Microtubule Interactions with the Cell Cortex Causing Nuclear Movements in *Saccharomyces cerevisiae*

Neil R. Adames and John A. Cooper

Department of Cell Biology and Physiology, Washington University School of Medicine, St. Louis, Missouri 63110

Abstract. During mitosis in budding yeast the nucleus first moves to the mother-bud neck and then into the neck. Both movements depend on interactions of cytoplasmic microtubules with the cortex. We investigated the mechanism of these movements in living cells using video analysis of GFP-labeled microtubules in wild-type cells and in EB1 and Arp1 mutants, which are defective in the first and second steps, respectively. We found that nuclear movement to the neck is largely mediated by the capture of microtubule ends at one cortical region at the incipient bud site or bud tip, followed by microtubule depolymerization. Efficient microtubule interactions with the capture site require that microtubules be sufficiently long and dynamic to probe the cortex. In contrast, spindle movement into the neck is mediated by microtubule sliding along the bud cortex, which requires dynein and dynactin. Free microtubules can also slide along the cortex of both bud and mother. Capture/shrinkage of microtubule ends also contributes to nuclear movement into the neck and can serve as a backup mechanism to move the nucleus into the neck when microtubule sliding is impaired. Conversely, microtubule sliding can move the nucleus into the neck even when capture/shrinkage is impaired.

Key words: mitosis • dynein • dynactin • EB1 • *Saccharomyces cerevisiae*

Introduction

Cells coordinate the position of the mitotic spindle with the site of cytokinesis. In many cells, the site of cytokinesis is determined by the position of the mitotic spindle. In the budding yeast *Saccharomyces cerevisiae*, cell division occurs at the mother-bud neck, and cells must position the spindle within the neck. Spindle positioning in all cells, including yeast, is thought to depend on the interaction of astral/cytoplasmic microtubules with the cell cortex (Stearns, 1997).

In yeast, the nucleus initially moves to the mother-bud neck and then maintains its position at the neck (Stearns, 1997). Nuclear position at the neck depends on cytoplasmic microtubules, the kinesin-related protein Kip3p, Kar9p, the formin Bni1p, the actin interacting protein Bud6p/Aip3p, and actin (Cottingham and Hoyt, 1997; DeZwaan et al., 1997; Miller and Rose, 1998; Lee et al., 1999; Miller et al., 1999). There is a correlation between the orientation of microtubules into the bud and nuclear position, suggesting that proper positioning of nuclei at the neck depends on the interaction of cytoplasmic microtubules with cortical capture sites in the bud (Miller and Rose, 1998). Kar9p appears to be important for microtubule capture (Miller and Rose, 1998). Bni1p, Bud6p, and actin presumably affect nuclear positioning through their effects on Kar9p localization (Miller et al., 1999). Kip3p might affect nuclear positioning by pulling on cytoplasmic microtubules at the cortex or through its effects on cytoplasmic microtubule lengths (Cottingham and Hoyt, 1997). Despite the identification of proteins involved in nuclear positioning, the nature of the microtubule–cortex interactions and associated nuclear movements to the neck affected by these proteins is not clear.

Next, the spindle moves into the neck. This movement requires dynein, its regulator dynactin, and cytoplasmic microtubules (McMillan and Tatchell, 1994; Muhua et al., 1994; Kahana et al., 1998). Since dynein is a minus end–directed microtubule motor, a favored hypothesis is that dynein is anchored in the bud cortex and pulls on the plus ends of cytoplasmic microtubules (Carminati and Stearns, 1997). Microtubule–cortex interactions during movement of the spindle into the neck have not been described.

Thus, nuclear positioning appears to involve two distinct, and apparently sequential, steps: Kar9p/Kip3p-dependent movement to the neck and dynein/dynactin-dependent movement into the neck (Cottingham and Hoyt, 1997; DeZwaan et al., 1997; Miller et al., 1998). Based on null mutants, loss of one step is deleterious but not lethal, and the loss of both steps is lethal (Cottingham

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Address correspondence to John A. Cooper, 660 S. Euclid Ave., Box 8228, St. Louis, MO 63110. Tel.: (314) 362-3964. Fax: (314) 362-0098. E-mail: jcooper@cellbio.wustl.edu

and Hoyt, 1997; DeZwaan et al., 1997; Miller et al., 1998; Lee et al., 1999).

In this study, we examine microtubule interactions with the cell cortex, the association of those interactions with nuclear movements, and the influence of microtubule dynamics on the interactions. To examine the role of microtubules in the first step, nuclear movement to the neck, we use a strain deficient in yeast EB1 (Yeb1p), encoded by the gene *YEB1/BIM1*. (Another name for the yeast gene *YEB1* [Muhua et al., 1998] is *BIM1*, based on the protein's ability to bind microtubules [Schwartz et al., 1997]. The *BIM1* gene in yeast should not be confused with *bim* genes in *Aspergillus*, defined by their mutant phenotype, blocked in mitosis [Morris, 1975].) Fixed populations of *yeb1*Δ cells show defects in nuclear positioning at the neck (Schwartz et al., 1997; Tirnauer et al., 1999), and double mutants lacking Yeb1p and dynein or dynactin Arp1p are inviable (Muhua et al., 1998). Moreover, *yeb1*Δ *kar9*Δ double mutants are viable and have no additive nuclear positioning defects, indicating that Yeb1p is in the Kar9p/Kip3p class of proteins (Korinek et al., 2000; Lee et al., 2000). The *yeb1*Δ mutant has altered microtubule dynamics during G1; the major effect is increased time spent in pause (Tirnauer et al., 1999). Here we use green fluorescent protein (GFP)[1]-labeled microtubules in the *yeb1*Δ mutant to test the hypothesis that reduced microtubule growth and lengths affect microtubule–cortex interactions required for nuclear movement to the neck.

To study the second step of nuclear movement in which the spindle enters the neck, we use a strain containing a conditional mutation in *ARP1/ACT5* (the protein previously called Act5p [Muhua et al., 1994] has been renamed Arp1p [Poch and Winsor, 1997]), encoding dynactin Arp1p, which is necessary for dynein function (Clark and Meyer, 1994; Muhua et al., 1994). This conditional mutation, *ts-arp1*, is the only existing conditional mutation for any component of the dynein/dynactin pathway. Phenotypes of the *arp1* null to date are identical to those of dynein null mutants (Muhua et al., 1994, 1998).

Materials and Methods

Strains, Media, and Genetic Techniques

Strains were transformed with pAFS92 (provided by Aaron Straight, Harvard University, Cambridge, MA) to integrate, at the *ura3* locus, a GFP fusion to the α-tubulin gene *TUB1* under control of the *MET3* promoter. All strains were isogenic with the wild-type strain, YJC1560 (*MAT*a *ade2-1 ade3 lys2-801 his3*-Δ200 *leu2-3,112 ura3-52::URA3-GFP::TUB1*). The *ts-arp1*, *yeb1*Δ, and *arp1*Δ mutations were as described (Muhua et al., 1998) and yielded the following strains, differing from wild-type as indicated: YJC1562 (*trp1-1 yeb1Δ::HIS3*), YJC1588 (*leu2-3,112::LEU2-ts-arp1 arp1::HIS3*), YJC1589 (*yeb1Δ::HIS3 leu2-3,112::LEU2-ts-arp1 arp1::HIS3*). The plasmid for overexpression of *YEB1* from the *GAL1* promoter (pBJ819) was made by inserting a PCR fragment of *YEB1* into pBJ246 (ATCC 77452). pBJ819 was transformed into YJC1687 and YJC1659, which were derived from YJC1560 and YJC1588, respectively, by making these strains *ura3*. Media, genetic manipulations, and lithium acetate transformation were performed as described (Kaiser et al., 1994).

Fluorescence Microscopy

Movies were used to examine nuclear migration, cytoplasmic microtubule growth/shrinkage, spindle elongation, and timing of cell cycle events. To induce GFP-Tub1p expression, mid-logarithmic cultures were resuspended in SC-methionine for 2 h. Induction did not alter cell growth based on doubling times in liquid medium. The level of GFP-Tub1p was uniform in the cell population. Cells were incubated at 37°C for 1 h to inactivate Arp1p (assayed by phenotype) and allow time for recovery from nonspecific effects of heat shock. Cells were placed on a slide with a thin agarose pad (Waddle et al., 1996). An Air Therm heater (World Precision Instruments, Sarasota, FL) kept the system at the appropriate temperature (37°C unless otherwise noted).

Movies were collected as described (Waddle et al., 1996). For experiments examining nuclear movement, a Z-series of 10 focal planes 0.5 μm apart was collected over ~8 s and projected onto a single two-dimensional image. Z-series of both bright-field and fluorescent images were collected every minute for 3 h. Large nuclear movements were transient, lasting ~2–5 s. Approximately 3–6 movements were required to position the nucleus at the neck. Therefore, nuclei were rarely moving during image acquisition, and nuclear position could be accurately determined from the two-dimensional projections. Bright-field images were used to determine the time of bud emergence and cell separation. Photobleaching was minimized with an excitation shutter, neutral density filters, and an aperture diaphragm.

To examine cytoplasmic microtubules, we acquired images at 5 frames/s in a single focal plane. Movies lasted 4 min before photobleaching was noticeable. Since microtubules are motile, their ends can move up and down. We discarded these data, restricting our analysis to microtubules with both ends in focus at all times. Analysis of microtubule turnover during sliding was by fluorescent speckle microscopy (Maddox et al., 2000). To examine free microtubules detached from the SPB, cells containing pBJ819 (*GAL1-YEB1*) were grown in raffinose to mid-logarithmic cell density and Yeb1p overexpression was induced by the addition of galactose for 4 h.

Movie Analysis

Lengths, angles, and the timing of nuclear movements and cell cycle events were analyzed with NIH Image 1.62 (written by Wayne Rasband at NIH). Comparisons of statistical significance were by *t* test.

Microtubule dynamics were calculated from plots of microtubule lengths at 1-s intervals. Linear regression analysis was performed on segments of the plots. Polymerization (growth) or depolymerization (shrinkage) phases were defined as a line with at least three time points, an R^2 value ≥0.8, and a minimum change in length of 0.5 μm. Pauses were defined as no significant growth or shrinkage for ≥5 s.

Online Supplementary Material

The online version of this article includes movies that accompany the figures. Movies are in QuickTime format and are available at http://www.jcb.org/cgi/content/full/149/4/863/DC1.

Videos 1–5. These videos depict Fig. 1, nuclear movement caused by microtubule growth, capture/shrinkage and sweeping. In unbudded (G1 phase) cells, capture at the cortex and subsequent shortening of cytoplasmic microtubules coincides with nuclear movements toward the capture sites in wild-type cells (Fig. 1 B, wild-type, video 1) and in *yeb1*Δ cells (Fig. 1 B, *yeb1*Δ, video 2). Movies are shown at six times the real speed. In small-budded (S-phase) cells, the nucleus moves to and from the neck as a microtubule captured at the bud tip shortens and grows, respectively. Six times real speed (Fig. 1 C, video 3). In pre-anaphase (G2/M-phase) cells with short spindles, the end of the spindle close to the neck pivots to and from the neck as microtubules in the bud sweep along the cortex in wild-type cells (Fig. 1 D, *yeb1*Δ, video 4). In *yeb1*Δ cells the frequency of sweeping is reduced (Fig. 1 D, wild-type, video 5). Six times real speed.

Videos 6–11. These videos depict Fig. 4. In a wild-type cell, a cytoplasmic microtubule laterally interacts with the bud cortex and slides along the cortex as the spindle moves into the neck. Six times real speed (Fig. 4 A, video 6). Microtubule sliding along the cortex causes bending of the spindle in a wild-type cell. Six times real speed (Fig. 4 B, video 7). In a wild-type cell, a cytoplasmic microtubule slides along the mother cortex as the spindle moves back toward the mother. Microtubule sliding along the mother cortex was observed only after the spindle moved into the neck. Six times real speed (Fig. 4 C, video 8). A *yeb1*Δ cell showing microtubule sliding along the bud cortex during spindle movement into the neck. The spindle is initially misaligned but aligns along the mother-bud axis and moves into the neck when a microtubule grows into the bud and slides along the cortex. Six times real speed (Fig. 4 D, video 9). Video 10 depicts

[1]*Abbreviations used in this paper*: GFP, green fluorescent protein; SEP, standard error of proportion; SPB, spindle pole body.

Fig. 4 E, top. Video 11 depicts Fig. 4 E, bottom. Capture/shrinkage events during anaphase in wild-type cells occur at the mother (video 9) and bud (video 10) cortices. During anaphase, capture/shrinkage events were observed only after the spindle moved into the neck. Six times real speed.

Videos 12 and 13. These videos depict Fig. 5. Free microtubules stabilized by overexpression of Yeb1p laterally associate with the cortex and slide. Yeb1p overexpression apparently caused cytoplasmic microtubules to pull out of the spindle pole body. Occasionally free microtubules had apparent remnants of the SPB with a second short microtubule at their trailing end (video 12). Free microtubules could slide along both the mother and bud cortex, passing between the cells through the neck (video 13). 15 times real speed.

Videos 14 and 15. These videos depict Fig. 7. In this *ts-arp1* cell, microtubules do not make lateral associations with the bud cortex and do not slide, but do sweep. The spindle does not move into the neck. Later in the movie a microtubule grows very long and buckles as the spindle is pushed out of the neck. 15 times real speed (Fig. 7 A, video 14). In this *ts-arp1* cell, a capture/shrinkage event in the bud occurs as the spindle moves into the neck. Here, the spindle movement into the neck was not caused solely by spindle elongation since both ends of the spindle moved toward the bud. 15 times real speed (Fig. 7 B, video 15).

Results

How Do Nuclei Move to the Bud Neck?

Nuclear movement to the neck has been described as random (Shaw et al., 1997) or directed (DeZwaan et al., 1997; Lee et al., 1999). During random movements, nuclei appear to be pushed by the growth of microtubules against the cortex (Fig. 1 A; Shaw et al., 1997). Once the nucleus is next to the neck, a microtubule enters the bud and is cap-

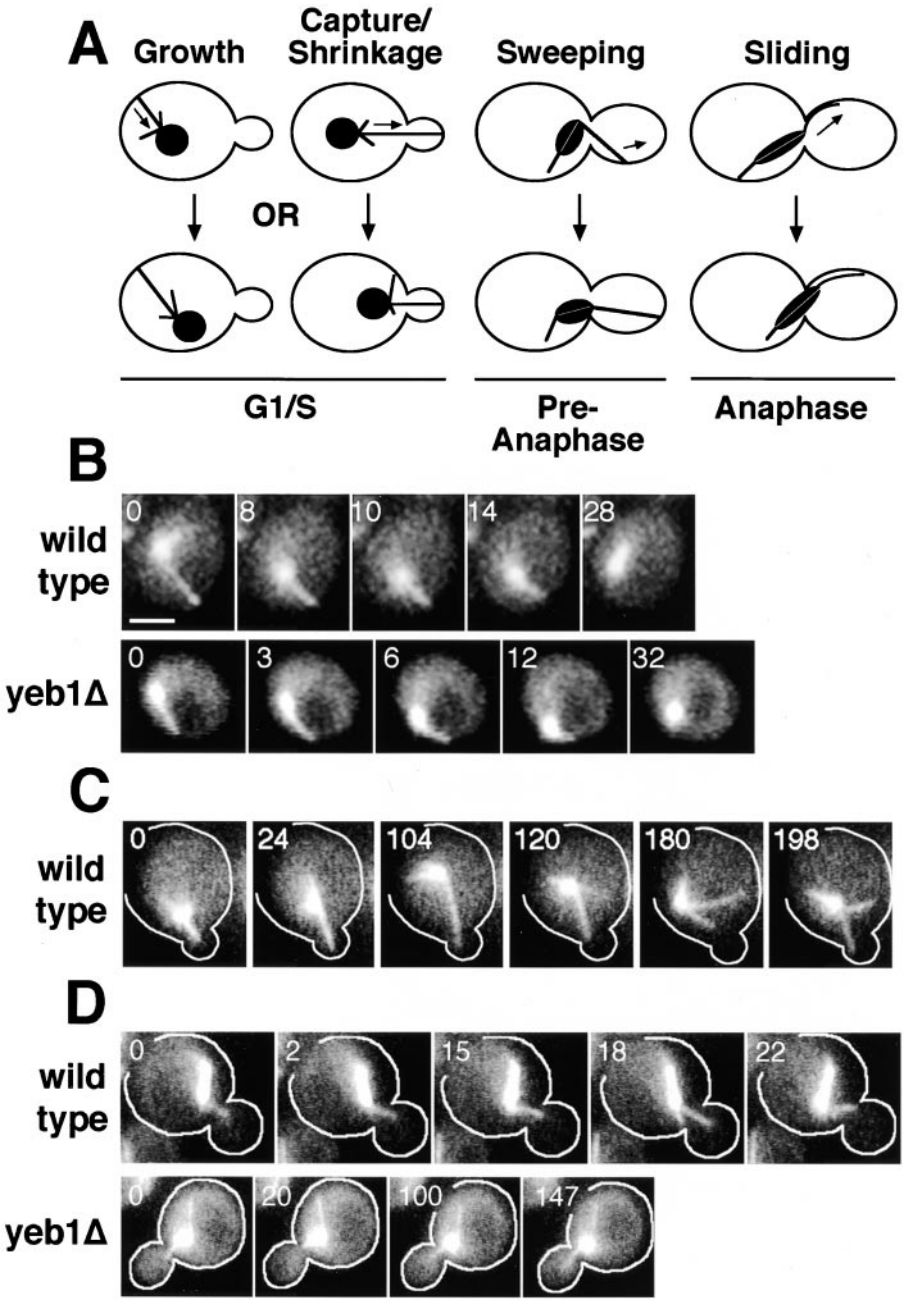

Figure 1. Nuclear movement caused by microtubule growth, capture/shrinkage, sweeping and sliding. (A) This schematic illustrates the different microtubule behaviors and associated nuclear movements described in the paper. (B) Nuclear movements to the incipient bud site accompanied by microtubule capture/shrinkage in G1. (C) Nuclear movements in a budded cell accompanied first by microtubule growth and later by capture/shrinkage. (D) Microtubule sweeping and spindle movement during G2/M before anaphase. The end of the spindle closer to the neck pivots as the microtubule sweeps in the direction of the mother-bud axis. Spindles in the *yeb1*Δ strain do not make the large movements seen in the wild-type strain. The spindle shown is at a large angle relative to the mother-bud axis and seen end-on. Budded cells are outlined for clarity. Time (s) is indicated. See supplemental videos 1–5 at http://www.jcb.org/cgi/content/full/149/4/863/DC1. Bar, 2 μm.

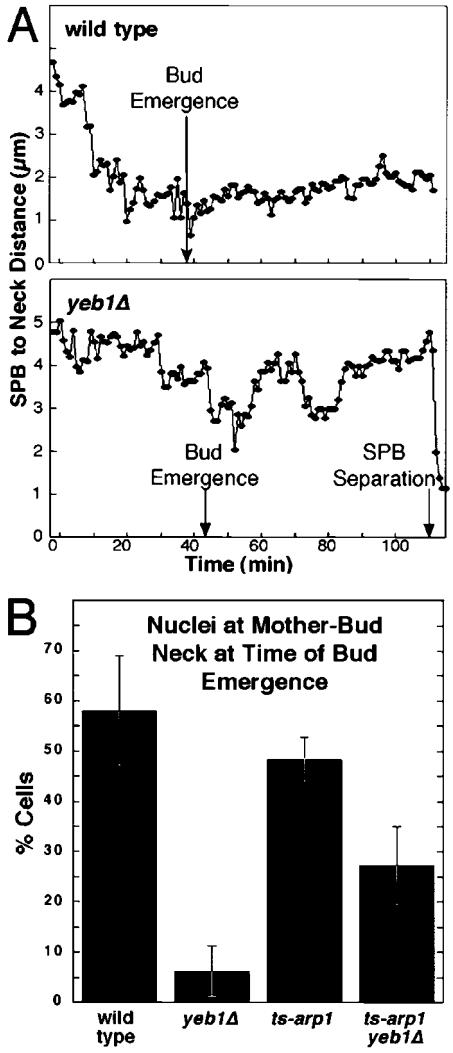

Figure 2. Nuclear movement to the neck. (A) Representative plots of the distance of the SPB from the bud neck vs. time for a wild-type cell and a *yeb1*Δ cell. (B) The percentage of cells in which the nucleus was positioned at the bud neck when the bud formed. Nuclei were considered to be at the bud neck if they were within the third of the mother cell nearest the neck (~1.5–2 μm from the neck). Numbers of cells in each group: wild-type, 54; *yeb1*Δ, 61; *ts-arp1*, 122; *ts-arp1 yeb1*Δ, 33. Error bars represent standard error of proportion (SEP).

tured at the bud tip cortex. Then the captured microtubule shortens and the nucleus moves closer to the neck (Fig. 1, A and C; Shaw et al., 1997). Here, we refer to the latter microtubule–cortex interaction and its associated nuclear movement as a capture/shrinkage event. In studies describing directed nuclear movement to the neck, microtubule interactions with the cortex have not been described (DeZwaan et al., 1997; Lee et al., 1999).

In our wild-type strain, nuclei did not move randomly. Instead, nuclei moved in a directed manner from one end of the cell to the bud site, usually before a bud was visible (Fig. 2; Table I). These movements occurred in several large steps (Fig. 2 A). The nuclei then remained within 2 μm of the neck until mitosis.

We examined microtubule–cortex interactions during these directed nuclear movements. Most nuclear movements were associated with microtubule capture/shrinkage events (Fig. 1 B; Table I). Microtubule capture/shrinkage was associated with nuclear movement approximately twice as often as was microtubule growth against the cortex (Table I).

Nuclei often moved to the neck before bud emergence, and 90% (27/30) of nuclear movements to the neck before bud emergence were accompanied by microtubule capture/shrinkage at the incipient bud site. In several cells (3/49), repeated microtubule capture/shrinkage events occurred at the same cortical position; the microtubule shrank or moved away from the capture site between capture/shrinkage events. In time-lapse movies, nuclear movement to the neck was usually accompanied by multiple microtubule capture/shrinkage events at the incipient bud site or bud cortex (Table I). Microtubule growth and capture/shrinkage also occurred at the bud tip even after nuclei moved to the neck (Fig. 1 C) and microtubules interacted with the bud tip 70% of the time (14/20 min, 5 cells). Therefore, the cortical capture site appears to be solitary and persist at the bud tip.

Microtubule capture/shrinkage was observed exclusively at the site of the future bud or the bud cortex (208/208 events); microtubule capture/shrinkage at the mother cortex never occurred, even when microtubules lost contact with the bud tip and moved out of the bud (Fig. 1 C). Microtubule capture/shrinkage also did not occur at the mother cortex in the *yeb1*Δ mutant, even when the nucleus was grossly mispositioned (10/10 cells, 2,400 s), allowing

Table I. Nuclear Migration and Microtubule–Cortex Interactions during G1

Strain	Time nucleus is positioned at neck relative to bud emergence	% Nuclear movements associated with microtubule shrinkage at bud cortex or incipient bud site	Frequency of microtubule capture/shrinkage	Frequency of microtubule growth and pushing
	min		min^{-1}	min^{-1}
Wild-type	0.8 ± 3.0 before (19)	70.4 ± 6.2 (54)	0.126 (13)	0.078 (8)
*yeb1*Δ	**36.7 ± 4.6 after (16)**	**42.6 ± 6.3 (61)**	0.066 (6)	0.036 (3)
ts-arp1	1.8 ± 1.1 before (122)	57.4 ± 5.8 (62)	0.036 (3)	0.060 (5)
*ts-arp1 yeb1*Δ	**15.1 ± 5.3 after (33)**	58.1 ± 8.9 (31)	0.048 (3)	0.048 (3)

Data for the first two columns were obtained from time-lapse movies (1-min intervals). Data for the last two columns were obtained from real-time movies (5 fps). Values for times are mean ± SEM. Values for percentages are mean ± SEP. For times and percentages, numbers of cells are in parentheses. For frequencies, numbers of events are in parentheses. Frequencies are of microtubule shrinkage or growth, associated with nuclear movements, and were obtained from 27 wild-type cells, 22 *yeb1*Δ cells, 21 *ts-arp1* cells, and 16 *ts-arp1 yeb1*Δ cells. Numbers in bold type are significantly different from wild-type values, with $P < 0.01$.

additional opportunity for microtubules to interact with the mother cortex.

In cases where the nucleus moved to the neck after bud formation, 54% (13/24) of nuclear movements were associated with microtubule growth against the mother cortex. Therefore, both mechanisms for nuclear movement to the neck were present and active; which mechanism dominated depended on when nuclear movement occurred relative to bud formation.

Why Do yeb1Δ Mutants Have Mispositioned Nuclei?

Yeb1p, Kip3p, Bni1p, and Kar9p are required for nuclear positioning at the neck, based on observations of fixed cells from asynchronous populations (Cottingham and Hoyt, 1997; DeZwaan et al., 1997; Schwartz et al., 1997; Miller et al., 1998, 1999; Lee et al., 1999). Abnormal nuclear position might be caused by poor nuclear movement to the neck or failure to maintain the position of the nucleus at the neck. *bni1*Δ mutants may be defective for nuclear movement to the neck (Lee et al., 1999). In contrast, nuclei in *kip3*Δ mutants move to and from the neck and are thus unable to maintain the nucleus at the neck, probably due to excessive microtubule growth (DeZwaan et al., 1997). We asked whether *yeb1*Δ cells behaved like *bni1*Δ or *kip3*Δ cells.

In movies of living *yeb1*Δ cells, nuclei exhibited many short movements toward and away from the bud site (Fig. 2 A). The end result was that few cells had nuclei positioned at the neck when buds were visible (Fig. 2 B). Most nuclei arrived at the neck long after bud emergence (Table I). After nuclei arrived at the neck, they remained close to it. Therefore, the nuclear positioning defect in the *yeb1*Δ mutant is due to inefficient movement to the neck and not a failure to maintain position at the neck.

Because nuclear movement to the neck is associated with microtubule capture/shrinkage events in wild-type cells, we asked if the *yeb1*Δ mutant was defective for capture/shrinkage. Real-time analysis of *yeb1*Δ cells showed that nuclei moved in association with apparent pushing by microtubule growth against the cortex and apparent pulling by capture/shrinkage events (Fig. 1 B). The frequencies of both events, growth and capture/shrinkage, were reduced in *yeb1*Δ vs. wild-type cells (Table I). Consistent with these observations, time-lapse movies showed that fewer nuclear movements to the neck were associated with microtubule capture/shrinkage at the bud cortex (Table I).

We considered two roles for Yeb1p in microtubule capture/shrinkage. First, Yeb1p might affect microtubule polymerization dynamics and thereby the frequency with which microtubule ends encounter cortical capture sites. Shorter or less dynamic microtubules should reduce the probability of a microtubule end encountering a capture site. Second, Yeb1p might affect the probability that a microtubule that encounters a capture site will be captured and shortened, causing nuclear movement. During G1/S, microtubules in *yeb1*Δ cells were shorter, had a reduced growth rate, and spent more time in pause (Table II). *yeb1*Δ cells also had fewer cytoplasmic microtubules (0.89 ± 0.05 per SPB, $n = 720$) than did wild-type cells (2.47 ± 0.04 per SPB, $n = 515$). Most of the nuclei in the *yeb1*Δ mutant moved to the neck when the cells entered G2/M, 38.5 ± 3.0 min ($n = 25$) after bud emergence (Table I), at which time microtubule dynamics were normal (Table II). These data suggest that the frequency with which microtubules encounter cortical capture sites is impaired in the *yeb1*Δ mutant.

If the primary cause of the nuclear movement defect in the *yeb1*Δ mutant is fewer, shorter microtubules, a second mutation that increases the number and length of microtu-

Table II. Microtubule Dynamics

Strain	Cell cycle phase	Growth rate	Shrinkage rate	Catastrophe frequency	Rescue frequency	% Time in pause	Ratio time growing vs. shrinking	Mean Mt length over time
		$\mu m\ min^{-1}$	$\mu m\ min^{-1}$	s^{-1}	s^{-1}			μm
Wild-type 26°C	G1	4.6 ± 0.8 (51)	4.8 ± 0.4 (53)	0.026 (49)	0.019 (37)	33.4 ± 1.3	1.21	1.44 ± 0.01
Wild-type	G1	14.4 ± 1.2 (63)	16.2 ± 1.2 (68)	0.036 (65)	0.030 (54)	31.8 ± 1.1	1.44	2.62 ± 0.03
	S	17.4 ± 1.8 (43)	16.2 ± 1.8 (54)	0.042 (53)	0.034 (43)	24.4 ± 1.2	1.20	3.02 ± 0.05
	G2/M	12.6 ± 1.2 (77)	12.0 ± 0.6 (76)	0.042 (85)	0.034 (70)	26.5 ± 1.0	1.07	2.04 ± 0.03
*yeb1*Δ	G1	**10.2 ± 1.2 (28)**	14.4 ± 1.8 (51)	0.026 (50)	0.015 (28)	**66.1 ± 1.1**	0.96	**1.19 ± 0.01**
	S	16.8 ± 3.0 (35)	13.8 ± 2.4 (36)	0.022 (33)	0.020 (30)	**39.3 ± 1.3**	0.37	**2.89 ± 0.04**
	G2/M	13.8 ± 0.6 (136)	13.2 ± 0.6 (137)	0.033 (136)	0.035 (145)	**31.2 ± 0.7**	1.02	**3.79 ± 0.03**
ts-arp1	G1	**10.2 ± 1.2 (68)**	15.6 ± 1.8 (68)	0.035 (69)	0.039 (76)	34.0 ± 1.1	1.42	**2.22 ± 0.04**
	S	**9.6 ± 1.8 (18)**	12.0 ± 1.8 (16)	0.034 (19)	0.038 (21)	**42.0 ± 2.1**	1.10	**1.50 ± 0.03**
	G2/M	**20.4 ± 1.2 (92)**	**21.6 ± 1.2 (84)**	0.056 (102)	0.056 (102)	**18.0 ± 0.9**	1.06	**5.94 ± 0.06**
*ts-arp1 yeb1*Δ	G1	15.6 ± 2.4 (26)	**12.0 ± 1.8 (37)**	0.025 (38)	0.021 (31)	**37.8 ± 1.2**	1.04	**2.17 ± 0.03**
	S	12.6 ± 1.8 (15)	12.6 ± 2.4 (21)	0.037 (18)	0.031 (15)	**13.1 ± 1.5**	1.20	**2.34 ± 0.05**
	G2/M	12.6 ± 2.4 (25)	**16.8 ± 2.4 (30)**	0.030 (32)	0.028 (30)	**20.1 ± 1.2**	1.33	**6.19 ± 0.13**

Plots of individual microtubules, at 1-s intervals, were used to calculate rates, frequencies, percentages, and lengths. Values for rates, frequencies, and lengths are mean ± SEM. Numbers in parentheses are the number of events. Bold type indicates a statistical difference from wild-type at 37°C, with $P < 0.05$. Percentages are mean ± SEP over the total time observed. Microtubule lengths are also averaged over the total time observed. Our absolute values for the rates and frequencies in wild-type and *yeb1*Δ cells are 5–10-fold higher than were obtained by Tirnauer et al. (1999) because our experiments were performed at 37°C, the restrictive temperature for the *ts-arp1* mutant. At 26°C, our rates of microtubule growth and shrinkage in wild-type cells were only slightly greater than those of Tirnauer et al. (1999). Wild-type (26°C): G1, 33 microtubules, 9 cells, 1,917 s. Rates and frequencies were similar during S and G2/M. Wild-type (37°C): G1, 35 microtubules, 9 cells, 1,815 s; S, 17 microtubules, 5 cells, 1,263 s; G2/M, 29 microtubules, 11 cells, 2,032 s. *yeb1*Δ mutant: G1, 38 microtubules, 15 cells, 1,908 s; S, 18 microtubules, 8 cells, 1,470 s; G2/M, 42 microtubules, 17 cells, 4,163 s. *ts-arp1* mutant: G1, 38 microtubules, 18 cells, 1,949 s; S, 10 microtubules, 3 cells, 560 s; G2/M, 30 microtubules, 10 cells, 1,813 s. *ts-arp1 yeb1*Δ double mutant: G1, 16 microtubules, 9 cells, 1,493 s; S, 6 microtubules, 4 cells, 483 s; G2/M, 9 microtubules, 5 cells, 1,056 s.

bules should alleviate the defect. Dynein and dynactin null mutants have longer microtubules than wild-type cells (Muhua et al., 1994; Carminati and Stearns, 1997). We found that in a *ts-arp1 yeb1*Δ double mutant, microtubule length, time in pause, and time growing vs. shrinking were intermediate between the values for *yeb1*Δ and wild-type strains (Table II). As predicted, the frequencies of microtubule growth and shrinkage events at the cell cortex in the *ts-arp1 yeb1*Δ double mutant were intermediate between these frequencies in the *yeb1*Δ and wild-type strains (Table I). Also as predicted, nuclear migration to the neck in the double mutant was more efficient than in *yeb1*Δ cells (Fig. 1 B; Table I).

How Is Pre-Anaphase Spindle Orientation Achieved?

As cells enter G2/M, a short spindle forms in the nucleus. Normally, the short spindle is oriented along the mother-bud axis via microtubule interactions with the cell cortex, which is thought to promote spindle movement into the bud neck. (DeZwaan et al., 1997; Lee et al., 1999; Tirnauer et al., 1999).

To understand how the spindle becomes properly oriented, we observed the behavior of the cytoplasmic microtubules and the spindle in G2/M. We found that when the spindle rotated, the end of the spindle farthest from the neck remained relatively stationary, the end of the spindle close to the neck moved, and cytoplasmic microtubules in the bud performed a characteristic sweeping motion (Carminati and Stearns, 1997; Fig. 1, A and D). Sweeping consisted of a microtubule moving laterally, pivoting at its connection with the SPB, its distal end maintaining contact with the bud cortex. Most spindle rotations occurred when there was a net displacement of the sweeping microtubule's distal end along the mother-bud axis (Fig. 2 C). Neither microtubule capture/shrinkage nor growth contributed substantially to spindle rotations.

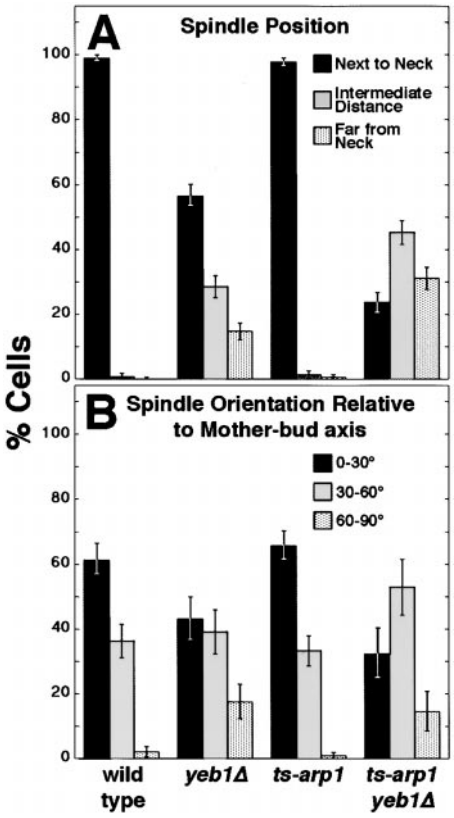

Figure 3. Spindle position and orientation before anaphase. (A) Position of the short spindle relative to the neck in pre-anaphase cells. Numbers of cells: wild-type, 112; *yeb1*Δ, 189; *ts-arp1*, 134; *ts-arp1 yeb1*Δ, 190. Error bars represent SEP. (B) Angle of the short spindle from the mother-bud axis in cells with spindles positioned at the neck. Numbers of cells: wild-type, 88; *yeb1*Δ, 51; *ts-arp1*, 102; *ts-arp1 yeb1*Δ, 34. Error bars represent SEP.

Why Do yeb1Δ Mutants Have Misoriented Spindles?

If sweeping microtubules promote spindle orientation, then cells with defective spindle orientation may be defective in sweeping. *yeb1*Δ and other Kar9p/Kip3p-class mutants have misoriented pre-anaphase spindles (DeZwaan et al., 1997; Miller et al., 1998; Lee et al., 1999). Therefore, we examined microtubule and spindle movements in *yeb1*Δ cells. To address the issue of spindle orientation alone, we restricted our analysis to cells with spindles properly positioned at the neck (Fig. 3). Relative to wild-type cells, spindles were not as motile in *yeb1*Δ cells (Fig. 1 D) and the frequency with which spindles moved in association with sweeping was only 0.25 min^{-1} (12 events/48 min) compared with 1.94 min^{-1} (62 events/32 min) in the wild-type strain. Therefore, spindle orientation correlated with microtubule sweeping.

We asked whether the sweeping defect in the *yeb1*Δ mutant resulted from inability of microtubules to reach the bud cortex. Microtubule lengths, rates of growth and shrinkage and other dynamic parameters were essentially normal in *yeb1*Δ cells during G2/M (Table II). Therefore, Yeb1p's role in microtubule sweeping appears to involve making microtubule–cortex contact events productive for force generation and spindle movement, rather than just promoting the frequency with which microtubules interact with the bud cortex.

How Do Spindles Move into the Neck?

To move the nucleus into the neck, dynein at the bud tip might capture and pull the ends of microtubules (Carminati and Stearns, 1997). We tested this hypothesis by observing microtubule–cortex interactions, in real-time, during movement of the spindle into the neck.

During spindle movement into the neck in wild-type cells, microtubules associated laterally with the bud cortex along their complete length, not just at their ends (Fig. 4 A). The lateral association of a cytoplasmic microtubule with the bud cortex occurred within a few minutes of the start of spindle elongation. The microtubule appeared to slide along the bud cortex (12/13 cells) during spindle movement into the neck; sliding ensued within 8–40 s of the lateral interaction. In 10/12 cells, the microtubules slid without shrinking (Fig. 4 A). Nearly the entire length of a microtubule was curved along the inside of the cell and applied to the cortex. Sometimes, sliding microtubules were associated with bending of the spindle, suggesting that sliding can exert considerable force (Fig. 4 B). Therefore, the spindle is pulled into the neck due to interactions of

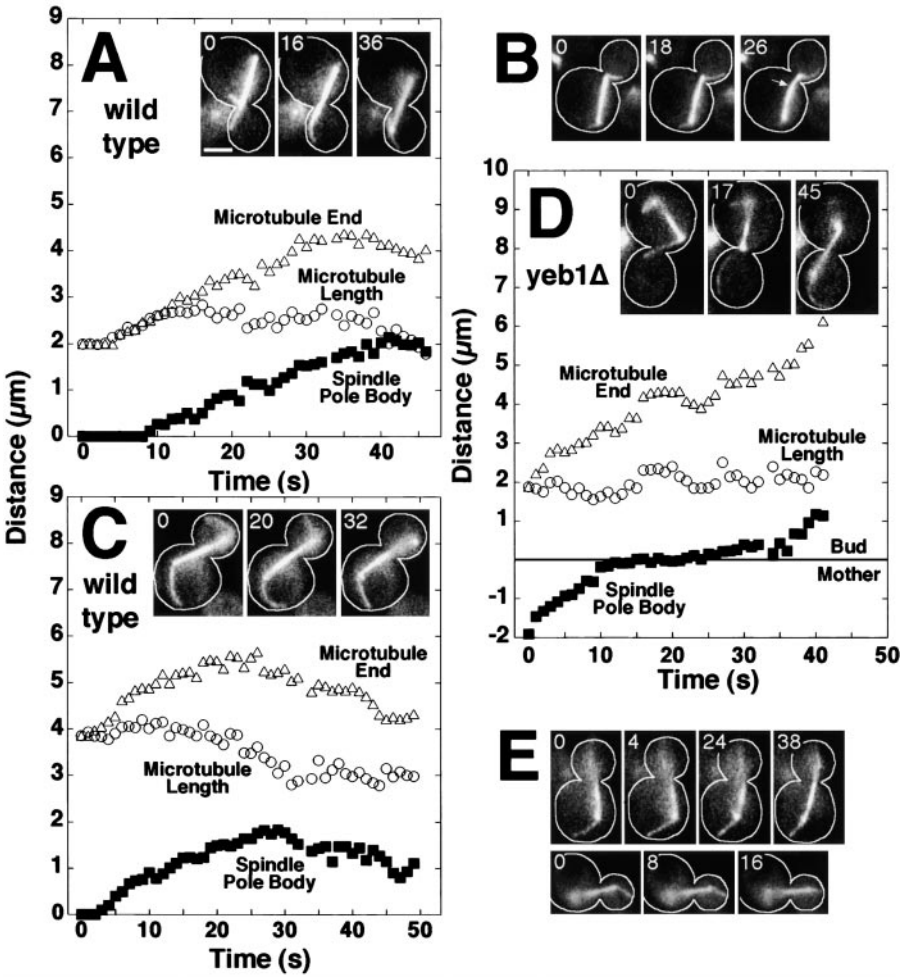

Figure 4. Microtubule sliding and capture/shrinkage during anaphase spindle movements. Microtubules made lateral associations with the bud cortex before and during spindle movements into the bud neck. For A, C, and D, the position of the distal end of a cytoplasmic microtubule (open triangles) and its SPB (filled squares) are plotted vs. time (s). Zero distance is defined as the position of the SPB at $t = 0$. The length of the microtubule (open circles) is also plotted. The insets show three frames from movies, with time (s) indicated. (A) In a wild-type cell, a cytoplasmic microtubule slides along the bud cortex as the spindle moves into the neck. (B) Bending of a spindle in a wild-type cell (arrow). (C) In a wild-type cell with the spindle in the neck, a cytoplasmic microtubule slides along the mother cortex as the spindle moves back toward the mother. (D) A *yeb1*Δ cell showing microtubule sliding along the bud cortex and spindle movement into the neck. (E) Capture/shrinkage events in wild-type cells with the spindle in the neck. (Top) Capture/shrinkage in the mother and (bottom) in the bud. Time (s) is indicated. See supplemental videos 6–11 at http://www.jcb.org/cgi/content/full/149/4/863/DC1. Bar, 2 μm.

the sides, not the ends, of microtubules with the bud cortex. After the spindle entered the neck, microtubule sliding also occurred in the mother (Fig. 4 C). Thus, the spindle appears to be kept in the neck by a balance of pulling forces caused by microtubule sliding in the mother and bud.

As alternative mechanisms that might push the spindle into the neck, we considered spindle elongation and microtubule growth in the mother. Both spindle pole bodies moved together toward the bud in all cases in wild-type cells (13/13 cells; Fig. 4); therefore, spindle elongation can be excluded. Microtubules from the SPB opposite the neck did not grow against the mother cortex while the spindle moved into the neck (7/7 cells in which microtubules in the mother were visible; Fig. 4 A), thus excluding microtubule growth as a mechanism.

Free microtubules not attached to an SPB slid along the cortex in cells overexpressing Yeb1p (Fig. 5, A–C). These microtubules slid for long distances and times, occasionally slowing or stopping, then resuming their previous velocities. The mean sliding speed was 5.12 ± 0.65 μm/min ($n = 9$ sliding events, 3 cells, 795 s). Fluorescent speckle analysis shows that sliding free microtubules move as a unit and do not treadmill (Fig. 5 C). Sliding microtubules in cells with normal levels of Yeb1p also do not treadmill (Fig. 5 D). These observations demonstrate that the cortex is able to exert force on a microtubule independent of any possible forces exerted through the SPB, which supports the hypothesis that the cortex pulls on microtubules to effect spindle movement.

If the minus end motor dynein moves these microtubules along the cortex, the microtubules should lead with their plus end. An occasional free microtubule had an apparent spindle fragment at its trailing end, suggesting that the plus end was leading (Fig. 5 A).

Why Do Dynactin Mutants Have Defects in Spindle Movement into the Neck?

To determine how dynactin affects spindle movement into the neck, we examined microtubules and spindle movement in a *ts-arp1* strain. Like dynein null mutants, *ts-arp1* cells showed no defects in nuclear positioning at the neck or in pre-anaphase spindle orientation (Fig. 1 B; Table I; Fig. 3). In many *ts-arp1* cells, the mid-anaphase spindle failed to enter the bud and then became misaligned (Fig. 6). *ts-arp1* spindles took longer than wild-type spindles to move into the neck (2.2 ± 0.3 min after the start of spindle elongation, $n = 109$, for wild-type and 8.5 ± 1.3 min, $n = 74$, for *ts-arp1*; $P < 0.01$) and moved into the neck at a slower speed (0.64 ± 0.12 μm min^{-1}, $n = 18$, compared with 1.18 ± 0.11 μm min^{-1}, $n = 19$, in the wild-type strain, $P < 0.01$).

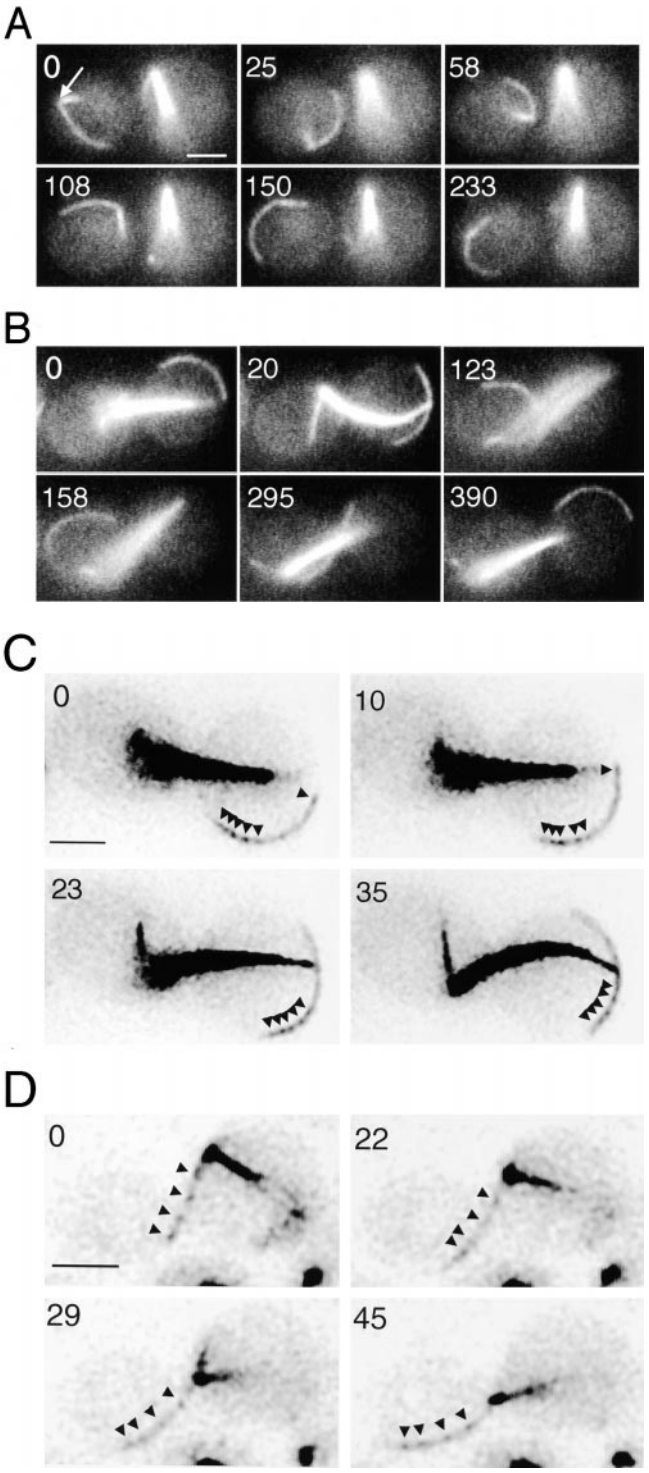

Figure 5. Sliding of free microtubules and fluorescent speckle analysis. Free microtubules stabilized by overexpression of Yeb1p laterally associated with the cortex and slid. (A) Occasionally free microtubules had apparent remnants of the SPB at their trailing end (arrow). (B) Free microtubules could slide along both the mother and bud cortex, passing between the cells through the neck. (C) Fluorescent speckles (arrowheads) serve as fiduciary markers on a free moving microtubule. The speckles move with the microtubule, indicating that the microtubule is not treadmilling. (D) Fluorescent speckles (arrowheads) on SPB-attached microtubules move with the microtubule as the spindle moves into the neck. This cell is a *yeb1*Δ mutant. Speckles were readily apparent in *yeb1*Δ cells with misoriented spindles because microtubules would grow long before reaching the bud cortex and sliding. In C and D, images were inverted to reveal speckles more readily. Time (s) is indicated. See supplemental videos 12 and 13 at http://www.jcb.org/cgi/content/full/149/4/863/DC1. Bars, 2 μm.

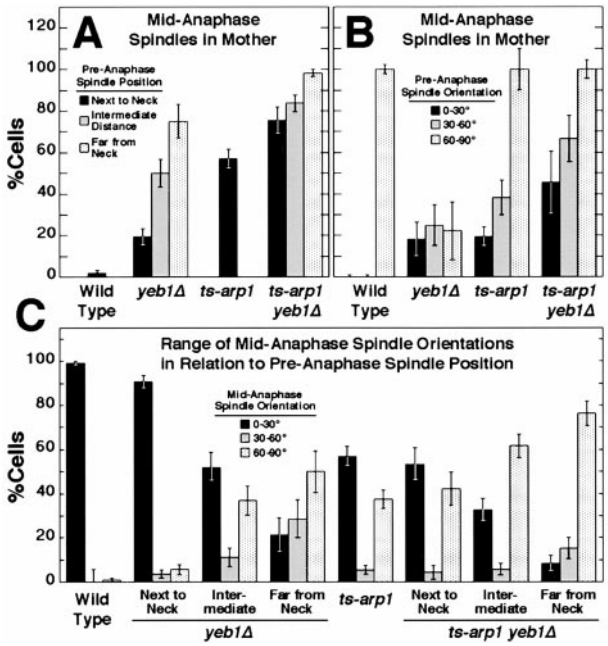

Figure 6. Movement and orientation of mid-anaphase spindles in relation to pre-anaphase spindle position and orientation. Time-lapse movies were analyzed for movement of spindles into the neck and orientation of spindles along the mother-bud axis. Each bar is the proportion of cells in the indicated pre-anaphase category showing delayed spindle movement into the neck. (A) The percentage of mid-anaphase spindles that failed to move into the neck is plotted in relation to their pre-anaphase position. Mid-anaphase was defined as half of the mean time from start of spindle elongation to spindle breakdown in wild-type cells. In wild-type and *ts-arp1* cells, nearly all spindles were at the bud neck before anaphase. Numbers of cells: wild-type, 112; *yeb1*Δ next to neck, 107; *yeb1*Δ intermediate distance, 54; *yeb1*Δ far from neck, 28; *ts-arp1*, 128; *ts-arp1 yeb1*Δ next to neck, 45; *ts-arp1 yeb1*Δ intermediate distance, 86; *ts-arp1 yeb1*Δ far from neck, 59. (B) The percentage of mid-anaphase spindles that failed to move into the neck, in relation to their pre-anaphase orientation. Results are grouped by pre-anaphase spindle orientation in increments of 30°. Only spindles positioned at the bud neck were included. Numbers of cells: wild-type 0–30°, 54; wild-type 30–60°, 32; wild-type 60–90°, 2; *yeb1*Δ 0–30°, 22; *yeb1*Δ 30–60°, 20; *yeb1*Δ 60–90°, 9; *ts-arp1* 0–30°, 67; *ts-arp1* 30–60°, 34; *ts-arp1* 60–90°, 1; *ts-arp1 yeb1*Δ 0–30°, 11; *ts-arp1 yeb1*Δ 30–60°, 18; *ts-arp1 yeb1*Δ 60–90°, 5. (C) Orientation of mid-anaphase spindles. Results are grouped by the range of angles of the mid-anaphase spindles from the mother-bud axis in increments of 30°, in addition to the pre-anaphase spindle position, as in A. Percentages are of mid-anaphase spindles with the indicated orientation and are derived from all spindles having the indicated pre-anaphase position. Numbers of cells are as in A. Error bars are SEP.

Microtubules in *ts-arp1* cells during early anaphase never showed lateral association with the cortex or sliding (0/24 cells, 96 min; Fig. 7). Microtubules in *ts-arp1* cells had opportunity to interact with the bud cortex. Microtubules were oriented properly (Fig. 8) and always pene-

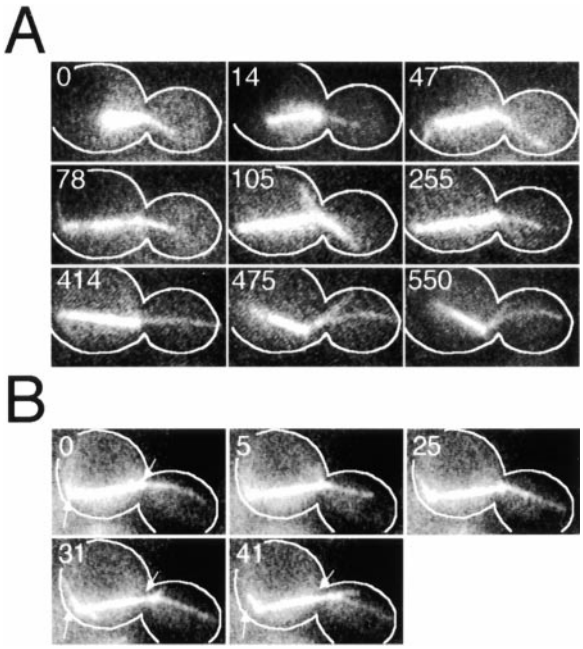

Figure 7. Microtubule sweeping and capture/shrinkage during anaphase spindle movements in *ts-arp1* cells. (A) In this cell, microtubules do not make lateral associations with the bud cortex and do not slide but do sweep. The spindle does not move into the neck. Later a microtubule grows very long and buckles as the spindle is pushed out of the neck. (B) In this cell, a capture/shrinkage event in the bud occurs as the spindle moves into the neck. Here, the spindle movement into the neck was not caused solely by spindle elongation since both ends of the spindle moved toward the bud. The starting positions of the SPBs are indicated with arrows. Time (s) is indicated. See supplemental videos 14 and 15 at http://www.jcb.org/cgi/content/full/149/4/863/DC1. Bar, 2 μm.

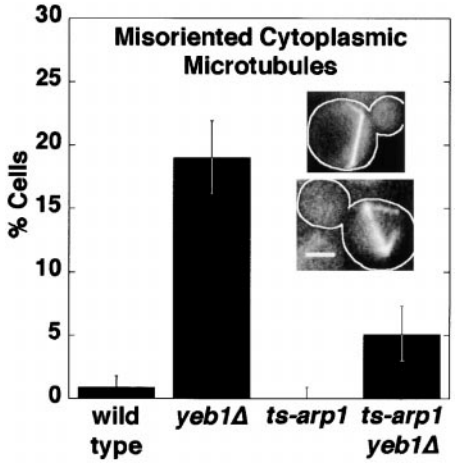

Figure 8. Misorientation of cytoplasmic microtubules during anaphase. In some cells, the cytoplasmic microtubules from both SPBs were temporarily directed toward the rear of the mother, as shown in the inset. The percentage of cells in which this occurred is plotted. Numbers of cells: wild-type, 112; *yeb1*Δ, 189; *ts-arp1*, 128; *ts-arp1 yeb1*Δ, 190. Error bars are SEP. Bar, 2 μm.

trated into the bud (128/128 cells). Furthermore, microtubules frequently made sweeping end-on interactions with the bud cortex (Fig. 7 A). After prolonged failure of spindle movement into the neck in *ts-arp1* cells, microtubules often grew long and then buckled, becoming pressed against the bud cortex (Fig. 7 A). Even in these cases, sliding did not occur (16 mid-anaphase cells, 64 min). Increased microtubule growth rate and rescue frequency probably account for the increase in microtubule length (Table II).

Other dynactin null mutants and a dynein heavy chain null mutant also showed the absence of lateral association and sliding. In a dynein null mutant, 0/4 anaphase cells (observed for 41 min) demonstrated lateral interactions of microtubules with the bud cortex or sliding. This was also the case for a *jnm1*Δ mutant (0/5 cells, 20 min) and for a *nip100*Δ mutant (0/6 cells, 24 min). *jnm1*Δ and *nip100*Δ cells have dynein-like nuclear segregation phenotypes, and Jnm1p and Nip100p coprecipitate with Arp1p (Kahana et al., 1998).

We asked whether free microtubules, stabilized by Yeb1p overexpression, could slide along the cortex of *ts-arp1* cells. We observed no free microtubules in *ts-arp1* cells at the restrictive temperature and even microtubules attached to SPBs did not slide (0/24 cells). Therefore, the force produced by sliding may be necessary to generate free microtubules by detaching them from the SPB. Microtubules also did not interact laterally with the cell cortex unless they grew very long and buckled against the cortex due to space constraints, suggesting that dynein/dynactin is necessary for the lateral microtubule–cortex interactions seen during sliding.

Yeb1p was not required for microtubule sliding. In *yeb1*Δ cells spindle movement into the neck still occurred. Even 41.5 ± 5.4% (n = 82) of mispositioned spindles and 78.3 ± 5.0% (n = 69) of all delayed spindles moved into the neck relatively well. Microtubule sliding might be the mechanism that corrects the nuclear movement defect in *yeb1*Δ cells (Tirnauer et al., 1999). We found that during spindle movement into the neck, microtubules always slid along the bud cortex regardless of the spindle position or orientation (7/7 cells; Fig. 4 D).

How Do Spindles Move into the Bud Neck in the Absence of Dynactin?

Nuclei do eventually segregate properly in dynein and *ts-arp1* mutants (Muhua et al., 1994; Yeh et al., 1995), indicating the existence of an alternative mechanism for nuclear movement into the neck (Yeh et al., 1995). First, we asked if spindle elongation was the mechanism. If elongation alone causes spindle penetration of the neck, the end of the spindle in the mother should not move toward the bud. In contrast, we found that the entire spindle, including the end in the mother, moved toward the bud in 14/16 cells in which the spindle moved into the neck. Spindle elongation was always coincident with spindle movement into the neck, but the rate of spindle movement in the *ts-arp1* cells (0.64 ± 0.12 μm min^{-1}, n = 16) was faster than the spindle elongation rate (0.13 ± 0.03 μm min^{-1}, n = 16), indicating that spindle elongation alone does not move the spindle into the neck.

In 2/16 cells, the force for spindle movement into the neck did appear to come entirely from spindle elongation, since the end of the spindle in the mother remained sta-

tionary as the other end moved into the neck. Even in these cases, the interaction of microtubule ends with the bud cortex appeared to contribute to spindle penetration of the neck by maintaining contact with the bud cortex and aiding spindle orientation along the mother-bud axis.

Second, we asked whether growth of microtubules in the mother pushed the spindle into the neck. We did not observe growth of microtubules against the mother cortex during movement of the spindle into the neck in any *ts-arp1* cells (seven cells in which microtubules in the mother were visible). Third, we asked whether microtubule sweeping in the bud pulled spindles into the neck. Microtubules did sweep along the bud cortex, but did not appear to move the spindle into the neck by sweeping toward the bud tip (10 cells).

Fourth and finally, we asked if microtubule capture/shrinkage caused spindle movement in *ts-arp1* cells. Spindle movement into the neck was associated with a microtubule capture/shrinkage event in 5/6 cells in real time observations (Fig. 7 B). These capture/shrinkage events were transient, and the spindle movements were small (0.5–1 μm) relative to wild-type. The rate of spindle movement in *ts-arp1* cells (0.64 ± 0.12 μm min^{-1}, $n = 16$) was less than that in wild-type cells (1.18 ± 0.11 μm min^{-1}, $n = 19$; $P < 0.01$). Also, successful spindle penetration required that the spindle be well oriented (Fig. 5 B). All these results suggest that this mechanism for spindle movement exerts less force on the spindle than does microtubule sliding.

We asked whether microtubule capture/shrinkage events also contributed to movement or positioning of the spindle in the neck of wild-type cells. After spindles moved into the neck, we sometimes observed shortening of microtubules while their distal ends were anchored at the cortex; this occurred in both the mother and the bud. These capture/shrinkage events pulled the spindle further into the neck and bent the spindle if the microtubule was at an appreciable angle away from the spindle axis (Fig. 4 E).

Since microtubule capture/shrinkage events are the alternate mechanism for spindle movement, then Kar9p/Kip3p-class proteins, such as Yeb1p, may be involved. To test this hypothesis, we asked if Yeb1p contributed to spindle movement into the neck. In *yeb1*Δ cells selected for normal spindle position, spindles showed delayed penetration of the bud (Fig. 5 A; 4.6 ± 1.1 min, $n = 81$, vs. 2.2 ± 0.3 min, $n = 109$, for wild-type; $P < 0.01$). This delay did not stem from a pre-anaphase spindle orientation defect; there was no correlation of the delay with spindle orientation (Fig. 5 B).

This delay in spindle movement into the neck in the *yeb1*Δ mutant might be caused by reduced ability of microtubules to make productive interactions with the bud cortex, as seen during pre-anaphase spindle orientation in *yeb1*Δ cells. As discussed above, microtubule sliding occurred normally in the *yeb1*Δ mutant. However, in some *yeb1*Δ cells, microtubules became misoriented, temporarily failing to enter the bud; such events were very rare in wild-type cells (Fig. 8). All cells with misoriented microtubules showed delayed spindle movement into the neck ($n = 36$), as expected. However, only 52% (36/69) of spindles with delayed neck penetration had misoriented microtubules. In these cases, poor efficiency of capture may cause delayed spindle movement. Cases of misoriented microtubules might represent cells where microtubule capture was very poor.

Do Spindles Move into the Neck in ts-arp1 yeb1Δ Cells?

If microtubule capture/shrinkage serves as the alternative to microtubule sliding for nuclear movement into the neck, then a mutant lacking both microtubule capture/shrinkage and sliding may not move the spindle into the neck at all. To test this prediction, we examined spindle movement into the bud neck in a *ts-arp1 yeb1*Δ mutant. In a wild-type strain, $99 \pm 0.9\%$ of anaphase cells ($n = 112$) successfully moved the spindle into the neck. In *yeb1*Δ and *ts-arp1* mutants, $92 \pm 2.0\%$ ($n = 189$) and $65 \pm 4.5\%$ ($n = 112$) of anaphase cells, respectively, moved the spindle into the neck. The spindle did move into the neck in many of the *ts-arp1 yeb1*Δ cells that entered anaphase ($43.3 \pm 3.7\%$, $n = 178$). Therefore, spindle movement into the neck was less frequent but not abolished in the double mutant.

We asked if the *ts-arp1 yeb1*Δ mutant also had an additive delay in time of spindle movement. The proportion of spindles in the double mutant that delayed movement into the neck was higher than in either single mutant, even when there was no prior positioning or orientation defect (Fig. 5). Furthermore, when nuclei were properly positioned at the neck in the double mutant, spindle movement into the neck required 19.5 ± 3.8 min ($n = 25$), much more than in the *yeb1*Δ and *ts-arp1* single mutants (4.6 ± 1.1 min, $n = 81$, and 8.5 ± 1.3 min, $n = 74$, respectively; $P < 0.001$). Therefore, the double mutant did show an additive delay in spindle movement.

We have shown that Yeb1p affects the efficiency of microtubule interactions with the bud cortex, and that Arp1p is required for microtubule sliding along the bud cortex. We asked whether defects in these microtubule–cortex interactions could account for the additive effect of the *ts-arp1* and *yeb1*Δ mutations on nuclear segregation. In terms of microtubule sliding, the *ts-arp1 yeb1*Δ double mutant was the same as the *ts-arp1* single mutant. Microtubule sliding did not occur even when long microtubules buckled and pressed against the cortex (5 cells, 1,200 s). The *ts-arp1 yeb1*Δ double mutant also showed a defect in microtubule capture, as suggested by the presence of misoriented microtubules (Fig. 8). This defect was less severe than in the *yeb1*Δ single mutant (Fig. 8). However, fewer misoriented microtubules in the double mutant might be due to longer microtubules that are unable to leave the bud (Table II), rather than more efficient capture of microtubule ends. The observation that there were fewer misoriented microtubules in the *ts-arp1* single mutant than in wild-type cells supports this view (Fig. 8).

Taken together, these observations suggest that the backup mechanism for spindle movement is less efficient but intact in *ts-arp1 yeb1*Δ cells. Spindle movement was associated with transient microtubule capture/shrinkage events (2/2 cells in real time). We ruled out spindle elongation as a major contributor to spindle movement; the entire spindle moved into the neck (8/9 cells), and the rate of spindle movement (0.93 ± 0.19, $n = 9$) was greater than the spindle elongation rate (0.39 ± 0.06, $n = 9$). Therefore, Yeb1p may not be absolutely required for microtubule capture/shrinkage.

Discussion

Spindle Movement into the Neck Is Mediated by Microtubule Sliding

In this study, we asked how the yeast cell moves and positions the spindle during mitosis. Our most novel and important conclusion is that the spindle is pulled into the mother/bud neck by microtubules that slide along the cortex of the bud. Dynein and dynactin are required for microtubule sliding; in their absence, microtubules do not associate laterally with the cortex and do not slide even if pressed against the cortex. Previous work suggested that dynein/dynactin might pull on the ends of microtubules at the cortex (Carminati and Stearns, 1997). However, the microtubule–cortex interactions described by Carminati and Stearns (1997) occurred before or after nuclei moved into the neck. Our results show that this model does not explain how dynein affects nuclear movement into the neck.

A major piece of evidence supporting the conclusion that microtubule sliding exerts force on the spindle is that free microtubules, not attached to a SPB, associated laterally with the cortex and slid. Therefore, the cortex is able to apply force to a microtubule and cause it to slide; thus moving the spindle. Moreover, dynactin was required for the lateral association of microtubules with the cortex and the production of free microtubules in cells overexpressing Yeb1p. Free microtubules were only stable in cells overexpressing Yeb1p, which should prevent microtubules from depolymerizing. On rare occasions, we saw microtubules break free of SPBs in cells with normal levels of Yeb1p. These microtubules depolymerized completely in a few seconds (Adames, N.R., and J.A. Cooper, unpublished results).

We can envision three models in which dynein/dynactin contributes to microtubule sliding. First, dynein/dynactin might be anchored at the cortex, bind the sides of microtubules, and walk in the minus end direction. Second, dynein/dynactin might regulate microtubule dynamics at the plus end. In this model, loss of dynein/dynactin would cause microtubules to grow so long that they impede spindle entry into the neck. This model is suggested by the observation that dynein and dynactin null mutants sometimes have very long cytoplasmic microtubules (Muhua et al., 1994; Carminati and Stearns, 1997). Third, dynein/dynactin might cause microtubule depolymerization at the SPB, allowing microtubules to treadmill. In this model, other proteins at the cortex would bind the microtubule laterally.

All of our data are consistent with the first model. Our results here with a conditional *arp1* allele demonstrate that loss of sliding and lateral association with the cortex are the primary defects associated with poor spindle movement, not the excessive microtubule growth seen in null mutants. This observation rules out the second model. Our speckle analysis of free microtubules sliding along the cortex shows that microtubules move as a whole and do not treadmill. This result is also consistent with the first model and argues against the third model. Although the polymerization dynamics of these microtubules are clearly not normal, the fact that microtubules slide without treadmilling shows that the cortex can exert force on the microtubule and should not simply bind to the sides of microtubules as predicted in the third model. Another piece of data consistent with the first model, but inconsistent with the other two models, is the observation that dynein/dynactin is required for the lateral association of microtubules with the cortex.

Spindles remain in the neck and are not pulled completely into the bud. Also, spindles in the neck oscillate along the mother-bud axis (Yeh et al., 1995). These results suggest that forces in the mother may pull backward on the spindle. We found evidence for microtubule sliding in the mother to account for this pulling force. First, free microtubules slid along the mother cortex as well as the bud cortex. Second, after spindles moved into the neck, microtubules in the mother slid along the mother cortex and the spindle moved backward. Perhaps dynein/dynactin undergoes spatial and temporal regulation during spindle movement, activated first in the bud to pull the spindle into the neck and later in the mother to pull backwards and keep the spindle in the neck.

Why do yeast need microtubule sliding for spindle movements into the neck? One possibility is that a relatively large force is needed to move the nucleus into the neck because the nucleus is larger than the opening of the neck. Lateral interaction of microtubules with the bud cortex should provide a stronger attachment to the cortex and allow more motor molecules to act on the microtubule, relative to microtubule end interactions.

Dynamic Microtubules Probe the Cortex for Microtubule Capture/Shrinkage Sites

Because some proteins involved in nuclear positioning at the neck have been localized to the bud tip, it has been hypothesized that microtubule capture/shrinkage sites are restricted to bud tips (Amberg et al., 1997; Evangelista et al., 1997; Miller and Rose, 1998). Consistent with this hypothesis, microtubule capture/shrinkage events at the bud tip, pulling the nucleus to the neck, have been described (Shaw et al., 1997). We found that, in budded cells, microtubule capture/shrinkage events never occurred on the mother cortex before anaphase, even when the nucleus was mispositioned and remained well within the mother. Therefore, microtubule capture/shrinkage is, indeed, restricted to the bud.

Here, we also found evidence that functional capture sites exist earlier than some studies have suggested. We found that most nuclei moved to the incipient bud site, and this movement coincided with microtubule capture/shrinkage at this site. We found much less of a role for microtubule growth pushing the nucleus about the mother, in contrast to the results of Shaw et al. (1997). Strain background may account for this difference. Nuclear movements associated with microtubule growth may be more prevalent after bud formation because microtubules must pass through the narrow neck to interact with capture sites at the bud tip.

In our current model for nuclear movement to the neck, the dynamic growth properties of microtubules allow microtubules to encounter functional capture/shrinkage sites. Our results here with the *yeb1*Δ mutant support this view. We found that nuclear movement to the neck was im-

paired in *yeb1*Δ cells, as suspected from studies of nuclear positioning (Tirnauer et al., 1999). More important, microtubule growth rates and lengths were reduced, leading to decreased frequency of microtubule interactions with the cortex.

A Mechanism for Nuclear Movement in the Absence of Dynein/Dynactin

Although dynein/dynactin is required for efficient spindle movement into the neck, spindles do eventually enter the neck in dynein/dynactin mutants (Yeh et al., 1995; Muhua et al., 1998). We found that the backup mechanism for spindle movement into the neck is mainly microtubule capture/shrinkage events, similar to the mechanism for nuclear movement to the neck. Our results show that Yeb1p contributes to microtubule capture and thereby plays a minor role in spindle movement into the neck in wild-type cells and a major role in the dynactin *arp1* mutant. Since we found that microtubule capture/shrinkage occurs in wild-type cells after the spindle has moved into the bud neck, the backup mechanism may be a normal but minor part of spindle movement into the neck, which is normally dominated by dynein/dynactin-dependent microtubule sliding. Yeb1p and other Kar9p/Kip3p-class proteins probably also contribute to the backup mechanism for spindle movement by maintaining spindle orientation, a major factor in the efficiency of nuclear movement into the neck in the *ts-arp1* mutant. Proper spindle orientation should allow spindle elongation to contribute to spindle penetration of the neck.

The backup mechanism for nuclear movement into the neck is impaired, but not abolished, in the *ts-arp1 yeb1*Δ double mutant. The backup mechanism depends largely on the stochastic capture of microtubule ends. During G1/S the primary defect in the *yeb1*Δ mutant was reduced microtubule length and number, leading to decreased capture. However, during G2/M the loss of Yeb1p reduced the frequency of productive microtubule capture events without affecting microtubule dynamics. In this case, Yeb1p appears to function at the cortical capture site to convert an encounter between a microtubule end and a capture site into an event that produces force. Yeb1p was recently shown to physically interact directly with the cortical protein Kar9p and the frequency with which microtubule ends interact with Kar9p cortical spots is reduced in *yeb1*Δ cells (Korinek et al., 2000; Lee et al., 2000; Miller et al., manuscript submitted for publication). Our results show that Yeb1p is not involved in the lateral association of microtubules with the cortex and sliding, indicating that the cortical microtubule capture sites involved in capture/shrinkage are distinct from those involved in sliding. We also show that the Yeb1p-Kar9p interaction is important (but not absolutely necessary) for capture of microtubule ends and nuclear movements during mitosis. This role for Yeb1p in anaphase nuclear movement is particularly important as a backup mechanism for nuclear movement into the neck in the absence of dynein/dynactin.

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