Fang, G., Kapoor, T.M., and Salmon, E.D. (2003). Determining the position of the cell division plane. Nature *424*, 1074–1078.