Role of Astral Microtubules and Actin in Spindle Orientation and Migration in the Budding Yeast, *Saccharomyces cerevisiae*

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Abstract. In the yeast *Saccharomyces cerevisiae*, before the onset of anaphase, the spindle apparatus is always positioned with one spindle pole at, or through, the neck between the mother cell and the growing bud. This spindle orientation enables proper chromosome segregation to occur during anaphase, allowing one replicated genome to be segregated into the bud and the other to remain in the mother cell. In this study, we synchronized a population of cells before the onset of anaphase such that >90% of the cells in the population had spindles with the correct orientation, and then disrupted specific cytoskeletal elements using temperature-sensitive mutations. Disruption of either the astral microtubules or actin function resulted in improper spindle orientation in ∼40–50% of the cells. When cells with disrupted astral microtubules or actin function entered into anaphase, there was a 100–200-fold increase in the frequency of binucleated cell bodies. Thus, the maintenance of proper spindle orienta-

tion by these cytoskeletal elements was essential for proper chromosome segregation. These data are consistent with the model that proper spindle orientation is maintained by directly or indirectly tethering the astral microtubules to the actin cytoskeleton.

After nuclear migration, but before anaphase, bulk chromosome movement occurs within the nucleus apparently because the chromosomes are attached to a mobile spindle. The frequency and magnitude of bulk chromosome movement is greatly diminished by disruption of the astral microtubules but not by disruption of the nonkinetochore spindle microtubules. These results suggest that astral microtubules are not only important for spindle orientation before anaphase, but they also mediate force on the spindle, generating spindle displacement and in turn chromosome movement. Potential roles for this force in spindle assembly and orientation are discussed.

C ELL division in the yeast, *Saccharomyces cerevisiae*, is an asymmetric process in which a bud emerges from a discrete region of the periphery of the parental, "mother," cell. Before anaphase the nucleus migrates from a location in the center of the mother cell to a position at the isthmus, or neck, between the mother cell and the growing bud (Pringle and Hartwell, 1981), and the nuclear envelope becomes extended between the mother cell and bud. Embedded in the nuclear envelope are the two separated spindle pole bodies (SPBs)[1] (Byers and Goetsch, 1974; Byers and Goetsch, 1975). Emanating from the lumenal face of each SPB into the nucleus are microtubules that form the bipolar spindle (Byers and Goetsch, 1974). Radiating away from the cytoplasmic face of each SPB towards the cell periphery are astral or cytoplasmic microtubules. Before the onset of anaphase, the bipolar spindle is always positioned with one SPB at, or through, the neck (Byers

and Goetsch, 1975; Pringle and Hartwell, 1981). At the onset of anaphase, the spindle and nuclear envelope elongate such that one spindle pole passes through the neck. Concomitant with spindle elongation, the replicated sister genomes segregate from each other with one genome moving into the bud and the other moving to the periphery of the mother cell.

By positioning the spindle so that one SPB is at the neck before anaphase, the yeast cell apparently ensures that during anaphase the spindle will elongate through the narrow opening of the neck. We are interested in identifying and characterizing the proteins that are involved in maintaining proper spindle position before anaphase. Since spindles are asymmetrically positioned in diverse cell types such as blastomeres of invertebrate embryos (Wilson, 1925), neuroblast cells (Carlson, 1977), and plant cells (Staiger and Lloyd, 1991), the elucidation of the mechanism of spindle position in yeast may help to understand aspects of spindle position and asymmetric cell division in other cell types.

Some insight into the process that maintains proper spindle position before anaphase has come from studying chro-

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1. *Abbreviations used in this paper*: DIM, digital imaging microscopy; HU, hydroxyurea; SPB, spindle pole body.

mosomes and spindles in populations of cells that were synchronized after nuclear migration but before anaphase (Palmer et al., 1989). In these cells, chromosomes migrated as a group within the lumen of the nucleus that extended between the mother cell and bud. This bulk chromosome migration was referred to as nuclear DNA transits. Nuclear DNA transits were observed frequently, were rapid and bidirectional, and had no effect on subsequent anaphase (Palmer et al., 1989). In cells undergoing nuclear DNA transits, the position of the spindle was variable within the lumen of the nucleus. It was located in the mother cell, the bud, or spanning the neck between the mother cell and bud (Palmer et al., 1989). However, whether the spindle was in the mother cell or bud, it was always positioned with one pole at the neck. Finally, the chromosomes were always colocalized with the spindle. From these results we conclude: (a) spindles are mobile after nuclear migration but before anaphase; (b) the bulk chromosome movement during nuclear DNA transits can be easily explained by the attachment of the chromosomes to the migrating spindle; and (c) maintenance of spindle position in budding yeast involves a dynamic process in which correct spindle orientation is maintained despite significant spindle displacement.

Analysis of yeast cells with conditional-lethal mutations in the β-tubulin (TUB2), and actin (ACTI) genes has shown that microtubules are required for nuclear migration and mitosis (Huffaker et al., 1988), and actin function is necessary for vectorial protein transport and bud formation (Novick and Botstein, 1985). In this study, we addressed whether these cytoskeletal components are also required for maintaining proper spindle orientation and movement before anaphase. For this purpose, we synchronized populations of cells after nuclear migration but before anaphase. We used different mutations and cell cycle inhibitors to disrupt microtubules or actin function and then examined spindle migration and position.

Materials and Methods

Reagents

The antibodies used in this study were gifts from the following individuals: rabbit anti-alpha and anti-beta tubulin antibodies were provided by Dr. Frank Solomon (Massachusetts Institute of Technology, Cambridge, MA); rabbit anti-KAR2 antibody was provided by Dr. Mark Rose (Princeton University, Princeton, NJ); and an affinity-purified rabbit anti-yeast actin antibody was a gift from Dr. David Drubin (University of California, Berkeley, CA). A rat anti-yeast tubulin mAb, YOL1/34, was purchased from Serotec (Indianapolis, IN). FITC-conjugated goat antiserum to rabbit IgG and a rhodamine-conjugated goat antiserum to rat IgG were obtained from Cappel (West Chester, PA). Calcofluor White M2R was obtained from Polysciences Inc. (Cellufluor, Worthington, PA), and DAPI (2,6,-diamidino-phenylindole) was obtained from Boehringer Mannheim Biochemicals (Indianapolis, IN). Zymolyase (100T) was purchased from ICN immunobiologicals (Lisle, IL).

Strains

All yeast strains used in this study are listed in Table I. Construction of the tub2-401 cold-sensitive strain was performed using the "pop-in"/"pop-out" protocol (Rothstein, 1991). Plasmid integration was directed into the TUB2 chromosomal locus by linearizing the plasmid pTH18 (Huffaker et al., 1988) at the KpnI site within the coding region of the TUB2 locus, and transforming the strain 4506-9-4 (Ito et al., 1983). The plasmid pTH18 contains the tub2-401 mutation on a ~4.0-kb region of genomic DNA. Integration resulted in Ura[+] transformants with the intact TUB2 gene and the tub2-401 mutant allele flanking the URA3 selectable marker. Excision of the

plasmid sequences was selected by growing the transformants on 5-fluoro-orotic acid plates to select against URA3 (Boeke et al., 1984). Since the excision event resulted in either a wild-type TUB2 gene or the tub2-401 mutant, the 5-fluoro-orotic acid–resistant papillae were replica plated to 14°C to check for cold sensitivity. The cdc16 tub2-401 double mutant was constructed by crossing the tub2-401 integrant strain with the cdc16-2 strain 5016-5-2, the diploid strain was sporulated and spores with mutations in both genes were isolated based on their cold and temperature sensitivity. A diploid cdc16-2 tub2-401 double mutant was constructed using standard yeast genetics.

Fluorescence Microscopy

Cells were processed for fluorescence and indirect immunofluorescence microscopy using protocols from Palmer et al. (1989) and Kilmartin and Adams (1984), respectively. Cells were fixed for 2 h by adding formaldehyde to a final concentration of 3.6% (vol/vol). For each strain, cells were collected by centrifugation, washed twice in SCE (1 M sorbitol, 10 mM sodium citrate, 60 mM EDTA, pH 7.0), and then resuspended in 1 ml of SCE plus 0.1% 2-mercaptoethanol and spheroplasted for 4–10 min at 37°C with zymolyase (final concentration 30 μg/ml). Antibodies were diluted in solution F (Kilmartin and Adams, 1984) and were used at the following dilutions: rabbit anti-alpha and beta tubulin 1/1,000, rabbit anti-KAR2 1/10,000, rabbit anti-actin 1/50, rat anti-tubulin 1/50, goat anti–rabbit IgG 1/2,000, and goat anti–rat IgG 1/200. For each experiment, between 100 and 300 cells were examined, and representative cells were photographed with a 35-mm camera (Zeiss) with Kodak TMAX 400 film (Eastman Kodak Co., Rochester, NY).

Role of Astral Microtubules in Maintaining Proper Spindle Position

The diploid cdc16 tub2-401 strain 5092 was grown at 23°C in YPD (Sherman et al., 1986) to a cell density of 4×10^6/ml then shifted to 36°C for 3 h to arrest cells after nuclear migration but prior to anaphase. An aliquot of cells was fixed and prepared for indirect immunofluorescence. The remaining cells were shifted to 18°C, cells were removed every 20 min, fixed, and prepared for indirect immunofluorescence. For each time point, the percentage of spindles with no discernable astral microtubules was determined. The orientation of the spindles was determined for the cells without astral microtubules. Mis-oriented spindles were defined as those in which the axis defined by the spindle failed to pass through the neck (i.e., if the spindle were to elongate, it would fail to pass through the neck). To determine whether the mis-oriented spindles resulted in abnormal mitoses, we determined the frequency of binucleate cell bodies in the cdc16 tub2-401 double mutant. The diploid cdc16 tub2-401 strain 5092 was grown at 23°C to a cell density of 4×10^6/ml then shifted to 36°C for 3 h. An aliquot was removed and prepared for indirect immunofluorescence to examine both microtubule structures and nuclear envelope position. The remaining cells were transferred to 18°C. After 2 and 3 h at 18°C, aliquots were removed and prepared for indirect immunofluorescence. The frequency of binucleate cell bodies (two separated genomes in one cell body, i.e., mother cell or bud) was calculated by dividing the number of binucleate cells by the total number of cells that had completed anaphase.

Role of Cytoplasmic Actin in Maintaining Proper Spindle Orientation

All strains were grown at 23°C to a cell density of 4×10^6/ml. Cells were synchronized after nuclear migration but before anaphase by adding hydroxyurea (HU) to final concentration of 100 mM for 3 h at 23°C. An aliquot of cells was removed and prepared for indirect immunofluorescence. While still in HU, cells were then shifted to the restrictive temperature of 36°C for 2 h to disrupt the actin cytoskeleton and a sample was fixed and prepared for indirect immunofluorescence. The percent of cells with mis-oriented spindles was determined as above. The actin cytoskeleton was examined by indirect immunofluorescence of fixed cells (see above). Cells with actin cables traversing between the mother cell and bud, and asymmetric distribution of actin cortical patches in the bud were considered to have a normal actin cytoskeleton. Cells were considered to have a disrupted actin cytoskeleton when they had similar numbers of cortical patches in the mother cell and the bud, diffuse background staining of the cytoplasm, and no cables. To determine whether the mis-oriented spindles resulted in abnormal mitoses, we determined the frequency of binucleate cell bodies in the act1-4 mutant. The remaining cells were washed twice with fresh YPD

medium and cultured at 23°C for 2 h to allow the cells to enter into mitosis. This same experimental design was performed with wild-type strains and a strain with a temperature-sensitive mutation in the gene *CDC3*.

A spontaneous *act1*-4 revertant was isolated by growing eight individual isolates of the strain TDY42 (Dunn and Shortle, 1990) to saturation at 23°C and then plating ~5 × 10⁸ cells on YPD plates at 36°C. The frequency of revertants was ~1 × 10⁸. The revertants were streaked to 23°C then retested for growth at 36°C. The growth rates of the *ACT1*, *act1*-4, and *act1*-4 revertant were determined by monitoring cell number (with a hemocytometer) as a function of time.

Digital Imaging Microscopy of Nocodazole-treated, cdc16-synchronized Cells

We treated cells undergoing nuclear DNA transits with the microtubule depolymerizing drug nocodazole to determine the role of microtubules on nuclear DNA transits. A *cdc16*-1 ρ° strain was used in order to minimize the background mitochondrial DNA staining. The *cdc16*-1 ρ° culture was grown in 1% DMSO (Jacobs et al., 1988) at 23°C to a cell density of 4 × 10⁶/ml then shifted to 36°C for 5 h to arrest cells, instead of 3 h, the normal procedure for ρ+ strains. The increased time at the restrictive temperature for the ρ° strain was needed to get it to arrest homogeneously. The longer time needed to arrest this *cdc16*-1 ρ° strain was because the growth rate of this strain was slower than a ρ^+ *cdc16*-1 strain. Cells were examined with phase-contrast microscopy to insure that >95% of the cells were arrested. The nocodazole-treated cells were processed as follows: after 4 h and 15 min at 36°C nocodazole was added to the cultures at a final concentration of 30 µg/ml. After 35 min DAPI was added (2.5 µg/ml), and cells were shifted to 23°C for 20 min. Control cells were treated identically to nocodazole-treated cells except nocodazole was omitted. Digital imaging microscopy (DIM) was then used to examine the movement of DAPI stained chromosomal DNA in both nocodazole-treated and control cells (Shero et al., 1991). For both treatments 16 cells were examined. Cells were observed for 1 h and the fraction of cells that exhibited chromosomal movements was determined. For the nocodazole treatment, an aliquot of cells was taken before and after the DIM, and microtubule and nuclear envelope structures were visualized by indirect immunofluorescence to determine whether nocodazole treatment had depolymerized all microtubules and whether the position of the nucleus had changed.

Position of Nuclear DNA in the Mother Cell and Bud in cdc31 Mutant

Using a fixed cell assay (Palmer et al., 1989) the position of the chromosomal DNA was examined in the *cdc31*-1 mutant to determine whether nuclear DNA transits had occurred. The strain 5071 was grown at its permissive temperature (23°C) in YPD to a density of 4 × 10⁶/ml, an aliquot of cells was removed and fixed, the remainder of the cells were synchronized by shifting the culture to 36°C for 3 h. The cells were fixed and stained with DAPI (1 µg/ml) to visualize nuclear DNA and calcofluor (50 µg/ml) to visualize the bud scar. Both dyes were observed simultaneously with the same filter combination on a Zeiss axiophot microscope with a 100× neofluar objective. Since the bud scar is located exclusively on the surface of the mother cell and not on the bud, we could define three distinct nuclear staining patterns: the nuclear DNA was either in the mother cell or the bud, was positioned equally in the isthmus (neck) between the mother cell and the bud, or had already segregated, with one genome in the mother cell and the other in the bud. Since chromosomes are positioned in the mother cell at the beginning of the cell cycle, cells in which the nuclear DNA was located in the bud represent cells where the bulk chromosomes had migrated from the mother cell to the bud, i.e., a nuclear DNA transit. Tubulin structures were visualized in the *cdc31*-1 mutant using indirect immunofluorescence with anti-tubulin antibodies, and the position of the spindle in relation to the nuclear DNA and cell surface was determined. The monopolar spindle was discernable from astral microtubules based on the fact that it was shorter and thicker than the astral microtubules. The SPB was observed as a brightly stained spot that had astral microtubules radiating out toward the cell periphery.

Analysis of Nuclear DNA Transits in Cells Lacking Astral Microtubules

Exponential cultures of the diploid *cdc16 tub2*-401 strain 5092 were grown at 23°C in YPD to a cell density of 4 × 10⁶/ml. These cells were shifted to 36°C for 3 h. An aliquot of cells was removed and prepared for indirect immunofluorescence to examine both microtubule structures and nuclear envelope position. An aliquot of cells was shifted to 23°C and stained with DAPI (2.5 µg/ml). Chromosome movement was followed by DIM (Shero et al., 1991). This was a control to ensure that nuclear DNA transits were capable of occurring in this strain at 23°C. The remainder of the cells were then shifted to 18°C for 40 min; an aliquot was removed and prepared for indirect immunofluorescence and processed for DIM. For these DIM experiments the room temperature was maintained at 17°C. A thermometer (Sensortek, Clifton, NJ) was attached to the microscope stage to monitor the temperature. The stage temperature was kept between 17.6 and 18.0°C. Cells were examined with DIM for 1.5 h. The same experiments were performed with the diploid *cdc16* strain 5055 to address if nuclear DNA transits can occur at 18°C.

We also examined the extent of bulk chromosome movement within one cell body for the *cdc16* strain (5055) and the *cdc16 tub2*-401 strain (5092) incubated at 18°C. This entailed measuring the displacement of the bulk chromosomes between each real-time image. Real-time images were taken ever 3 min. On each image a mark was placed in the center of the DAPI-stained chromosomes using the "draw" function of the Image-1/AT image processing system (Universal Imaging Corporation, Media, PA). The subsequent image was then overlaid on the first image and another mark was placed in the center of the DAPI-stained chromosomes. The distance between the two marks was measured with the "measure with caliper" function of the Image-1/AT program.

Results

Experimental Rationale

An inherent problem in using asynchronously dividing cells to investigate spindle movement and orientation after nuclear migration, but before anaphase, is that only a small fraction of cells are at this stage of the cell cycle. To circumvent this problem, asynchronously dividing cultures were synchronized after nuclear migration but before anaphase by arresting them transiently with mutations in the cell division cycle gene, *CDC16*, or with the DNA synthesis inhibitor hydroxyurea. We genetically, or pharmacologically, disrupted microtubules or actin function in these arrested cells, and then analyzed their chromosome movement, spindle migration, and spindle orientation. To facilitate these cytological analyses, diploid strains were used. Transient arrest of cells by either of these two methods is innocuous; when transferred back to permissive conditions >90% of the arrested cells survive (Koshland and Hartwell, 1987).

Spindle Orientation

Role of Astral Microtubules in Maintaining Proper Spindle Orientation before Anaphase. Recent analysis of yeast strains with the *tub2*-401 mutation showed that these strains have phenotypically normal microtubules when grown at 23°C, but when grown at 18°C the cells preferentially lose their astral microtubules (Sullivan and Huffaker, 1992). To determine whether the astral microtubules were required for spindle orientation before anaphase, we constructed the strains that contained the *cdc16*-2 mutation (5055) or both the *cdc16*-2 and *tub2*-401 mutations (5092) (Table I). These strains were grown at 23°C and then shifted to 36°C to arrest cells after nuclear migration but before anaphase, i.e., at the *cdc16* block. The arrested cells were then shifted either to 23 or to 18°C. Cells were fixed at various times after the shift to 18 or 23°C and the microtubule structures in these cells were examined by indirect immunofluorescence.

After 3 h at 36°C almost all the *cdc16* and *cdc16 tub2*-401 cells had an identifiable spindle and >93% of these cells had astral microtubules. When *cdc16 tub2*-401 cells were shifted

Table I. Yeast Strains Used in This Study

Strain	Genotype	Source
5050	*MATa/MATα ade2/ADE2 ade3/ADE3 his7/his7 hom3/HOM3 leu2/LEU2 can1/can1 sap3/sap3 CYC2/cyc2*	(Palmer et al., 1989)
208-1	*MATa/MATα cdc16-1/cdc16-1 ade2/ADE2 ade3/ADE3 his7/his7 hom3/HOM3 leu2/LEU2 ura1/URA1 can1/can1 sap3/sap 3 rho⁰*	(Palmer et al., 1989)
5071	*MATa/MATα cdc31-1/cdc31-1 his7/his7 HOM3/hom3 leu2/leu2 trp1/trp1 ura3/ura3*	(this study)
5055	*MATa/MATα cdc16-2/cdc16-2 ADE3/ade3 LEU2/leu2 TRP1/trp1 ura3/URA3 can1/can1 cyh2/chy2 sap3/sap3*	(this study)
5092	*MATa/MATα cdc16-2/cdc16-2 tub2-401/tub2-401 his3/HIS3 leu2/leu2 ura3/ura3*	(this study)
5105	*MATa/MATα ac1-4/ac1-4 HIS3/his3 leu2/LEU2 ura3/ura3*	(this study)
LH104-HO1	*MATa/MATα cdc3-1/cdc3-1*	(Adams and Pringle, 1984)
4506-9-4	*MATa his3 leu2 ura3*	(this study)
4506-9-46	*MATa tub2-401 his3 leu2 ura3*	(this study)
5016-5-2	*MATα cdc16-2 ade2 leu2 trp1 ura3*	(this study)
TDY42	*MATα act1-4 ade2 ura3*	(Dunn and Shortle, 1990)

to 18°C, there was a rapid loss of astral microtubules such that after 40 min only 21% of the cells had identifiable astral microtubules (Figs. 1 and 2 *a*). In addition, in cells that lacked the astral microtubules, spindle length transiently shrunk relative to the cells at 36°C (Figs. 1 and 2 *c*). In contrast, the shift to 18°C had no effect on astral microtubules or spindle length in the *cdc16* cells. These results demonstrated that *tub2-401* mutation in the 5092 strain caused permanent depletion of most astral microtubules and may have also caused a subtle, transient, effect on spindle microtubules at 18°C.

The position of the spindle was also examined in the *cdc16* strain and *cdc16 tub2-401* double mutant strain. When these strains were arrested at the *cdc16* block (36°C), >95% of the spindles were oriented properly, i.e., one SPB was positioned at, or through, the neck (Figs. 1 and 2 *a*). Similar results were obtained after shifting these strains to 23°C (data not shown). Thus, when the *cdc16* and *cdc16 tub2-401*

strains were grown under conditions where their astral microtubules were normal, spindle orientation was also normal. However, after shifting the *cdc16 tub2-401* strains from 36 to 18°C, there was a rapid increase in the percentage of cells with mis-oriented spindles (Figs. 1 and 2 *b*) that was not observed in the *cdc16* strain at 18°C (Fig. 2 *b*). This increase in misoriented spindles correlated with the frequency of cells that lacked astral microtubules but not with the transient shrinkage of the spindle. By 40 min at 18°C, 21% of the *cdc16 tub2-401* cells that still had astral microtubules also had properly oriented spindles. However, of the 79% of cells that had lost astral microtubules, two-thirds of the cells had improperly oriented spindles (Figs. 1 and 2 *b*). These results suggested that astral microtubules were important, if not essential, for maintaining proper spindle orientation after nuclear migration but before anaphase. The fact that all spindles were not mis-oriented was not surprising. If spindle orientation was random in cells without astral microtubules,
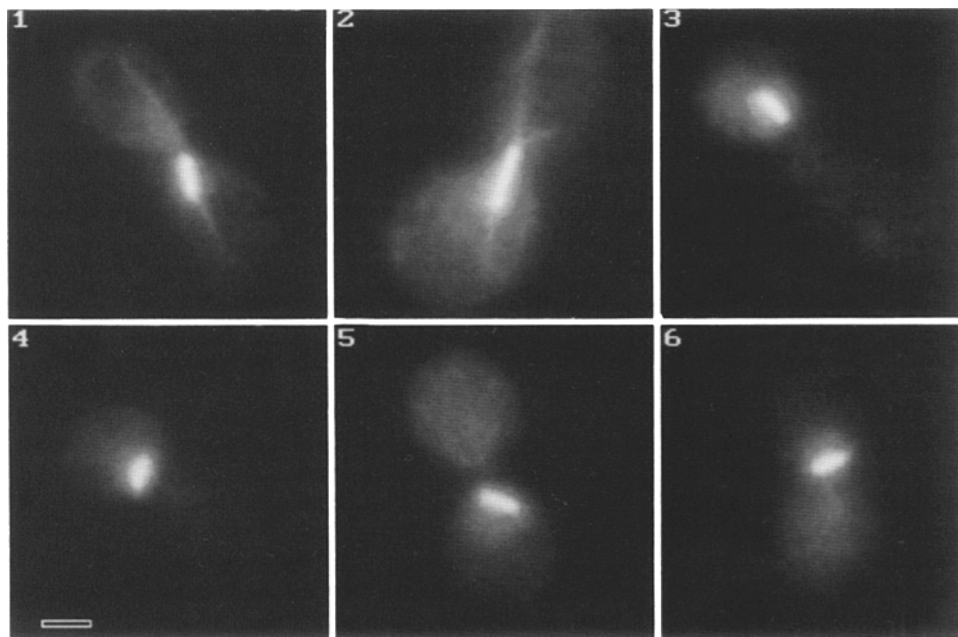

Figure 1. Micrographs of arrested *cdc16 tub2-401* double mutant cells. The *cdc16 tub2-401* strain 5092 was grown at 23°C then shifted to the nonpermissive temperature of 36°C for 3 h. An aliquot was removed, fixed, and prepared for indirect immunofluorescence microscopy (see Materials and Methods). The remaining cells were shifted to 18°C for 40 min and then fixed, and prepared for indirect immunofluorescence microscopy with anti-tubulin antibodies to examine microtubule structures. Micrographs of representative cells were prepared with DIM. The spindle is the brightly stained bar, and the astral microtubules are the fainter arrays that emanate from both the ends of the spindle. Note that the images were contrast enhanced to ensure the detection of the cytoplasmic microtubules resulting in an over exposure of the central spindle. (*1* and *2*) *cdc16 tub2-401* cells after 3 h at 36°C. (*3–6*) Cells that were shifted from 36 to 18°C for 40 min. Note in two cells (*4* and *6*) the position of the spindle was different; it was positioned perpendicular to the mother cell-bud plane. Bar, 2 μm.
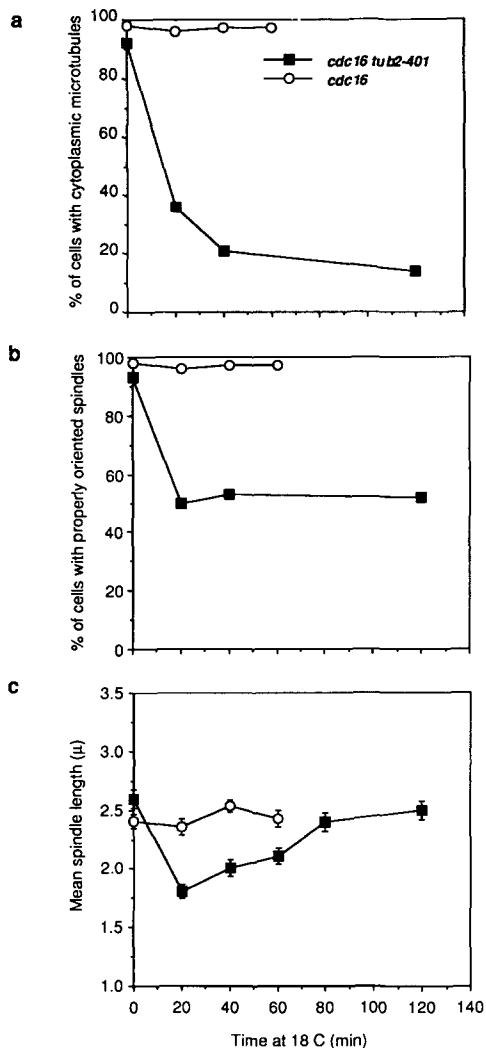
Figure 3. Indirect immunofluorescence micrographs of hydroxyurea-arrested *ACT1* and *act1*-4 cells. The *ACT1* strain 5050 and the *act1*-4 strain 5105 were grown at 23°C for 3 h in the presence of 100 mM hydroxyurea then shifted to the nonpermissive temperature of 36°C for 2 h. An aliquot was removed, fixed, and prepared for indirect immunofluorescence microscopy (see Materials and Methods). An *ACT1* (*a* and *b*) and *act1*-4 (*c* and *d*) cell are shown stained with anti-tubulin antibodies to examine the position of the spindle (*a* and *c*), and an anti-actin antibody to visualize actin distribution (*b* and *d*). The spindle is the brightly stained bar, and the astral microtubules are the fainter arrays that emanate from both the ends of the spindle. Bar, 5 μm.

Figure 2. Spindle characteristics of arrested *cdc16* and *cdc16 tub2-401* double mutant cells. The *cdc16 tub2-401* strain 5092 and the *cdc16* strain 5055 were grown at 23°C, and then shifted to the non-permissive temperature of 36°C for 3 h. An aliquot was removed, fixed, and prepared for fluorescence and indirect immunofluorescence microscopy (see Materials and Methods). The remaining cells were shifted to 18°C for 20, 40, 60, 80, and 120 min, fixed, and prepared for fluorescence and indirect immunofluorescence microscopy. The position of the nuclear DNA was determined with fluorescence microscopy of DAPI stained cells. For cells in which the nuclear DNA was on one side of the neck the following spindle characteristics were determined: the percent of cells with detectable astral microtubules, and the percent of cells that did not have their spindle oriented properly with one SPB positioned at or through the neck between the mother cell and bud. The percentages were determined from four different experiments, and for each experiment ~100-200 cells were examined per time point. Spindle lengths were measured from fixed cells using the "measure with caliper" program of the Image-1/AT image processing system (Universal Imaging Corporation, Media, PA). Values are the mean length ± the SEM, and are based on three experiments in which for each experiment 40-50 spindles were measured per time point.

one would expect a certain fraction of spindles to be fortuitously oriented properly.

Role of Actin in Maintaining Proper Spindle Orientation before Anaphase. We also examined the role of cytoplasmic actin in maintaining proper spindle orientation since a potential role for the cell cortex and actin in spindle orienta-
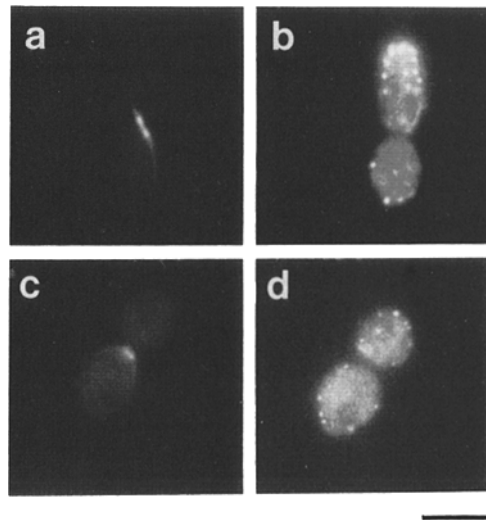
tion had been inferred from studies of mitoses in other systems (Hyman, 1989; Lutz et al., 1988). Our strategy was to use HU, the DNA synthesis inhibitor, to synchronize cells after spindle formation and nuclear migration but before anaphase (Hartwell, 1976; Byers and Goetsch, 1974) and then to inactivate actin function. For this purpose, diploid strains were constructed that were either homozygous for a temperature-sensitive mutation (*act1*-4) (Dunn and Shortle, 1990) in the single actin structural gene or homozygous *ACT1*. Exponentially dividing cultures of these strains were treated with hydroxyurea for 3 h at 23°C. In multiple experiments, the percent of cells synchronized by HU after nuclear migration but before anaphase was more variable and less complete for the *act1*-4 diploid strain (30-60%) than for the *ACT1* diploid strain (70-80%). Synchronized cells were then incubated for an additional 2 h in HU at 36°C (the nonpermissive temperature for *act1*-4 function). Cells were fixed before and after shift to 36°C and actin and microtubule structures were analyzed by indirect immunofluorescence (see Materials and Methods).

Actin is present in two morphologically distinct structures in the yeast cell. It is present as cortical patches that are asymmetrically distributed with a higher concentration of patches localized in the bud, and it is also found as cytoplasmic cables that traverse from the mother cell to the bud (Adams and Pringle, 1984). After 2 h at 36°C, >85% of our *ACT1* diploid cells had cables, and cortical patches preferentially localized in the bud (Fig. 3 *b*). In contrast, after 2 h at 36°C >90% of our *act1*-4 diploid cells showed complete disruption for all the actin cables and most of the cortical patches; the remaining cortical patches became randomly distributed between the mother cell and bud (Fig. 3 *d*). The

disruption of the actin cytoskeleton demonstrated that there was significant loss of actin function in the *actl*-4 mutant under these experimental conditions.

We then examined the position of the spindle in cells with normal and disrupted actin function. Before shifting the *actl*-4 or *ACTl* cells to 36°C, >90% of these cells had their spindles oriented properly, with one SPB positioned at or through the neck. However, after 2 h at 36°C ∼40% of the *actl*-4 cells had their spindles mis-oriented (Fig. 3) while only 3% of the *ACTl* cells had mis-oriented spindles. These results were consistent with the idea that actin function was required for maintenance of proper spindle orientation. To determine whether the improper spindle orientation was due to the absence of the astral microtubules (see above) we counted the number of spindles that had discernable astral microtubules. After 2 h at 36°C, >90% of the *actl*-4 cells had spindles with discernable astral microtubules. Thus, the increased frequency of mis-oriented spindles in the *actl*-4 mutant did not correlate with the absence of astral microtubules as it did for the *cdc16 tub2*-401 mutant. In most cells in which the spindle was mis-oriented there was a visible disruption of the actin cytoskeleton. However, there were a few cells in which the actin was completely disrupted and the spindle was oriented properly, and there were other cells in which the actin appeared phenotypically normal yet the spindle was not oriented properly. These rare cells do not contradict the hypothesis that actin function is required for proper spindle orientation because if spindle orientation was random in cells with a disrupted actin cytoskeleton then a certain fraction of spindles would be oriented properly. In addition, even though actin structure appeared normal in a few cells with mis-oriented spindles there may have been subtle changes in the actin cytoskeleton that were not detected at the resolution of indirect immunofluorescence.

To provide additional evidence for the linkage between spindle mis-orientation and the *actl*-4 mutation, we isolated spontaneous revertants of a temperature-sensitive haploid *actl*-4 strain. The growth rate at 36°C of all the revertants we isolated was slower than an *ACTl* strain, suggesting that the revertants we isolated were all partial revertants. One of these revertants, the parental *actl*-4 haploid and a wild-type *ACTl* strain were synchronized in HU and then shifted to 36°C. The frequency of mis-oriented spindles was determined (Table II). 14% of the *actl*-4 revertant cells had mis-oriented spindles, compared to 47% for *actl*-4 cells and 1% for *ACTl* cells. Thus, the partial restoration of actin function led to a partial decrease in the frequency of mis-oriented spindles. These data substantiate that before the onset of yeast mitosis, the disruption of actin function perturbs proper spindle orientation.

To address whether the increased frequency of mis-oriented spindles was a general feature of disruption of cytoskeletal elements, we used the same experimental design to examine spindle orientation in a strain with a temperature-sensitive *cdc3* mutation. The *CDC3* gene encodes a protein that is a component of a third yeast cytoskeletal element besides microtubules and microfilaments (Kim et al., 1991). After 2 h at 36°C, only 1% of *cdc3* cells exhibited mis-oriented spindles. Thus, the increased frequency of mis-oriented spindles was not a general property of disrupting any cytoskeletal component.

We examined the time-course of actin disruption and spindle mis-orientation in *actl*-4 and *ACTl* diploid cells to deter-

Table II. Frequency of Mis-oriented Spindles in ACT1, actl-4, and act 1-4 Revertant Cells Synchronized after Nuclear Migration but before Anaphase with Hydroxyurea then Shifted to 36° C for 2 h

Strain	Temperature	Mis-oriented spindles
	°C	%
actl-4	23	10
	36	47
actl-4 rev.	23	6
	36	14
ACTl	23	9
	36	1

Strains were grown at 23°C for 3 h in the presence of 100 mM hydroxyurea. An aliquot was removed, fixed, and prepared for fluorescence and indirect immunofluorescence microscopy. The remaining cells were shifted to 36°C for 2 h, and an aliquot of cells was removed, fixed, and prepared for fluorescence and indrect immunofluorescence microscopy.

mine whether the inactivation of actin function resulted in an immediate effect on spindle orientation or whether there was a lag between the inactivation of the actin function and the spindle mis-orientation. Hydroxyurea-arrested *actl*-4 or *ACTl* cells were shifted to 36°C, and at various times cells were fixed and double labeled with an anti-actin and an anti-tubulin antibody to examine actin and microtubule structure. In *actl*-4 diploid cells, concomitant with the disruption of the actin cytoskeleton there was an immediate increase in the percentage of cells in the population with mis-oriented spindles (Fig. 4 *a*). It has been observed that shifting *ACTl* cells to 36°C transiently disrupts the actin cytoskeleton (John Pringle, personal communication). A similar "heat shock" effect on the actin cytoskeleton was observed in our *ACTl* diploid cells (Figs. 4 *b* and 5 *d*). In addition, there was a transient increase in the percent of *ACTl* cells with mis-oriented spindles (Figs. 4 *b* and 5 *c*). Thus, there was no detectable lag between the disruption of the actin cytoskeleton and the onset of spindle mis-orientation in both the *actl*-4 and *ACTl* strains.

Disruption of Actin or Astral Microtubules before Mitosis Leads to Aberrant Mitoses

Our results demonstrated that in cells synchronized before anaphase both the astral microtubules and actin function were required for maintaining proper spindle orientation. Was the maintenance of proper spindle orientation after nuclear migration important for subsequent chromosome separation; i.e., when anaphase occurred, were the separated sister chromatids segregated into the mother cell and bud? To address this question *cdc16 tub2*-401 and *cdc16* were exposed to a temperature regime that gave rise to cells synchronized before anaphase with mis-oriented spindles (36°C for 3 h followed by 40 min at 18°C). These cells were incubated for an additional 140 min at 18°C to allow them to complete anaphase. The frequency of cells in which sister chromatids were segregated within either the mother cell or bud (binucleated cell bodies) was determined.

After 180 min at 18°C, 68% of the *cdc16 tub2*-401 cells and 95% of the *cdc16* cells completed anaphase. Thus, in the *cdc16 tub2*-401 cells lacking astral microtubules, the spindle efficiently elongated and two equal but separated masses of DAPI stained material were observed. However, of the *cdc16 tub2*-401 cells that completed anaphase, 40% produced
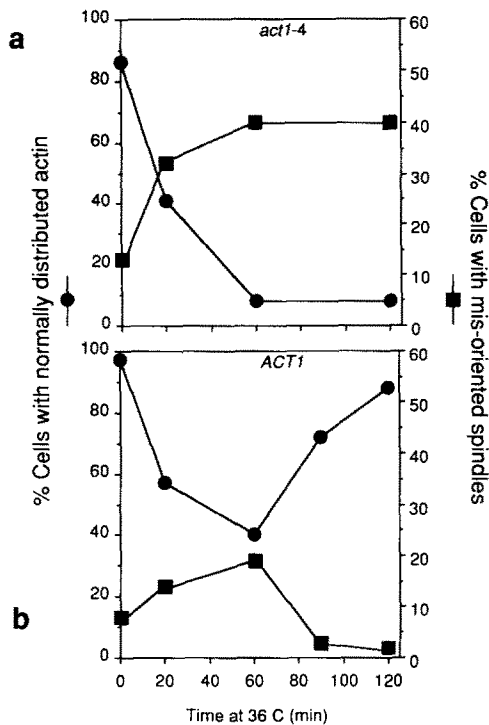
Figure 4. Spindle and actin characteristics of hydroxyurea synchronized *act1*-4 cells (*a*) and *ACT1* cells (*b*). The *act1*-4 strain 5105 and the *ACT1* strain 5050 were grown at 23°C for 3 h in the presence of 100 mM hydroxyurea. Aliquots were removed, fixed, and prepared for fluorescence and indirect immunofluorescence microscopy (see Materials and Methods). The remaining cells were shifted to 36°C for 20, 60, and 120 min, fixed, and prepared for fluorescence and indirect immunofluorescence microscopy. The position of the nuclear DNA was determined with fluorescence microscopy of DAPI stained cells. For cells in which the nuclear DNA was on one side of the neck the following characteristics were determined: (*1*) the percent of cells with the actin cortical patches preferentially distributed in the bud, i.e., correctly distributed (*circles*); and (*2*) the percent of cells that did not have their spindle oriented properly with one SPB positioned at or through the neck between the mother cell and bud (*squares*). The percentages were determined from at least two experiments, and for each experiment ~100–200 cells were examined per time point.

binucleate cell bodies as compared to only 0.5% binucleate cell bodies for the *cdc16* cells. The frequency of binucleate *cdc16 tub2*-401 cells was similar to the percent of these cells with mis-oriented spindles before anaphase (see above). This result suggests that the cells with the misoriented spindles gave rise to the binucleate cell bodies. Thus, the astral microtubules were important in maintaining proper spindle orientation in the nucleus before anaphase, and this orientation appeared to be functionally important to ensure that sister genomes were segregated properly during anaphase.

To address whether spindles mis-oriented because of actin disruption also gave rise to binucleate cells, we designed the following experiment. Haploid *act1*-4, *act1*-4 revertant and *ACT1* cells were arrested at 23°C after nuclear migration, but before anaphase with HU, and then shifted cells to 36°C for 2 h to inactivate actin. HU was removed and cells were incubated at 23°C for 2 h to allow them to complete DNA synthesis and enter mitosis. It was necessary to use 23°C to get expedient entry of the *act1*-4 cells into mitosis. After 2 h at 23°C, 50% of the *act1*-4, 75% of the *act1*-4 revertant, and
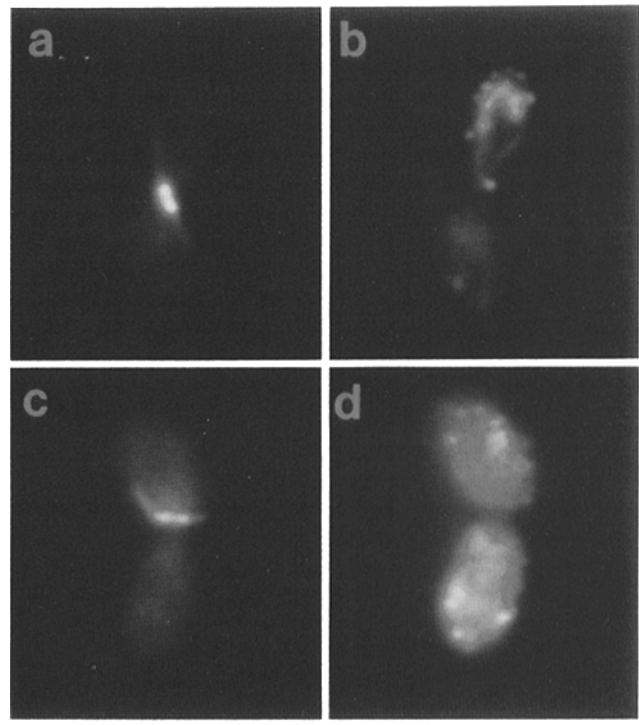

Figure 5. Indirect immunofluorescence images of hydroxyurea synchronized *ACT1* cells. The strain 5050 was grown at 23°C for 3 h in the presence of 100 mM hydroxyurea, an aliquot was removed, and the remaining cells were shifted to 36°C for 60 min. Cells were fixed, and stained with anti-tubulin antibodies (see Materials and Methods) to examine the position of the spindle (*a* and *c*), and an anti-actin antibody to visualize actin distribution (*b* and *d*). Micrographs of representative cells were prepared with DIM. The spindle is the brightly stained bar, and the astral microtubules are the fainter arrays that emanate from both the ends of the spindle. *a* and *b* show an *ACT1* cell grown at 23°C with normal actin distribution and spindle orientation. *c* and *d* are images of an *ACT1* cell that has been shifted to 36°C for 60 min in which both actin distribution and spindle orientation have been perturbed. Bar, 5 μm.

95% of the *ACT1* cells entered into anaphase. Of the *act1*-4 cells that entered into mitosis 29% formed binucleated cell bodies. In contrast, of the *ACT1* and *act1*-4 revertant cells that entered mitosis, the percent of binucleate cell bodies was only 0.3 and 1%, respectively. The percent of binucleate *act1*-4 cells compares favorably with the percent of these cells with mis-oriented spindles before anaphase (47%, Table II). The fact that not all of the cells with mis-oriented spindles gave rise to binucleate cells was probably because efficient re-entry into mitosis required 23°C. At this temperature, actin function may have recovered sufficiently in some cells to allow re-orientation of the spindle, thus, resulting in proper anaphase. In summary, actin function was important to maintain proper spindle orientation before anaphase, and without proper spindle orientation the sister genomes were not segregated properly.

Spindle Movements

Nuclear DNA Transits Are a Consequence of the Migration of the Spindle. We showed that the chromosomes could migrate as a group between the mother cell and bud in cells that were arrested after nuclear migration but before anaphase, or in cells that were released from arrest and were en-

tering into anaphase (Palmer et al., 1989). This bulk chromosome migration was referred to as nuclear DNA transits. We suggested that the bulk migration of chromosomes during a nuclear DNA transit was a consequence of their attachment to a migrating spindle (Palmer et al., 1989). If this were the case, nuclear DNA transits should be inhibited when spindles are disrupted by nocodazole, a microtubule-depolymerizing drug. To test this hypothesis, the cdc16-1 strain, 208-1, was synchronized after nuclear migration but before anaphase (conditions where they undergo frequent nuclear DNA transits [Palmer et al., 1989]), and then incubated in the presence or absence of nocodazole. Chromosome movement in these cells was followed for 1 h with DIM. Aliquots of untreated (control) and nocodazole-treated cells were removed before and after DIM analysis, fixed, and examined for the presence of microtubules by indirect immunofluorescence.

Of the 16 control cells examined with DIM, 25% exhibited nuclear DNA transits. This frequency of nuclear DNA transits was similar to the frequencies we observed in other strains synchronized after nuclear migration but before anaphase (Palmer et al., 1989). Of the 16 nocodazole-treated cells analyzed with DIM, no nuclear DNA transits were observed. In fact, no detectable chromosome movement (<0.2 μm) was observed in these cells. The difference between the frequencies of nuclear DNA transits in the nocodazole-treated and control cells was statistically significant ($P = 0.004$). Cytological analysis of the control and nocodazole-treated cells revealed that the position of the nucleus was not altered by nocodazole treatment. While spindles and astral microtubules were observed in >95% of the control cells, they were totally absent in all nocodazole-treated cells. The correlation between the absence of microtubules and the lack of nuclear DNA transits in nocodazole-treated cells suggested that microtubules were required for nuclear DNA transits, and was consistent with the model that nuclear DNA transits were a consequence of spindle migration.

Nuclear DNA Transits and Spindle Migration Require Astral Microtubules but Not Interdigitating Non-kinetochore Microtubules. Before anaphase, the yeast mitotic apparatus is bipolar with two half spindles that contain kinetochore, non-kinetochore, and astral microtubules. There is a region of overlap between the two half spindles where non-kinetochore microtubules from one SPB interdigitate with non-kinetochore microtubules from the other SPB (Peterson and Ris, 1976). We wanted to determine whether the interdigitating non-kinetochore microtubules were required for spindle migration. For this purpose, we used a strain with a temperature-sensitive mutation in the *CDC31* gene. At the nonpermissive temperature, the cdc31-1 mutant arrests cells after nuclear migration but before anaphase. However, unlike other cdc mutants that arrest cells at this point in the cell cycle, this mutant has a monopolar spindle that contains an unduplicated SPB, cytoplasmic and kinetochore microtubules, but no interdigitating non-kinetochore microtubules (Byers, 1981). By monitoring nuclear DNA transits in cdc31-1 arrested cells (see Materials and Methods), we could infer whether the monopolar spindles in these cells were able to migrate back and forth between the mother cell and bud.

35% of the cdc31-1 arrested cells had undergone at least one nuclear DNA transit from mother cell to bud. This frequency of nuclear DNA transits was similar to the frequencies we observed in other strains synchronized after nuclear

migration but before anaphase (Palmer et al., 1989). In 10 of 11 cdc31-1 cells examined by EM, the nuclear envelope was positioned spanning the neck between the mother cell and bud (data not shown). This result indicated that chromosome movement from mother cell to bud was due to bulk chromosome migration within the lumen of an extended nucleus rather than by the migration of the entire nucleus. When the microtubule structure was analyzed by indirect immunofluorescence, all cdc31-1 cells had spindles with a single vertex (data not shown), unlike bipolar spindles that have two vertices (at each end of the spindle) (see Fig. 1 a). The presence of a single vertex was consistent with these arrested cells having a monopolar spindle (Byers, 1981). The position of the monopolar spindle was always located with the chromosomes whether the chromosomes were in the mother cell or the bud (data not shown). The presence of the monopolar spindles in the bud demonstrated that these spindles must have been able to migrate from the mother cell to bud. In summary, nuclear DNA transits could occur in cdc31-1 arrested cells apparently as a consequence of the migration of the monopolar spindle. Thus, spindle migration did not require interdigitating non-kinetochore microtubules.

To determine whether the astral microtubules were required for spindle migration before anaphase, we examined the frequency of nuclear DNA transits in the cdc16 tub2-401 double mutant strain, 5092. The double mutant strain was grown at 23°C and then shifted to 36°C to arrest cells after nuclear migration but before anaphase, i.e., at the cdc16 block. The arrested cells were then shifted either to 23°C where they had normal microtubules, or to 18°C where most of the cells had lost their astral microtubules (see above). We then followed the extent of preanaphase spindle migration at 18 and 23°C by examining the frequency of nuclear DNA transits in these cells using DIM.

Of the cells shifted from 36 to 23°C, 36% ($n = 19$) exhibited nuclear DNA transits. This frequency of nuclear DNA transits in the cdc16 tub2-401 double mutant was in good agreement with what we had previously reported for a cdc16 mutant (Palmer et al., 1989), as well as data presented in this study. Thus, the cdc16 tub2-401 double mutant exhibited normal frequencies of nuclear DNA transits under conditions where its astral microtubules were normal. In contrast, when the cells were shifted from 36 to 18°C, nuclear DNA transits were observed in only 8% of the cells ($n = 26$) examined with DIM. The fourfold reduction in the frequency of nuclear DNA transits between cells shifted from 36 to 23°C and 36 to 18°C was statistically significant ($P = 0.004$). The decreased frequency of nuclear DNA transits was not a general property of shifting yeast cells to 18°C. The frequency of nuclear DNA transits in the cdc16 strain, 5055, when shifted from 36 to 18°C was 37% ($n = 19$). This frequency of nuclear DNA transits was similar to what was observed for cdc16 or cdc16 tub2-401 cells when shifted to 23°C. From these data we conclude that the reduction in frequency of nuclear DNA transits observed in the cdc16 tub2-401 double mutant after shifting the cells from 36 to 18°C correlated with the reduction in the number of cells with astral microtubules (Fig. 2 a). These results strongly suggested that the astral microtubules were necessary for nuclear DNA transits and by inference necessary for spindle migration from one cell body to the other.

We also followed the change in bulk chromosome position within one cell body in cdc16 and cdc16 tub2-401 cells at

18°C. During ~1 h of observation, *cdc16* cells exhibited bulk chromosome movement 60% of the time, while *cdc16 tub2-401* cells exhibited bulk chromosome movement only 30% of the time (Fig. 6). Furthermore, when bulk chromosome movement was observed in *cdc16* cells, the average displacement was 0.8 μm, and displacements as great as 2 μm were observed (Fig. 6). In contrast, in the *cdc16 tub2-401* cells the magnitude of bulk chromosomal displacement averaged 0.4 μm, and movements >0.7 μm were never observed (Fig. 6). These results strongly suggested that the astral microtubules were necessary for bulk chromosome movement within one cell body as well as for nuclear DNA transits and by inference necessary for spindle movement within one cell body, as well as, between cell bodies.

Discussion

Spindle Orientation

We describe two new roles for the astral microtubules and actin in the budding yeast, *S. cerevisiae*. Using conditional-lethal mutations in the β-tubulin and the actin genes we provide evidence that both astral microtubules and actin are required to maintain proper spindle orientation before the onset of anaphase. In cells synchronized after nuclear migration but before anaphase, the disruption of either astral microtubules or actin function resulted in spindles changing their position, often becoming perpendicular to the longitudinal axis of the cell. The effect on spindle orientation was immediate and correlated with the rapid disruption of the cytoskeletal structures. The effect on spindle orientation was functionally significant; sister chromatids were segregated in the same cell body rather than being separated in the mother cell and bud at a rate 100–200-fold greater than cells with properly oriented spindles. Thus, when spindle orientation was randomized after nuclear migration, aberrant anaphase often ensued.

Why have these newly identified roles of the astral microtubules and actin not been observed in previous genetic studies of these mutants? In the case of microtubules, their function in yeast has been assessed by mutants or drugs that abolished both cytoplasmic and spindle microtubules (Huffaker et al., 1988; Jacobs et al., 1988). Hence with these reagents, it was impossible to assess the role of just the astral microtubules in maintaining spindle orientation. The specific depolymerization of the astral microtubules in the *tub2-401* allele allowed us to circumvent this problem. In the case of cytoplasmic actin, its disruption leads to the accumulation of unbudded cells (Novick and Botstein, 1985) in which spindle orientation is impossible to assess. In our study, we bypassed these problems by synchronizing cells after bud emergence and nuclear migration but before anaphase, and then disrupted actin or microtubule function. While we cannot rule out the possibility that the requirement for astral microtubules and actin in the maintenance of spindle orientation is an artifact specific to synchronization with the *cdc16* mutation or hydroxyurea, we think this is unlikely for several reasons. First, cells synchronized by either of these methods efficiently enter anaphase. Second, the structure, orientation, and movement of the spindle after nuclear migration but before anaphase is the same in cells whether they are synchronized at this stage by the *cdc16* mutation, hydroxyurea
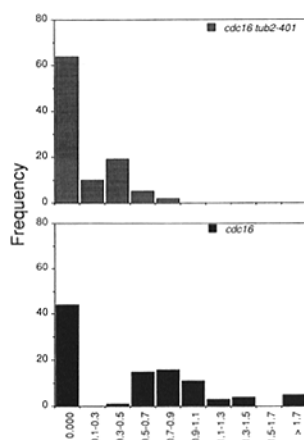

Figure 6. Displacement of bulk chromosomes (μm). Movement of chromosomes within one cell body for *cdc16 tub2-401* and *cdc16* cells. Exponential cultures of *cdc16 tub2-401* and *cdc16* cells were shifted to 36°C for 3 h, then shifted to 18°C for 40 min. Cultures were then prepared for DIM (see Materials and Methods). Cells were incubated at 18°C, and real-time images of chromosomal DNA were obtained from cells every 3 min for 1 h. The displacement (in μm) of the bulk chromosomes from one time interval to the next was determined by comparing the position of the chromosomal DNA in the two images as a function of time. Data was collected from nine cells.

or mutations in seven other *cdc* genes (Palmer et al., 1989; R. Palmer, unpublished results). These two results suggest that the spindles we observe in our synchronized cells are normal intermediates in mitosis. Finally, our results are consistent with observations in other eukaryotic cells (see below).

Previous studies have implicated both the astral microtubules and cytoplasmic actin in maintaining proper spindle orientation in other eukaryotes. Reorientation of metaphase spindles occurs in the P_1 blastomere of the nematode *Caenorhabditis elegans*. This reorientation was inhibited by laser ablation of a region of the cell containing the astral microtubules (Hyman and White, 1987). The treatment of different invertebrate embryos with ethyl ether resulted in the disruption of the astral rays and the subsequent formation of binucleated cells (Wilson, 1925; Dan and Tanaka, 1990). Finally, the treatment of *C. elegans* blastomeres with the inhibitor of microfilament assembly, cytochalasin D, resulted in changes in the position of the spindle (Hill and Strome, 1988, 1990). While these results with invertebrate embryos suggest that the astral microtubules and cytoplasmic actin might be important for maintaining proper spindle orientation, the lack of specificity of lasers, ethyl ether, and cytochalasin D allowed for other interpretations. However, these alternatives are eliminated by the results reported in this study in which defined mutations in the actin and tubulin structural genes also perturb spindle orientation.

A simple model to account for the role of both astral microtubules and actin in maintaining spindle orientation before anaphase is that spindle orientation is achieved by tethering of the astral microtubules to specific sites on the actin cytoskeleton. A similar model has been proposed for positioning of spindles in developing embryos (Hyman, 1989; Lutz et al., 1988). From our data, we cannot assess whether the astral microtubules are directly interacting with actin, or whether the actin is responsible for correctly positioning microtubule capturing proteins, which in turn interact with the astral microtubules. However, there are no reports of a direct microtubule–microfilament interaction. Therefore, we prefer the model that there are proteins or protein complexes that mediate the microtubule–microfilament interaction.

Previous analysis of actin in yeast and *Drosophila melanogaster* suggest several alternative hypotheses to explain the

role of the actin cytoskeleton in maintaining proper spindle orientation. An intact actin cytoskeleton is required for polarized growth of the yeast bud (Novick and Botstein, 1985). If spindle orientation requires maintenance of cell polarity, then disruption of actin cytoskeleton may disrupt cell polarity and lead to spindle mis-orientation. A second hypothesis is that actin is involved with proper spindle orientation by a direct interaction with the SPBs. In the Drosophila melanogaster embryo the in situ distribution f-actin localizes to a region near the SPBs during metaphase and anaphase (Karr and Alberts, 1986; Kellogg et al., 1988). Finally, since there are many pleiotropic phenotypes associated with the disruption of the actin cytoskeleton in yeast (Novick and Botstein, 1985) spindle mis-orientation in the actl-4 mutant may have been a nonspecific effect of disturbing cell physiology.

However, several results argue against these models. Several secretory mutants that are incapable of polarized cell growth had no effect on spindle orientation (data not shown), and at the level of indirect immunofluorescence, there does not appear to be a colocalization of actin structures and the SPBs. Finally, in wild-type or actin mutants there is no lag between the disruption of the actin cytoskeleton and spindle mis-orientation indicating that actin function plays an immediate role in maintaining proper spindle orientation. Furthermore, the transient disruption of the actin cytoskeleton does not affect cell viability or morphology, suggesting that overall cell physiology is not dramatically affected under these experimental conditions.

Spindle Movement before Anaphase

In yeast cells synchronized before anaphase bulk movement of chromosomes (nuclear DNA transits) occurs between the mother cell and the bud (Palmer et al., 1989). In cells that are undergoing nuclear DNA transits the position of the spindle always colocalizes with the position of the chromosomes (this study; Palmer et al., 1989), and the nuclear DNA transits were microtubule dependent (this study). These results suggest that nuclear DNA transits are the consequence of the oscillation of the spindle between the mother cell and the bud.

In cells synchronized before anaphase, a β-tubulin mutation (tub2-401) was used to deplete cells of their astral microtubules. In these cells the magnitude of bulk chromosome movement was reduced both between cell bodies and within one cell body suggesting that at least a component of the force that generates spindle movement (and hence bulk chromosome movement) before anaphase requires astral microtubules. Presumably, astral microtubules generate spindle movement by exerting force on spindle pole bodies. This conclusion is supported by the observations that spindle pole bodies can migrate away from each other when they contain astral but no central spindle microtubules (Aist and Berns, 1981; Aist et al., 1991; Winey et al., 1991). One mechanism for the astral microtubules to mediate force on the spindle pole bodies is by their interaction with mechanochemical proteins (Vallee et al., 1989; Vallee and Shpetner, 1990). In yeast, several genes have been identified that encode putative microtubule-dependent mechanochemical molecules (Meluh and Rose, 1990) (M. Rose, personal communication). A null mutation in one of these genes, KAR3, does not block nuclear DNA transits or spindle migration (R. Palmer, unpublished results), suggesting that KAR3 protein was not re-

quired for these processes. It will be interesting to determine whether any of the other putative microtubule-dependent mechanochemical proteins are required for spindle and chromosome movement before anaphase.

A caveat to these conclusions is that the tub2-401 mutation did cause the length of the spindle to shrink temporarily which may have reflected partial disassembly of non-kinetochore microtubules, kinetochore microtubules, or both. Thus, we cannot exclude the possibility that the reduction in the extent of spindle migration observed in the β-tubulin mutant was due to subtle effects on the spindle itself rather than the astral microtubules. However, since this β-tubulin mutation did not block spindle elongation (this paper, and Sullivan and Huffaker, 1992) the kinetochore and non-kinetochore microtubules must have remained functional. Furthermore, even if non-kinetochore microtubules were partially affected by this β-tubulin mutation, data presented for the cdc31 mutant suggests that the non-kinetochore microtubules are not required for nuclear DNA transits. Therefore, it seems likely that the reduction in the extent of spindle migration observed for the β-tubulin mutant was due to the depolymerization of the astral microtubules.

Many exponentially dividing cells never appear to undergo nuclear DNA transits before anaphase (Palmer et al., 1989), suggesting that extensive spindle movement at this stage is not an essential prerequisite to proper mitosis. If this is the case, how might the forces on astral microtubules that cause pre-anaphase spindle migration contribute to mitosis? It has been shown in other eukaryotes that opposing forces mediated by kinetochore and kinetochore microtubules create tension on metaphase chromosomes (Nicklas and Koch, 1969; Ault and Nicklas, 1989). This tension appears to be used to align chromosomes on the metaphase plate of the spindle. In an analogous way, forces might pull on the yeast astral microtubules that extend from one pole into the bud and from the other pole into the mother. These pulling forces could be used to create tension on the spindle which could be used to maintain its proper position at the neck. Nuclear transits may be a consequence of temporary imbalance of this tension. The increased frequency of nuclear DNA transits in arrested yeast cells compared to dividing cells may reflect that the tension on the spindle becomes less balanced as yeast "metaphase" is prolonged. In support of this idea, bidirectional oscillations of the yeast spindle and chromosomes during nuclear DNA transits are similar to the tension induced bidirectional oscillations of metaphase chromosomes which also become more severe as metaphase is prolonged.

In the second model, forces mediated by the astral microtubules prior to anaphase may help maintain spindle length. Forces that pull spindle poles together have been observed in yeast and other eukaryotes (Cassimeris and Salmon, 1991; A. Hoyt, personal communication). Therefore, to maintain spindle length before anaphase, additional forces that counterbalance these forces must exist. The forces on the astral microtubules may be a component of these counterbalancing forces. Interestingly, we observe a shrinkage of the yeast spindle when astral microtubules depolymerize.

Spindle orientation and migration are general features of cells undergoing asymmetric cell divisions. In this study we report striking similarities between the cytoskeletal elements involved in spindle orientation and migration in yeast and other eukaryotes. Thus, it is possible that the positioning of

the spindle in all asymmetrically dividing cells may be achieved by a common mechanism involving these structures. Since there is a remarkable conservation in the components that comprise the mitotic machinery and those that regulate mitosis between yeast and other eukaryotes, yeast should be an excellent model system to identify and characterize proteins that interact with actin and microtubules to maintain spindle orientation as well as those that generate force on astral microtubules.

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