Involvement of an Actomyosin Contractile Ring in *Saccharomyces cerevisiae* Cytokinesis

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Abstract. In *Saccharomyces cerevisiae,* the mother cell and bud are connected by a narrow neck. The mechanism by which this neck is closed during cytokinesis has been unclear. Here we report on the role of a contractile actomyosin ring in this process. Myo1p (the only type II myosin in *S. cerevisiae*) forms a ring at the presumptive bud site shortly before bud emergence. Myo1p ring formation depends on the septins but not on F-actin, and preexisting Myo1p rings are stable when F-actin is depolymerized. The Myo1p ring remains in the mother–bud neck until the end of anaphase, when a ring of F-actin forms in association with it. The actomyosin ring then contracts to a point and disappears. In the absence of F-actin, the Myo1p ring does not contract. After ring contraction, cortical actin patches congregate at the mother–bud neck, and septum formation and cell separation rapidly ensue. Strains deleted for *MYO1* are viable; they fail to form the actin ring but show apparently normal congregation of actin patches at the neck. Some *myo1Δ* strains divide nearly as efficiently as wild type; other *myo1Δ* strains divide less efficiently, but it is unclear whether the primary defect is in cytokinesis, septum formation, or cell separation. Even cells lacking F-actin can divide, although in this case division is considerably delayed. Thus, the contractile actomyosin ring is not essential for cytokinesis in *S. cerevisiae*. In its absence, cytokinesis can still be completed by a process (possibly localized cell–wall synthesis leading to septum formation) that appears to require septin function and to be facilitated by F-actin.

Key words: actin • cytokinesis • myosin • septins • yeast

The mechanisms of cytokinesis and its spatial and temporal control are important problems for both cell and developmental biology (Conrad and Schroeder, 1990; Wick, 1991; Miller and Kiehart, 1995; Rappaport, 1996). These mechanisms may be fundamentally different in different types of cells. In most plant cells, cytokinesis occurs through the construction of a new membrane-enclosed disc of cell wall, the cell plate, which forms in the cell interior and then grows centrifugally until it fuses with the plasma membrane (Wick, 1991; Staehelin and Hepler, 1996). In contrast, in most animal cells, actin and nonmuscle myosin II become concentrated in a band at the cortex that then contracts, invaginating the plasma membrane to form the cleavage furrow (Satterwhite and Pollard, 1992; Fishkind and Wang, 1995; Rappaport, 1996; Glotzer, 1997). Contraction of the actomyosin ring is sometimes followed directly by the completion of cell division, but in other cases the daughter cells remain connected for considerable periods by a narrow bridge with a central midbody, which is typically filled with residual spindle microtubules (Byers and Abramson, 1968; Mullins and Biesele, 1973, 1977; Mullins and McIntosh, 1982). The basis for eventual cell separation in these cases is unclear.

Like plant cells, typical fungal cells are surrounded by a rigid cell wall. However, in many fungi, cytokinesis appears to involve the centripetal growth of a septum from the cell cortex rather than the centrifugal growth of a cell plate (Hoch and Howard, 1980; Streiblová and Hasek, 1984; Roberson, 1992; Momany and Hamer, 1997). Actin is typically concentrated at the site of septation in the form of a cortical band, clustered cortical patches, or both (Girbardt, 1979; Marks and Hyams, 1985; Alfa and Hyams, 1990; Roberson, 1992; Momany and Hamer, 1997). In the fission yeast *Schizosaccharomyces pombe*, recent studies have shown that an actomyosin ring forms at the cell cor-

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tex and then contracts (Chang and Nurse, 1996; Gould and Simanis, 1997; Bezanilla et al., 1997; Kitayama et al., 1997; May et al., 1997). During or after ring contraction, actin patches cluster at the cytokinesis site and the septum grows inwards (Marks and Hyams, 1985; Jochová et al., 1991). These studies suggest that cytokinesis in fission yeast involves a step similar to that of animal cell cleavage, coupled to an additional mechanism for septum formation.

The situation has been less clear for the budding yeast *Saccharomyces cerevisiae,* in which cytokinesis occurs at the narrow neck that connects the mother cell and bud. Although early studies of actin localization in *S. cerevisiae* noted cells late in the cell cycle that had an apparent ring of actin at the neck (Adams and Pringle, 1984; Kilmartin and Adams, 1984), this structure was not observed consistently. In addition, although a type II myosin heavy chain (the *MYO1* gene product) was detected in early studies (Watts et al., 1985, 1987; Rodriguez and Paterson, 1990), these studies did not resolve either the localization or the function of this protein (Brown, 1997). Moreover, the narrowness of the mother–bud neck (at ∼1 μm, it is close to the diameter of a mammalian midbody) suggested that budding yeast cells might have no need for a contractile cytokinesis mechanism (Sanders and Field, 1994). The narrowness of the neck has also made it difficult to be certain whether the *S. cerevisiae* septum grows centripetally from the cell cortex or by fusion of vesicles in the midline of the neck (Byers and Goetsch, 1976; Cabib et al., 1982). Cortical actin patches congregate at the neck before division and have been thought to be involved in directing cell wall deposition to the septation site, but the details of actin function have remained unclear, a problem emphasized by the recent report that at least half of the budded cells in an asynchronous population can complete division in the absence of F-actin (Ayscough et al., 1997). Thus, it has remained unclear how closely the roles of actin and myosin in *S. cerevisiae* cytokinesis resemble those in other types of cells.

Another important question about cytokinesis concerns the role(s) of the septins, a family of proteins that was originally identified by the analysis of *S. cerevisiae* mutants that are defective in cytokinesis but otherwise continue to progress through the cell cycle (Hartwell, 1971; Pringle and Hartwell, 1981; Longtine et al., 1996). The *S. cerevisiae* septins are constituents of a filamentous ring that is found adjacent to the plasma membrane in the mother–bud neck. The diameter of this ring appears to remain constant (or even increase) during division (Byers and Goetsch, 1976; Byers, 1981; Kim et al., 1991; Ford and Pringle, 1991), indicating that it is not contractile. Although it appears that a primary role of the septins is to serve as a scaffold or template for the assembly of other proteins at the cell surface (Chant et al., 1995; DeMarini et al., 1997), their precise role in *S. cerevisiae* cytokinesis remains unknown. Septins have now also been found in other fungi and in many animal species, although not yet in plants. In animal cells, the septins are concentrated in the cleavage furrow and appear to be required for cytokinesis (Neufeld and Rubin, 1994; Fares et al., 1995; Kinoshita et al., 1997), but their precise role remains unknown.

In this paper, we report that there is indeed a contractile actomyosin ring in *S. cerevisiae* but that this ring is not essential for cytokinesis. Assembly of the actomyosin ring requires the septins, thus defining one role (although apparently not the only role) for the septins in *S. cerevisiae* cytokinesis.

Materials and Methods

Strains, Growth Conditions, and Genetic Methods

Strains used in this study are listed in Table I or described where appropriate below. Standard media (Guthrie and Fink, 1991) including synthetic complete (SC)[1] medium were used except where noted; in some cases, the rich, buffered medium YM-P (Lillie and Pringle, 1980) was used for growth of yeast in liquid culture. Glucose (2%) was used as carbon source. Cultures were grown at 23°C except where noted. Standard procedures (Sambrook et al., 1989; Guthrie and Fink, 1991) were used for genetic manipulations, polymerase chain reaction (PCR), and other molecular biological procedures except where noted.

Construction of Strains Carrying MYO1:GFP or MYO1 Deletions

A strain expressing Myo1p with green fluorescent protein (GFP) fused in frame at its COOH terminus (and not expressing untagged Myo1p) was constructed using a PCR method (Wach et al., 1997; Longtine et al., 1998) to tag the *MYO1* gene in its normal chromosomal location. The *GFP (S65T)–kanMX6* fragment from plasmid pFA6a–GFP(S65T)–kanMX6 was amplified together with sequences immediately flanking the stop codon of *MYO1* (GenBank/EMBL/DDBJ accession number U10399) using forward primer 5′-AAATATTGATAGTAACAATGCACAGAG-TAAAATTTTCAGTCGGATCCCCGGGTTAATTAA-3′ and reverse primer 5′-GGATATAAAGTCTTCCAAATTTTTAAAAAAAAGTT-CGTTAGAATTCGAGCTCGTTTAAAC-3′ (the sequences flanking the *MYO1* stop codon are underlined). The amplified fragment was transformed into strain YEF473, and the transformants were plated on YPD medium and incubated overnight at 30°C. The lawn of cells was then replica-plated onto YPD containing 200 μg/ml G418/geneticin (Life Technologies, Inc., Gaithersburg, MD) to select stable G418-resistant transformants. The success of the GFP tagging was confirmed by PCR using genomic DNA as template, the reverse primer described above, and a forward primer (5′-GAGATTGAGATGAAAAAATCCATTAGAGA-TAATTCT-3′) internal to *MYO1*. In addition, all clones that were positive by the PCR test showed Myo1p-GFP signal. *MAT**a*** and *MAT*α segregants from one transformant carrying the *MYO1:GFP* allele were mated to create diploid strain YEF1698. In addition, a *MAT**a** MYO1:GFP* segregant was crossed to strain M-238, and appropriate segregants were mated to create strain YEF1798.

Strains carrying deletions of the *MYO1* open reading frame (ORF) were constructed in two genetic backgrounds using the PCR method of Baudin et al. (1993). First, the *kanMX6* marker from plasmid pFA6a–kanMX6 (Wach et al., 1997) and the *His3MX6* marker from plasmid pFA6a–His3MX6 (Wach et al., 1997) were amplified together with sequences corresponding to the regions immediately upstream and downstream of the *MYO1* ORF using forward primer 5′-GAAGATC-ATAACAAAGTTAGACAGGACAACAACAGCAATACGGATCC-CCGGGTTAATTAA-3′ (the *MYO1* flanking sequence is underlined) and the same reverse primer as used in the GFP-tagging. The amplified fragments were transformed into strain YEF473, selecting for Kan^r or His$^+$ as appropriate, yielding strains YEF1750 and YEF1751. The success of the deletions was confirmed by PCR using the reverse primer described above and a forward primer (5′-ATGACCAGCTACTTTATTCATACTCTTC-CACAC-3′) corresponding to sequences 122–90 nucleotides upstream of the *MYO1* ORF. *myo1Δ* segregants from strains YEF1750 and YEF1751 were mated to create diploid strain YEF1820. Second, the *URA3* marker from plasmid pRS306 (Sikorski and Hieter, 1989) was amplified together with sequences near the ends of the *MYO1* ORF using forward primer 5′-

1. *Abbreviations used in this paper*: DIC, differential interference contrast; GFP, green fluorescent protein; LAT-A, latrunculin-A; ORF, open reading frame; SC, synthetic complete medium.

GCAGTCTTGCAGTTCTAATATGATCGTCTGGATACCAGATGG-CGCGTTTCGGTGATGAC-3′ and reverse primer 5′-CTTGGG-CCATTTCCTGCACTTTGTCGCGGTAAGAAGAATTTTTCCTGA-TGCGGTATTTTCTCCT-3′ (the *MYO1* sequences are underlined). The amplified fragment was transformed into strain DLY5, selecting for stable Ura$^+$ transformants. The success of the deletion was checked by PCR using two different combinations of primers. For one reaction, the forward primer was 5′-AAAAGGGTAATTGCGTAAACAT-3′ (corresponding to sequences 185–164 nucleotides upstream of the *MYO1* start codon), and the reverse primer was 5′-TTCAAACCGCTAACAATACC-3′ (corresponding to sequences internal to *URA3*). For the second reaction, the same forward primer was used, but the reverse primer was 5′-TAAAAATAAGCCGGAATAGGTG-3′ (corresponding to sequences within the *MYO1* ORF). This deletion removes all but 52 nucleotides at the 5′ end and 132 nucleotides at the 3′ end of the *MYO1* ORF. *MAT**a*** and *MATα* segregants from one transformant that carried the *myo1Δ* deletion were mated to create diploid strain JMY1318.

Morphological Observations

Except where noted, cells were fixed by adding formaldehyde directly to the growth medium (final concentration, 3.75%). Overall cell morphologies were observed by differential interference contrast (DIC) microscopy. In some cases, cell clumps were dispersed either by lightly sonicating the fixed cells (Pringle and Mor, 1975) or by digesting them for 60 min at 37°C with 35 U/ml of lytic enzyme (ICN Biochemicals, Inc., Cleveland, OH) to remove the cell walls. The latter procedure separates cells that have completed cytokinesis even if cell separation is incomplete (Hartwell, 1971; Pringle and Mor, 1975). Indirect immunofluorescence was performed as described previously (Pringle et al., 1991), using a rat monoclonal anti-tubulin antibody (YOL1/34; Accurate Chemical and Scientific, Westbury, NY) to visualize microtubule structures (Kilmartin et al., 1982; Kilmartin and Adams, 1984) and rabbit polyclonal antibodies against Cdc11p to visualize septin localization (Ford and Pringle, 1991). Actin rings and patches were visualized by staining cells with 0.2–20 U/ml of rhodamine-conjugated phalloidin (Molecular Probes, Inc., Eugene, OR) for 5–30 min (see also below). Nuclear DNA was stained by incorporating 1 μg/ml of bisBenzimide (Sigma Chemical Co., St. Louis, MO) in the mounting medium. The secondary antibodies used were FITC-conjugated goat anti–rat IgG and goat anti–rabbit IgG (both from Jackson ImmunoResearch Laboratories, West Grove, PA).

To visualize F-actin, Myo1p–GFP, and DNA in the same cells, exponentially growing cells were pelleted by centrifugation in a microfuge and then fixed by resuspending the cell pellet in ice-cold 70% ethanol and incubating on ice for 10 min. Cells were then pelleted by centrifugation for 20 s in a microfuge at top speed and incubated for 2 min at 23°C with various concentrations of rhodamine-phalloidin in PBS containing 10 mg/ml BSA: 0.2 U/ml to visualize the polarized actin cytoskeleton and Myo1p–GFP ring at the presumptive bud site (at higher concentrations, bleed-through rhodamine fluorescence obscured the GFP signal), 2 U/ml to visualize the actin ring and Myo1p-GFP ring at the neck, and 20 U/ml to visualize the general organization of the actin cytoskeleton. After the incubation with rhodamine-phalloidin, the cells were washed three times with PBS by centrifugation and then resuspended in mounting medium containing 1 μg/ml of bisBenzimide. 2–3 μl of this suspension were placed on a slide, covered with a coverslip, and then pressed with a weight (usually a centrifuge rotor) for 10 min before microscopic examination.

Time-lapse Microscopy

To visualize the Myo1p–GFP ring in side view, exponentially growing cells were spotted onto a thin layer of SC medium containing 25% gelatin (Yeh et al., 1995). To visualize the ring in an angled or en face view, cells were mixed with molten (but cooling) gelatin-containing medium, then spotted onto a slide at 23°–25°C. A computer-controlled microscope (model FXA; Nikon, Tokyo, Japan) with a cooled, slow-scan CCD camera (model C4880; Hamamatsu Photonics, Bridgewater, NJ) was used in time-lapse experiments (Shaw et al., 1997*a*,*b*). Five fluorescence images (3-s exposure) at different focal planes and a single DIC image corresponding to the central fluorescence image were taken at 1-min intervals. Excitation was attenuated to between 1 and 10% of the maximum to avoid phototoxicity (Shaw et al., 1997*b*). For presentation, the five fluorescence images were overlaid and paired with the corresponding DIC image. Contrast was enhanced using the MetaMorph (Universal Imaging Corp., West Chester, PA) and/or Photoshop (Adobe Systems Corp., San Jose, CA) systems.

Latrunculin Treatment

To examine formation of the Myo1p–GFP ring in the absence of F-actin, unbudded cells were isolated and treated with latrunculin A (LAT-A) (Molecular Probes, Inc.) essentially as described by Ayscough et al. (1997). A 200-μl aliquot of an overnight culture of strain YEF1698 was spread onto each of two YPD plates. The cells were then grown at 30°C for 24–30 h, scraped into YM-P liquid medium at 23°C, and immediately pelleted. After resuspension in 40 ml of YPD medium that had been diluted twofold with 2 M sorbitol, the cell suspension was centrifuged at 500 *g* for 3 min. The pellet was discarded, and the supernatant was centrifuged again at 500 *g* for 3 min. This step was repeated several times until essentially all of the remaining cells were unbudded. These cells were resuspended in fresh YM-P medium to a final concentration of $\sim 0.67 \times 10^7$. 0.6 ml of this cell suspension was treated with LAT-A (added to a final concentration of 200 μM from a 20-mM stock solution in DMSO) or with an equal volume of DMSO as a control.

For time-lapse analysis of the behavior of the Myo1p ring in the absence of F-actin, cells of strain YEF1698 growing exponentially in SC medium were treated with LAT-A (as above) for 10 min before spotting onto a thin layer of SC medium containing 25% gelatin and 200 μM LAT-A. Time-lapse microscopy was then performed as described for cells without LAT-A treatment.

Results

Assembly of an Actin Ring at the Mother–Bud Neck Late in the Cell Cycle and Its Contraction After Anaphase

During experiments done for other purposes, we serendipitously observed that brief incubation of fixed cells with low concentrations of rhodamine-phalloidin allowed the clear and consistent visualization of a distinct actin ring structure in a subset of the cells in an asynchronous population (Fig. 1, cells *3–6*). Although such a structure had been observed previously (Adams and Pringle, 1984; Kilmartin and Adams, 1984), it had been difficult to visualize consistently using the usual staining techniques. Thus, it had remained unclear whether a distinct actin ring structure was normally formed during a particular (perhaps brief) stage of the cell cycle or whether the structures visu-

Table I. S. cerevisiae Strains Used in This Study

Strain	Genotype	Source
YEF473	***a***/*α his3/his3 leu2/leu2 lys2/lys2 trp1/trp1 ura3/ura3*	Bi and Pringle, 1996
YEF1698	as YEF473 except *MYO1:GFP/MYO1:GFP*	See text
YEF1820	as YEF473 except *myo1Δ::kanMX6/myo1Δ::His3MX6*	See text
DLY5	***a***/*α ade1/ade1 his2/his2 leu2/leu2 trp1/trp1 ura3/ura3*	Lew and Reed, 1993
JMY1318	as DLY5 except *myo1Δ::URA3/myo1Δ::URA3*	See text
M-238	*α his3 leu2 trp1 ura3 cdc12-6*	Pringle lab stock
YEF1798	***a***/*α his3/his3 leu2/leu2 LYS2/lys2 trp1/trp1 ura3/ura3 cdc12-6/cdc12-6 MYO1:GFP/MY01:GFP*	See text

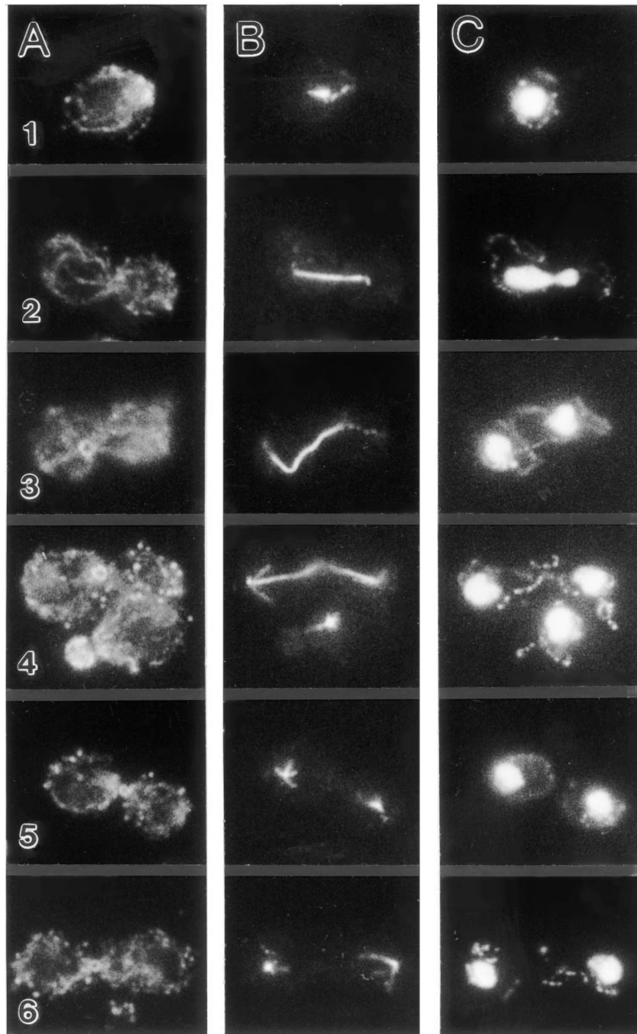

Figure 1. Formation and contraction of an actin ring during the cell cycle. Cells of strain YEF473 growing exponentially in SC medium were triple-stained for F-actin (*A*), tubulin (*B*), and DNA (*C*) as described in Materials and Methods. Cells shown are in G1 or S phase (*1*), S or G2 phase (*4, bottom cell*), early anaphase (*2*), late anaphase (*3* and *4, top cell*), or after anaphase (*5* and *6*). All cells are shown at the same magnification. The cells shown were chosen for presentation because they had been distorted enough during preparation (note also the bend in the spindle of cell *3*) to allow the actin rings to be seen more or less en face. More typical side views of the actin ring can be seen in Fig. 4.

alized might only represent occasional fortuitous arrangements of the well known actin cables and/or patches. With the lower phalloidin concentration used here, the cables and patches are less strongly stained, and the actin ring is much easier to see. Although it is not clear why the ring should be preferentially stained at lower phalloidin concentrations, it is clear from the work described here and from recent work by others (Epp and Chant, 1997; Lippincott and Li, 1998; see Discussion) that the ring is indeed a distinct structure and not an artifact.

To characterize the timing of actin ring appearance and its behavior during the cell cycle, wild-type cells were triple-stained to visualize F-actin, tubulin, and DNA. Actin rings were observed in ~5% of the cells in asynchronous populations of two different strains (YEF473 and M-238) grown in SC liquid medium at 23°C (>700 cells counted per strain). Rings were observed only in late-anaphase or post-anaphase cells with fully separated chromosomes (Fig. 1, compare cells *1* and *2* with cells *3–6*) and were seen in nearly all such cells. The diameter of the actin ring was not identical in every cell. The largest rings (~1 μm-diam) were consistently observed in cells with fully elongated spindles that were intact (Fig. 1, cell *3*) or just beginning to disassemble (Fig. 1, cell *4*). Smaller rings were seen in cells with disassembling or disassembled spindles (Fig. 1, cells *5* and *6*). Thus, the actin ring assembles near the end of anaphase and appears to contract during or after spindle disassembly.

Assembly of a Myo1p Ring Early in the Cell Cycle and Its Contraction After Anaphase

The behavior of the actin ring was reminiscent of that of the contractile actomyosin ring involved in animal cell cytokinesis. To ask if the yeast actin ring also contains a type II myosin, we fused GFP-encoding sequences to the

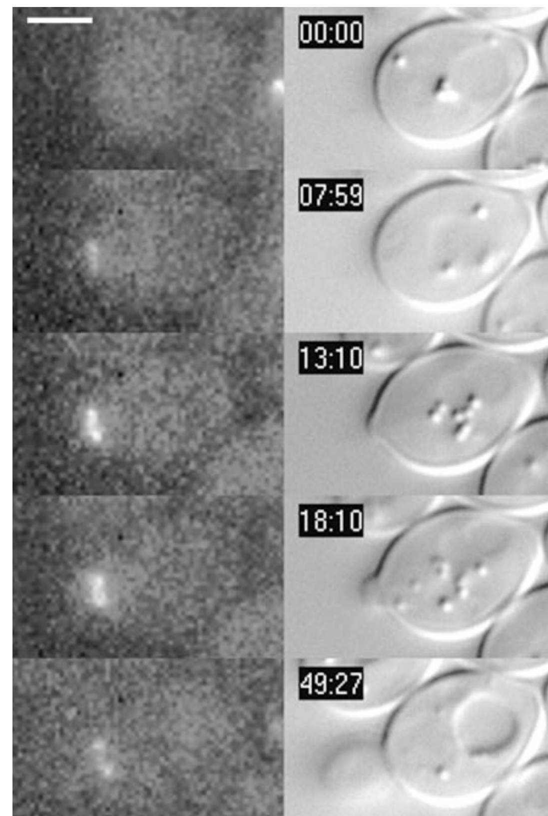

Figure 2. Formation of the Myo1p–GFP ring relative to bud emergence. Cells of strain YEF1698 growing exponentially in SC medium were spotted onto SC medium containing 25% gelatin and observed by time-lapse photomicroscopy as described in Materials and Methods. Pairs of GFP (*left*) and DIC (*right*) images were recorded at the indicated times. Similar series of images were obtained for five different cells undergoing bud emergence. Formation of the Myo1p ring before bud emergence was also supported by observations on fixed cells (Fig. 4, cell *1;* Fig. 7; and data not shown). Bar, 2 μm.

COOH-terminal end of *MYO1,* which encodes the only type II myosin in *S. cerevisiae*. The tagged Myo1p was expressed from its own promoter at its normal chromosomal locus and appeared to be fully functional by several criteria, including the normal morphology and cell separation of the strain harboring the tagged gene (see also below, and note that the strain background in which the tagging was done was that in which deletion of *MYO1* produced the more conspicuous phenotype). We then performed time-lapse analysis of living cells expressing Myo1p–GFP. Newborn unbudded cells did not display localized Myo1p (Fig. 2, *00:00*). Before bud emergence, a ring of Myo1p assembled at one pole of the cell (Fig. 2, *07:59*; that these structures were indeed rings was verified by observations on cells in suspension), and a bud emerged from that site ~5–6 min later (Fig. 2, *13:10*). As the bud grew, the ring of Myo1p remained visible at the mother–bud neck and retained a constant diameter of $\sim 1 \pm 0.1$ μm ($n = 6$) (Fig. 2, *18:10* and *49:27*; Fig. 3 *A, 00:00* and *02:59*) until nuclear division. (The timing of nuclear division is difficult to discern in Fig. 3 but was clear in other series.) The Myo1p ring then contracted to a point and disappeared (Fig. 3, *A, 02:59–09:59*, and *B*). Contraction of the ring took 7–9 min from the initiation of contraction to the disappearance of the Myo1p-GFP signal. After contraction of the Myo1p ring, a septum visible by DIC microscopy formed rapidly

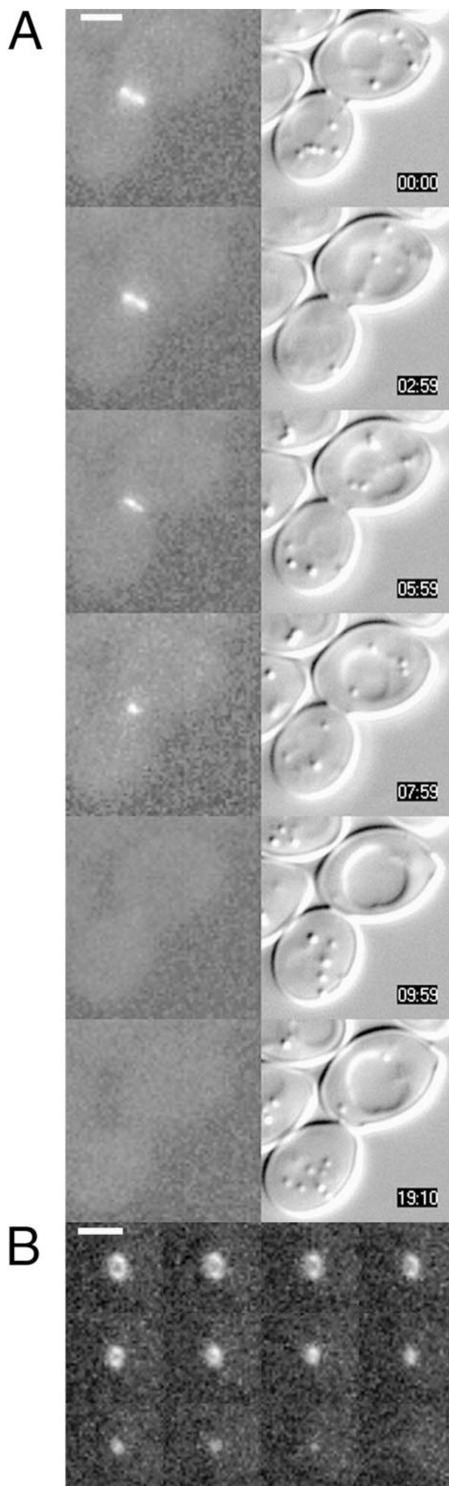

Figure 3. Contraction of the Myo1p–GFP ring late in the cell cycle. Time-lapse analysis of strain YEF1698 was performed as in Fig. 2. (*A*) Pairs of GFP fluorescence and DIC images were recorded at the indicated times from a cell positioned such that the Myo1p–GFP ring was seen from the side. Very similar series of images were obtained from four different cells. (*B*) GFP fluorescence images were recorded at 1-min intervals from a cell positioned such that its Myo1p–GFP ring was seen en face. Very similar series of images were obtained for two additional cells. Bars, 2 μm.

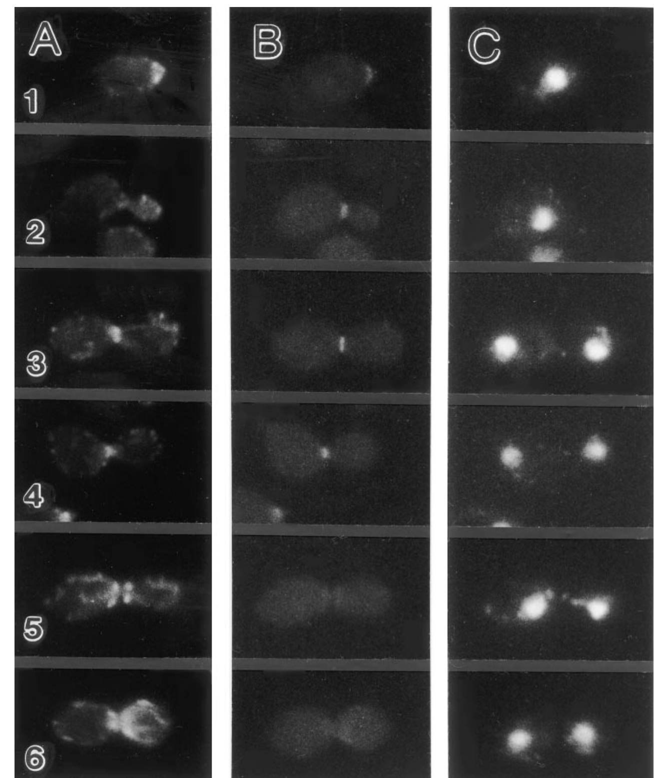

Figure 4. Coincidence of the actin and Myo1p rings. Cells of strain YEF1698 growing exponentially in YM-P medium were fixed with ice-cold 70% ethanol (refer to Materials and Methods) and then stained for F-actin (*A*) and DNA (*C*). The GFP signal was also recorded (*B*). Cells *1–6* show successive stages in the cell cycle. All cells are shown at the same magnification.

(Fig. 3 *A, 9:59*), and cell separation (detected as a rotation of the daughter cell relative to the mother; e.g., Fig. 3 *A, 19:10*) occurred ~10 min later.

Coincidence of the Actin and Myo1p Rings

To examine whether the actin and Myo1p rings were part of the same structure, exponentially growing cells expressing Myo1p–GFP were fixed with ice-cold 70% ethanol and stained with rhodamine-phalloidin and bisBenzimide. Before bud emergence, actin patches clustered at the presumptive bud site together with the Myo1p ring (Fig. 4, cell *1*), but no actin ring was detected. In budded preanaphase cells, the Myo1p ring was visible at the neck, while actin patches were concentrated in the bud (Fig. 4, cell *2*). In late-anaphase and post-anaphase cells, the Myo1p and actin rings appeared to coincide at the neck (Fig. 4, cells *3* and *4*). In all cells with a detectable actin ring (25 cells scored), the diameters of the Myo1p and actin rings were similar (Fig. 4, cells *3* and *4*). Thus, the actin ring assembles at the site of the preexisting Myo1p ring, and the actomyosin ring then contracts to a point and disappears. Using the Myo1p–GFP as a guide in these preparations, it was possible to observe smaller actin rings that had previously escaped detection and that represented the final stages of ring contraction; including these structures, ~9% of the cells (n = 373) in an asynchronous population contained an actomyosin ring. In addition, ~7% of the cells in the same population contained clusters of actin patches symmetrically disposed on both sides of the neck; none of these cells contained a Myo1p ring (Fig. 4, *cells 5* and *6*). This suggests that the actomyosin ring contracts and disappears before the actin patches congregate at the neck.

Dependence of Actin Ring Assembly on Myo1p

To determine whether Myo1p is important for the formation or contraction of the actin ring, we generated *MYO1* deletion strains in two genetic backgrounds (refer to Materials and Methods). These strains were viable, and their phenotypes are described in more detail below. We were unable to detect any cells containing actin rings in either strain YEF1820 or JMY1318 (>1,000 cells scored in each case). Examples of *myo1Δ* cells with fully elongated spindles are shown in Fig. 5, *D–F*; wild-type cells at this stage of the cell cycle almost always contained a detectable actin ring (Fig. 5, *A–C*). Thus, Myo1p appears to be required for formation of the actin ring. Myo1p–GFP supported actin ring formation and contraction (see above), indicating that it was functional in these regards.

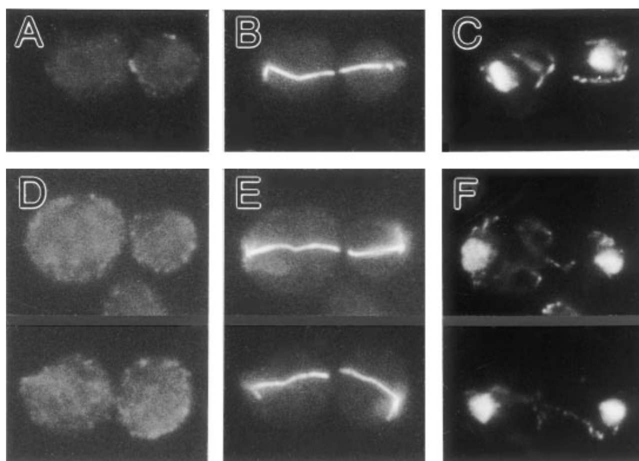

Figure 5. Dependence of actin ring formation on Myo1p. Cells of wild-type strain YEF473 (*A–C*) and *myo1Δ/myo1Δ* strain YEF1820 (*D–F*) growing exponentially in SC medium were triple-stained for F-actin (*A* and *D*), tubulin (*B* and *E*), and DNA (*C* and *F*).

Dependence of Myo1p Ring Contraction, but Not of Myo1p Ring Formation or Maintenance, on F-actin

To determine whether F-actin is important for the maintenance or contraction of the Myo1p ring, we treated exponentially growing cells of a Myo1p–GFP-expressing strain with 200 μM LAT-A and observed them by time-lapse video microscopy. At this concentration, LAT-A caused the loss of all detectable F-actin within 10 min (Fig. 6, *B* and *C*) (Ayscough et al., 1997). Most budded and some unbudded cells retained a strong Myo1p–GFP signal after LAT-A treatment (Fig. 6 *A, 03:38* and *16:37*). In five small-budded cells observed, the intensity of the Myo1p–GFP ring did not change significantly during 1–4.5 h of filming (Fig. 6 *A, right-hand cell*, and data not shown), indicating that maintenance of the Myo1p ring does not require F-actin. In contrast, observations on large-budded cells revealed that the Myo1p–GFP ring disappeared following anaphase (nuclei visualized by DIC microscopy), but that it did so without contracting (Fig. 6 *A, left-hand cell*, *21:37–27:37*). In the seven cells observed, the disappearance of the Myo1p ring took ~8 min, which was very similar to the time required for contraction of the ring when F-actin was present (see above). During its disappearance in the LAT-A–treated cells, the Myo1p–GFP ring appeared to remain constant in diameter at ~1 ± 0.1 μm (n = 7), indicating that F-actin is essential for contraction of the Myo1p ring.

To determine whether F-actin is essential for the initial formation of the Myo1p ring at the presumptive bud site, we isolated stationary phase cells of a Myo1p–GFP-expressing strain and inoculated them into fresh medium in the presence or absence of LAT-A. In the absence of LAT-A, most cells assembled detectable Myo1p–GFP rings (Fig. 7, *A, open circles*, and *B*) and budded (data not shown) during the 4-h time course. In the presence of LAT-A, cells did not form buds over the course of the experiment, but they did form Myo1p–GFP rings (Fig. 7, *A*, *closed circles*, and *C*). However, ring formation was significantly delayed: at 2 h, only 9% of the cells contained detectable Myo1p–GFP rings, compared with 41% of the cells incubated in the absence of LAT-A. In contrast, other (actin-independent) markers of the presumptive bud site appear with normal kinetics when cells are treated with LAT-A under these conditions (Ayscough et al., 1997), indicating that such treatment does not produce a nonspecific delay in reentry into the cell cycle. Thus, it appears that F-actin is not essential for the initial assembly of the Myo1p ring but does contribute to the efficiency and timing of ring formation.

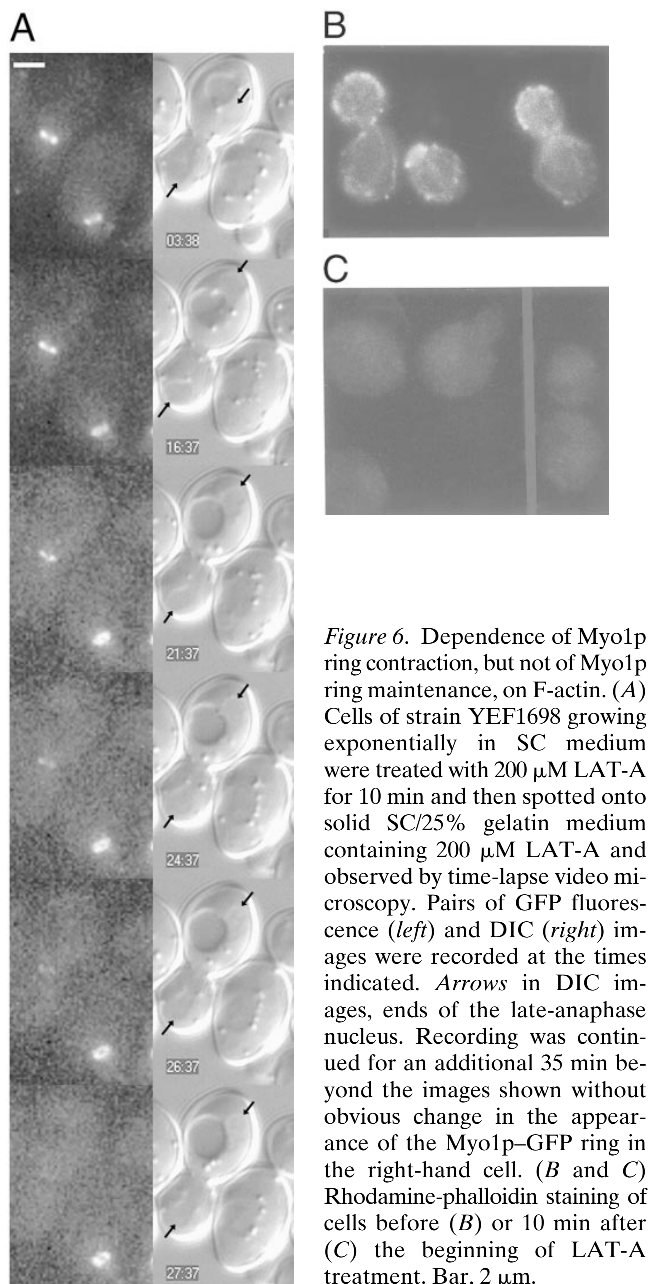

Figure 6. Dependence of Myo1p ring contraction, but not of Myo1p ring maintenance, on F-actin. (*A*) Cells of strain YEF1698 growing exponentially in SC medium were treated with 200 μM LAT-A for 10 min and then spotted onto solid SC/25% gelatin medium containing 200 μM LAT-A and observed by time-lapse video microscopy. Pairs of GFP fluorescence (*left*) and DIC (*right*) images were recorded at the times indicated. *Arrows* in DIC images, ends of the late-anaphase nucleus. Recording was continued for an additional 35 min beyond the images shown without obvious change in the appearance of the Myo1p–GFP ring in the right-hand cell. (*B* and *C*) Rhodamine-phalloidin staining of cells before (*B*) or 10 min after (*C*) the beginning of LAT-A treatment. Bar, 2 μm.

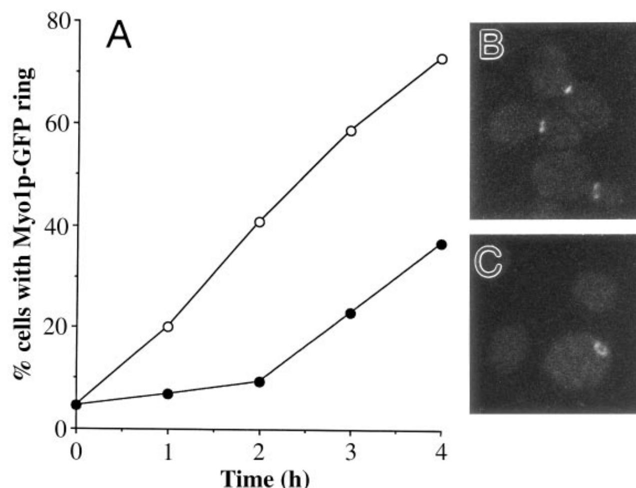

Figure 7. Formation of Myo1p–GFP rings in the absence of F-actin. Unbudded cells of strain YEF1698 were isolated as described in Materials and Methods and reinoculated into fresh YM-P medium in the absence or presence of 200 μM LAT-A. (*A*) Time-course of Myo1p–GFP ring formation in the absence (*open circles*) or presence (*closed circles*) of LAT-A. (*B* and *C*) Representative cells from the cultures without (*B*) and with (*C*) LAT-A, photographed 4 h after reinoculation.

Septin Dependence of Myo1p Ring Formation and Maintenance

To determine whether the formation and/or maintenance of the Myo1p ring requires the septins, we generated a diploid strain homozygous both for *MYO1–GFP* and for the temperature-sensitive *cdc12-6* septin mutation. Cells were fixed and processed for visualization of Myo1p–GFP or the septin Cdc11p during exponential growth at 23°C or 1 h after a shift to the restrictive temperature of 37°C. At 23°C, most cells displayed normal septin localization to the presumptive bud site or the bud neck, but some cells had aberrantly elongated buds and/or lacked detectable septin staining at the bud neck (Fig. 8 *C*), indicating a partial defect even at 23°C, as observed previously for other *cdc12-6* strains (Adams, 1984). At this temperature, Myo1p–GFP rings were seen in 43% of the cells (Fig. 8 *A*; $n = 207$). However, after a shift to 37°C for 1 h, no septin staining or Myo1p–GFP rings were detected (Fig. 8, *B* and *D*; $n = 229$). For comparison, wild-type cells expressing Myo1p–GFP showed a detectable Myo1p–GFP ring in 71% of cells ($n = 215$) at 23°C and 65% of cells ($n = 234$) at 37°C. Thus, assembly and maintenance of the Myo1p ring appear to require septin function.

Cytokinesis and Cell Separation in myo1 Mutants

Previous studies using disruptions or partial deletions of *MYO1* suggested that loss of Myo1p function caused a partially penetrant defect in cytokinesis and/or cell separation associated with aberrant septum deposition (Watts et al., 1987; Rodriguez and Paterson, 1990; Brown, 1997; Rodriguez, J., personal communication). To examine further the function of Myo1p, we generated complete or nearly complete deletions of the *MYO1* coding region in two different strain backgrounds. The *myo1Δ* cells displayed growth defects relative to wild-type cells that were variable but relatively mild in one strain background and variable but relatively severe in the other. In particular, diploid strain JMY1318 grew nearly as well as wild type, and although haploid *myo1Δ* segregants from this diploid formed colonies of variable size, many of these were comparable to wild-type colonies. In contrast, diploid strain YEF1820 grew significantly more slowly than wild type, and haploid *myo1Δ* segregants in this strain background were either inviable or formed small or medium-sized colonies. Much of this variability in colony size appeared to be epigenetic and/or to reflect differences in the efficiency of spore germination or the initial outgrowth of single cells, because in both strain backgrounds, restreaking cells from a single colony resulted again in a range of colony sizes.

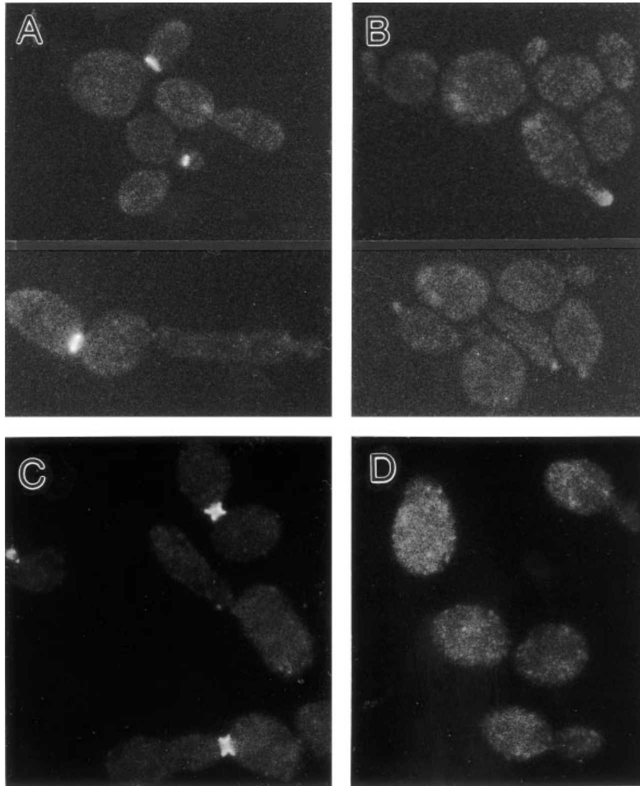

Figure 8. Dependence of Myo1p–GFP ring formation and maintenance on the septins. Cells of strain YEF1798 were fixed with 70% ethanol (refer to Materials and Methods) and processed for the observation of Myo1p-GFP (*A* and *B*) or fixed with formaldehyde and processed for the visualization of Cdc11p (*C* and *D*) during exponential growth in YM-P medium at 25°C (*A* and *C*) or 1 h after a shift to 37°C (*B* and *D*).

Phenotypes at the single cell level were also somewhat variable. In strain JMY1318, many cells appeared normal, but there were also chains of cells (Fig. 9 *B*) suggestive of a partially penetrant cytokinesis or cell separation defect. Light sonication readily separated the cells (Fig. 9 *C*), indicating that cytokinesis had in fact been completed. The defect was more severe in strain YEF1820, which formed longer chains (Fig. 9 *D*) that were resistant to sonication (data not shown). However, most cells could be separated by treating the fixed cells with lyticase to digest the cell walls (Fig. 9 *E*), indicating that the principal defect was in septum formation or cell separation and not in cytokinesis. Thus, it seems clear that cells can undergo cytokinesis and (with lesser and more variable efficiency) cell separation in the absence of Myo1p and hence of the contractile ring.

In addition to the defect in cell separation, some of the *myo1Δ* cells were aberrantly large and/or multinucleate (Fig. 9, *D*, *J*, and *K*; cf. *A*, *G*, and *H*). Cell shape was also aberrant in some of the cells in various ways: some cells were rounder than wild-type, whereas others were more elongated. Some cells also had abnormally wide mother–bud necks (Fig. 9 *D*). As with the growth defect, these phenotypes were considerably more severe for strain YEF1820 (e.g., 36% of the cells had two or more nuclei; $n = 234$) than for strain JMY1318, in which the large majority of the cells had a normal morphology. Although *myo1Δ* cells of either strain background completely lacked an actin ring (see above), the subsequent clustering of actin patches at the neck seemed to occur normally (Fig. 9, *F* and *I*), indicating that this process does not require the prior presence or contraction of the actomyosin ring.

Cytokinesis without F-actin

Ayscough et al. (1997) reported that many of the cells in an asynchronous population could undergo cell separation in the presence of 200 μM LAT-A, suggesting that cytokinesis in *S. cerevisiae* does not require F-actin. We have confirmed this result. Using strain YEF1698 growing exponentially in SC medium, and scoring budded cells under conditions (sonication after fixation) that minimize the number of cells that have completed cytokinesis but not cell separation (Pringle and Mor, 1975), we found that the proportion of budded cells decreased from 60 to 28% during a 4-h incubation in the presence of 200 μM LAT-A. Treatment of fixed cells from the population before LAT-A treatment with cell wall-digesting enzymes reduced the proportion of budded cells from 60 to 51%, implying that only 6% of the population had completed cytokinesis but not cell separation (recall that each budded cell produces two unbudded cells upon division); thus, most of the decrease in the percent budded cells during LAT-A treatment indeed involved the completion of both cytokinesis and cell separation. We also used time-lapse microscopy to examine possible cell separation in LAT-A–treated cells. As described above, untreated cells developed a visible septum shortly after contraction of the Myo1p ring, and cell separation was observed ~10 min later. In contrast, we observed neither septum formation nor cell separation during the 30 min after the disappearance of the Myo1p ring, in the LAT-A–treated cells. In one case, a cell that was before anaphase when first observed was seen to form a septum and undergo cell separation ~3 h after the beginning of LAT-A treatment. Thus, it appears that cytokinesis and/or septum formation is delayed or partially defective in the absence of F-actin.

Discussion

An Actomyosin Contractile Ring in S. cerevisiae

The timing and geometry of cytokinesis in budding yeast differ in several ways from those in other eukaryotes. First, the eventual site of cytokinesis is determined at the beginning of the cell cycle when the bud site is selected (Pringle et al., 1995; Drubin and Nelson, 1996). Second, a ring of septin proteins, which is essential for eventual cytokinesis (Hartwell, 1971; Longtine et al., 1996), appears at the presumptive bud site before bud emergence (Kim et al., 1991; Ford and Pringle, 1991) and remains in place at the mother–bud neck until the end of the cell cycle. Third, a ring of chitin is deposited in the cell wall at the presumptive bud site before bud emergence (Hayashibe and Katohda, 1973; Cabib et al., 1982; Kim et al., 1991); this chitin ring forms a constriction at the mother–bud neck throughout the period of bud growth and ultimately is extended

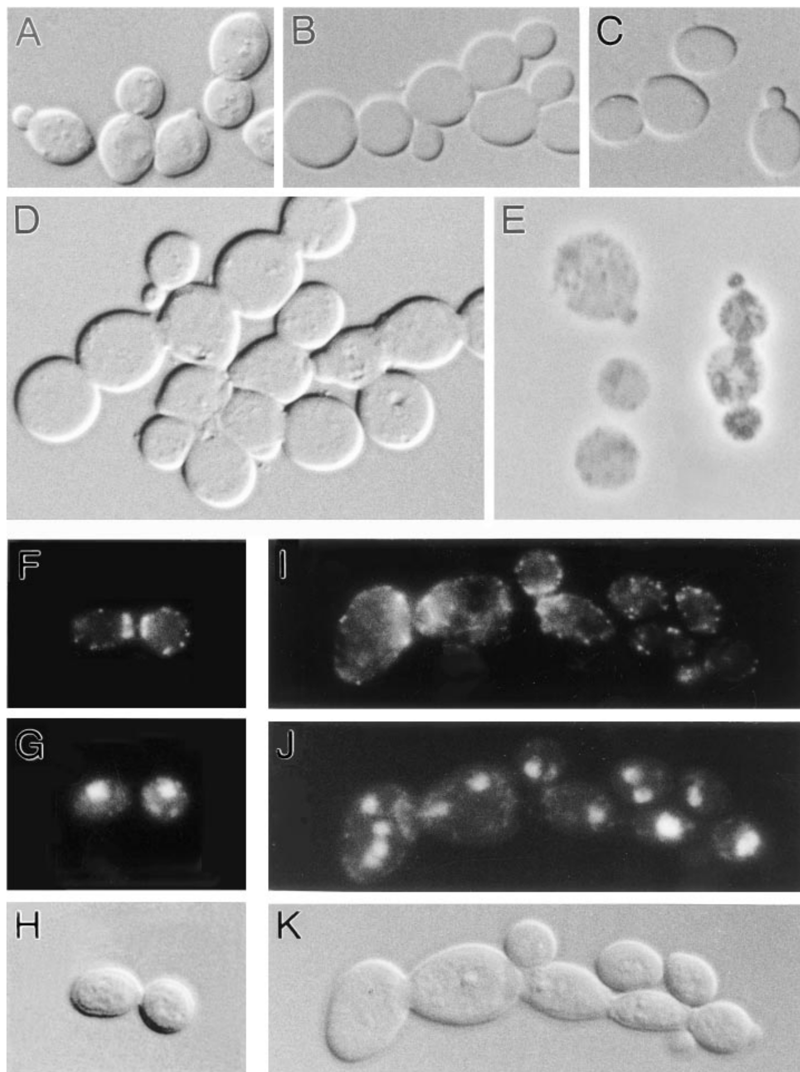

Figure 9. Phenotypic effects of *MYO1* deletions. (*A–E*) Cells of wild-type strain YEF473 (*A*) and of *myo1Δ/myo1Δ* strains JMY1318 (*B* and *C*) and YEF1820 (*D* and *E*) were fixed during exponential growth in SC medium and examined by DIC (*A–D*) or phase-contrast (*E*) microscopy. Cells were examined without additional treatments (*A*, *B*, and *D*), after light sonication (*C*), or after treatment with lytic enzyme to remove cell walls (refer to Materials and Methods) (*E*). (*F–K*) Cells of strains YEF473 (*F–H*) and YEF1820 (*I–K*) were fixed during exponential growth in YM-P medium, double stained for F-actin (*F* and *I*) and DNA (*G* and *J*), and observed also by DIC (*H* and *K*). All cells are shown at the same magnification.

across the neck to form the primary septum. In sum, it appears that the first steps toward eventual division are taken very early in the cell cycle, and cytokinesis after anaphase only needs to complete the process by closing the narrow neck. For this and other reasons (refer to Introduction), it has been unclear whether cytokinesis in budding yeast involves mechanisms similar to those used in other cells.

The results presented here, together with the recent results of others (Epp and Chant, 1997; Lippincott and Li, 1998), demonstrate that an actomyosin ring assembles and contracts after anaphase in *S. cerevisiae* cells. Although it is not yet proven that this contraction involves the production of force by actin–myosin interaction, the data suggest that cytokinesis in budding yeast involves the same broadly conserved cleavage furrow mechanism that is found in animal cells, slime molds, and fission yeast. As in fission yeast (refer to Introduction), the function of the contractile ring is presumably coupled to mechanisms for the localized synthesis of cell wall to form the septum.

Sequential Assembly of Myosin and Actin into the Contractile Ring

Myo1p forms a ring at the presumptive bud site in late G1 or early S phase, shortly before bud emergence. This Myo1p ring then remains at the mother–bud neck until the end of anaphase, when a corresponding ring of actin assembles and the resulting actomyosin ring contracts. In many other cell types, actin and myosin coassemble into the contractile ring near the end of the cell cycle, and it has generally been supposed that actin filaments are tethered to the plasma membrane and that the myosin filaments assemble by association with the actin. This is clearly not the case in *S. cerevisiae*. Not only does the Myo1p ring assemble at a time when no actin ring is detectable, but preexisting Myo1p rings persisted, and new Myo1p rings could even form, in cells lacking F-actin. In contrast, the actin ring failed to assemble in cells lacking Myo1p. Evidence from other organisms suggests that such a role for myosin II in the recruitment of actin may in fact be a common feature of contractile ring assembly. For example, *Dictyostel-*

ium cells lacking myosin II fail to concentrate actin filaments in the cleavage furrow (Neujahr et al., 1997), and a study on isolated contractile rings from sea urchin eggs found that myosin II remained in a ring even after actin was depolymerized (Schroeder and Otto, 1988).

Although F-actin is not required for formation of the Myo1p ring, ring formation is more efficient when F-actin is present. Given the motor function of myosins, it is possible that Myo1p molecules (perhaps assembled into filaments) are transported to the presumptive bud site by movement along the actin cables that become oriented towards that site before bud emergence (Adams and Pringle, 1984; Kilmartin and Adams, 1984; Ford and Pringle, 1991; Lew and Reed, 1993; Bretscher et al., 1994; Drubin and Nelson, 1996). However, the eventual formation of Myo1p rings in the absence of F-actin suggests that binding interactions between freely diffusing Myo1p molecules (or filaments) and other components at the cell cortex are sufficient to allow Myo1p ring assembly even in the absence of directed transport.

In most cell types, myosin II is thought to form bipolar filaments through interactions of its long coiled-coil tails. Studies using mutant forms of myosin II in *Dictyostelium* indicate that a myosin that cannot form bipolar filaments does not assemble at the cleavage furrow and cannot promote cytokinesis of nonadherent cells (Burns et al., 1995*a*,*b*). At present, it is unclear whether myosin II from yeasts forms bipolar filaments. Interestingly, the coiled-coil domains both of Myo1p (GenBank/EMBL/DDBJ accession number U10399) and of the two fission yeast myosin II heavy chains (Kitayama et al., 1997; Bezanilla et al., 1997; May et al., 1997) contain multiple proline residues that would be expected to form kinks in the tails. It may also be relevant that our Myo1p–GFP fusion appeared to retain full activity even though the GFP was fused to the Myo1p COOH terminus. Thus, the issues of whether yeast myosin tails mediate bipolar filament formation, and of whether bipolar filament formation is necessary for function in the contractile ring, require further investigation.

The assembly of the Myo1p ring early in the cell cycle suggests that it may play a role in the function of the actin cytoskeleton during bud formation in addition to its role in cytokinesis. Consistent with this hypothesis, many *myo1Δ* cells were large, aberrantly shaped, and/or multinucleate. The variable penetrance of these phenotypes has made it difficult to determine the underlying defect(s). However, it is difficult to envisage how these phenotypes could all arise simply as consequences of a primary defect in cytokinesis or septum formation, especially given that the cytokinesis-defective septin mutants do not show a similar constellation of phenotypes.

A Role for Septins in Contractile Ring Assembly

The Myo1p ring assembles at the same site as the septin ring. Moreover, new Myo1p rings failed to form and preexisting Myo1p rings disappeared when a temperature-sensitive septin mutant was incubated at restrictive temperature. These observations suggest that one role of the septins in budding yeast cytokinesis is to serve as a scaffold for the assembly of Myo1p, and hence subsequently of actin, into the contractile ring. It will be interesting to see whether this role of the septins in assembly of the actomyosin ring holds true for animal cells and explains the dependence of cytokinesis on septin function in such cells (Neufeld and Rubin, 1994; Kinoshita et al., 1997).

Contraction or Disassembly of the Ring in Response to a Cell Cycle Signal

After anaphase, the actomyosin ring contracted to a point and disappeared. Contraction of the ~1-μm-diam ring took 7–9 min. This rate is rather slow relative to that in *Dictyostelium* cytokinesis, where contraction of an ~12-μm-diam furrow takes ≤4 min (Sabry et al., 1997; Zang et al., 1997), but may be comparable to that in fission yeast, where contraction of an ~3-μm-diam ring is reported to take ~30 min (Kitayama et al., 1997). However, the exact rates in both yeasts must be regarded as uncertain, because it is not clear that the GFP-tagged myosins used in these studies constrict the ring at the same rate as normal myosin.

In the absence of F-actin, Myo1p rings failed to contract, as expected on the hypothesis that sliding of actin and myosin filaments provides the force for contraction. Instead, after anaphase, the Myo1p ring grew progressively fainter and soon disappeared; disappearance of the ring took about as long as ring contraction in cells containing F-actin. In contrast, the Myo1p rings were stable for long periods in cells arrested early in the cell cycle by the LAT-A treatment. It has long been presumed (Schroeder, 1972) that actin and myosin filaments must be released from the contractile ring during contraction of the cleavage furrow in order to maintain the approximately constant thickness of the ring. Thus, the gradual disassembly of Myo1p from the ring in post-anaphase cells lacking F-actin may reflect the action of this release mechanism occurring without concurrent contraction. In any case, these observations indicate that the behavior of Myo1p is subject to cell cycle regulation after anaphase, even in the absence of F-actin.

Cytokinesis and Cell Separation without a Contractile Ring

After contraction of the actomyosin ring, a septum visible by DIC developed within 1–2 min. Cell separation, reflecting degradation of the primary septum, occurred 10–11 min later. Cells lacking Myo1p were able to complete cytokinesis, septum formation, and cell separation. Because no actin rings were detected in the *myo1Δ* cells, we conclude that there is no redundant mechanism for generating an actomyosin ring in the absence of Myo1p. Instead, there must be an alternative pathway for completing cytokinesis without a contractile ring. Cytokinesis without myosin II has also been observed in *Dictyostelium*. In this case, the cells are able to complete a process that resembles normal cleavage provided that they have access to an adherent surface (DeLozanne and Spudich, 1987; Knecht and Loomis, 1987; Neujahr et al., 1997; Zang et al., 1997); traction generated by surface contact may play a role in this process (Spudich, 1989). In yeast, it seems likely that the localized synthesis of cell wall that normally produces the septum may be sufficient to drive cytokinesis of the narrow neck in the absence of the contractile ring.

In this regard, the behavior of the actin patches is of in-

terest. These patches appear to represent the points at which cytoplasmic actin cables contact the plasma membrane, and thus their distribution is thought to reflect the pattern in which new plasma membrane and cell wall materials are being delivered to the cell surface (Adams and Pringle, 1984; Kilmartin and Adams, 1984; Lew and Reed, 1993; Bretscher et al., 1994; Mulholland et al., 1994, 1997; Doyle and Botstein, 1996; Waddle et al., 1996; Brown, 1997). Congregation of these patches at the mother–bud neck follows closely upon contraction of the actomyosin ring, consistent with the hypothesis that a localized, actin-based delivery of new cell-surface materials plays a role in septum formation. Thus, the observation that these patches still congregate at the neck in the absence of Myo1p is consistent with the hypothesis that septum formation may drive cytokinesis under these conditions. In this regard, it is remarkable that many cells are able to complete cytokinesis and cell separation even in the absence of F-actin (Ayscough et al., 1997; this study). However, the time-lapse studies indicated that cell division was considerably delayed under these conditions, as if localized cell–wall synthesis could drive cell division, but only very inefficiently, in the absence of F-actin.

Although *myo1Δ* cells could complete cytokinesis efficiently, they displayed a partially penetrant defect in cell separation, forming short chains or clumps of connected cells. It seems unlikely that Myo1p plays a direct role in cell separation per se (i.e., in the dissolution of the primary septum), because the Myo1p ring has disassembled by the time when this occurs. Thus, the cell separation defect is more likely an indirect consequence of an earlier defect in cytokinesis or septum formation; for example, the lack of Myo1p might result in formation of an abnormal primary septum whose dissolution is then retarded. This possibility is supported by electron microscopic observations of abnormal septum structures in a *myo1* mutant strain (Rodriguez and Paterson, 1990), although these observations would bear repeating with the *myo1Δ* strains used here.

Unlike Myo1p and the actomyosin ring, the septins appear to be absolutely required for cytokinesis (Hartwell, 1971; Longtine et al., 1996). Thus, the septins presumably contribute to the alternative pathway for cytokinesis as well as to the formation of the actomyosin ring. One likely possibility is suggested by the role of the septins in localizing the chitin synthase III complex that forms the chitin ring before bud emergence (DeMarini et al., 1997). It would not be surprising if the septins play a similar role in localizing the activity of chitin synthase II (which appears to be primarily responsible for synthesis of the primary septum: Bulawa, 1993) or other proteins required for septum formation, such as proteins required for targeted secretion. Thus, the absolute dependence of cytokinesis on the septins in *S. cerevisiae* would reflect the dual roles of these proteins in serving as a scaffold both for cytoskeletal elements and for proteins involved in targeted secretion and/or cell–wall biosynthesis.

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References

Adams, A.E.M. 1984. Cellular morphogenesis in the yeast *Saccharomyces cerevisiae*. Ph.D. thesis. University of Michigan, Ann Arbor, MI. 179 pp.

Adams, A.E.M., and J.R. Pringle. 1984. Relationship of actin and tubulin distribution to bud growth in wild-type and morphogenetic-mutant *Saccharomyces cerevisiae*. *J. Cell Biol.* 98:934–945.

Alfa, C.E., and J.S. Hyams. 1990. Distribution of tubulin and actin through the cell division cycle of the fission yeast *Schizosaccharomyces japonicus* var. *versatilis*: a comparison with *Schizosaccharomyces pombe*. *J. Cell Sci.* 96:71–77.

Ayscough, K.R., J. Stryker, N. Pokala, M. Sanders, P. Crews, and D.G. Drubin. 1997. High rates of actin filament turnover in budding yeast and roles for actin in establishment and maintenance of cell polarity revealed using the actin inhibitor latrunculin-A. *J. Cell Biol.* 137:399–416.

Baudin, A., O. Ozier-Kalogeropoulos, A. Denouel, F. Lacroute, and C. Cullin. 1993. A simple and efficient method for direct gene deletion in *Saccharomyces cerevisiae*. *Nucleic Acids Res.* 21:3329–3330.

Bezanilla, M., S.L. Forsburg, and T.D. Pollard. 1997. Identification of a second myosin-II in *Schizosaccharomyces pombe*: Myp2p is conditionally required for cytokinesis. *Mol. Biol. Cell.* 8:2693–2705.

Bi, E., and J.R. Pringle. 1996. *ZDS1* and *ZDS2*, genes whose products may regulate Cdc42p in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* 16:5264–5275.

Bretscher, A., B. Drees, E. Harsay, D. Schott, and T. Wang. 1994. What are the basic functions of microfilaments? Insights from studies in budding yeast. *J. Cell Biol.* 126:821–825.

Brown, S.S. 1997. Myosins in yeast. *Curr. Opin. Cell Biol.* 9:44–48.

Bulawa, C.E. 1993. Genetics and molecular biology of chitin synthesis in fungi. *Annu. Rev. Microbiol.* 47:505–534.

Burns, C.G., D.A. Larochelle, H. Erickson, M. Reedy, and A. De Lozanne. 1995*a*. Single-headed myosin II acts as a dominant negative mutation in *Dictyostelium*. *Proc. Natl. Acad. Sci. USA*. 92:8244–8248.

Burns, C.G., M. Reedy, J. Heuser, and A. De Lozanne. 1995*b*. Expression of light meromyosin in *Dictyostelium* blocks normal myosin II function. *J. Cell Biol.* 130:605–612.

Byers, B. 1981. Cytology of the yeast life cycle. *In* The Molecular Biology of the Yeast *Saccharomyces*: Life Cycle and Inheritance. J.N. Strathern, E.W. Jones, and J.R. Broach, editors. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. 59–96.

Byers, B., and D.H. Abramson. 1968. Cytokinesis in HeLa: Post-telophase delay and microtubule-associated motility. *Protoplasma.* 66:413–435.

Byers, B., and L. Goetsch. 1976. A highly ordered ring of membrane-associated filaments in budding yeast. *J. Cell Biol.* 69:717–721.

Cabib, E., R. Roberts, and B. Bowers. 1982. Synthesis of the yeast cell wall and its regulation. *Annu. Rev. Biochem.* 51:763–793.

Chang, F., and P. Nurse. 1996. How fission yeast fission in the middle. *Cell*. 84: 191–194.

Chant, J., M. Mischke, E. Mitchell, I. Herskowitz, and J.R. Pringle. 1995. Role of Bud3p in producing the axial budding pattern of yeast. *J. Cell Biol.* 129: 767–778.

Conrad, G.W., and T.E. Schroeder, editors. 1990. Cytokinesis: mechanisms of furrow formation during cell division. Vol. 582. *Ann. NY Acad. Sci.*,11 New York. 327 pp.

De Lozanne, A., and J.A. Spudich. 1987. Disruption of the *Dictyostelium* myosin heavy chain gene by homologous recombination. *Science*. 236:1086–1091.

DeMarini, D.J., A.E.M. Adams, H. Fares, C. De Virgilio, G. Valle, J.S. Chuang, and J.R. Pringle. 1997. A septin-based hierarchy of proteins required for localized deposition of chitin in the *Saccharomyces cerevisiae* cell wall. *J. Cell Biol.* 139:75–93.

Doyle, T., and D. Botstein. 1996. Movement of yeast cortical actin cytoskeleton visualized *in vivo*. *Proc. Natl. Acad. Sci. USA*. 93:3886–3891.

Drubin, D.G., and W.J. Nelson. 1996. Origins of cell polarity. *Cell.* 84:335–344.

Epp, J.A., and J. Chant. 1997. An IQGAP-related protein controls actin-ring formation and cytokinesis in yeast. *Curr. Biol.* 7:921–929.

Fares, H., M. Peifer, and J.R. Pringle. 1995. Localization and possible functions of *Drosophila* septins. *Mol. Biol. Cell*. 6:1843–1859.

Fishkind, D.J., and Y. Wang. 1995. New horizons for cytokinesis. *Curr. Opin. Cell Biol.* 7:23–31.

Ford, S.K., and J.R. Pringle. 1991. Cellular morphogenesis in the *Saccharomyces cerevisiae* cell cycle: localization of the *CDC11* gene product and the timing of events at the budding site. *Dev. Genet.* 12:281–292.

Girbardt, M. 1979. A microfilamentous septal belt (FSB) during induction of cytokinesis in *Trametes versicolor* (L. ex Fr.). *Exp. Mycol.* 3:215–228.

Glotzer, M. 1997. The mechanism and control of cytokinesis. *Curr. Opin. Cell Biol.* 9:815–823.

Gould, K.L., and V. Simanis. 1997. The control of septum formation in fission yeast. *Genes Dev*. 11:2939–2951.

Guthrie, C., and G.R. Fink, editors. 1991. Guide to yeast genetics and molecular biology. *Methods Enzymol.* Vol. 194. 933 pp.

Hartwell, L.H. 1971. Genetic control of the cell division cycle in yeast. IV. Genes controlling bud emergence and cytokinesis. *Exp. Cell Res.* 69:265–276.

Hayashibe, M., and S. Katohda. 1973. Initiation of budding and chitin ring. *J. Gen. Appl. Microbiol.* 19:23–39.

Hoch, H.C., and R.J. Howard. 1980. Ultrastructure of freeze-substituted hyphae of the basidiomycete *Laetisaria arvalis. Protoplasma.* 103:281–297.

Jochová, J., I. Rupeš, and E. Streiblová. 1991. F-actin contractile rings in protoplasts of the yeast *Schizosaccharomyces*. *Cell Biol. Int. Rep*. 15:607–610.

Kilmartin, J.V., and A.E.M. Adams. 1984. Structural rearrangements of tubulin and actin during the cell cycle of the yeast *Saccharomyces*. *J. Cell Biol*. 98: 922–933.

Kilmartin, J.V., B. Wright, and C. Milstein. 1982. Rat monoclonal antitubulin antibodies derived by using a new nonsecreting rat cell line. *J. Cell Biol*. 93: 576–582.

Kim, H.B., B.K. Haarer, and J.R. Pringle. 1991. Cellular morphogenesis in the *Saccharomyces cerevisiae* cell cycle: localization of the *CDC3* gene product and the timing of events at the budding site. *J. Cell Biol*. 112:535–544.

Kinoshita, M., S. Kumar, A. Mizoguchi, C. Ide, A. Kinoshita, T. Haraguchi, Y. Hiraoka, and M. Noda. 1997. Nedd5, a mammalian septin, is a novel cytoskeletal component interacting with actin-based structures. *Genes Dev*. 11: 1535–1547.

Kitayama, C., A. Sugimoto, and M. Yamamoto. 1997. Type II myosin heavy chain encoded by the *myo2* gene composes the contractile ring during cytokinesis in *Schizosaccharomyces pombe*. *J. Cell Biol*. 137:1309–1319.

Knecht, D.A., and W.F. Loomis. 1987. Antisense RNA inactivation of myosin heavy chain gene expression in *Dictyostelium discoideum*. *Science*. 236:1081–1086.

Lew, D.J., and S.I. Reed. 1993. Morphogenesis in the yeast cell cycle: regulation by Cdc28 and cyclins. *J. Cell Biol*. 120:1305–1320.

Lillie, S.H., and J.R. Pringle. 1980. Reserve carbohydrate metabolism in *Saccharomyces cerevisiae*: responses to nutrient limitation. *J. Bacteriol*. 143: 1384–1394.

Lippincott, J., and R. Li. 1998. Sequential assembly of myosin II, an IQGAP-like protein, and filamentous actin to a ring structure involved in budding yeast cytokinesis. *J. Cell Biol.* 140:355–366.

Longtine, M.S., D.J. DeMarini, M.L. Valencik, O.S. Al-Awar, H. Fares, C. De Virgilio, and J.R. Pringle. 1996. The septins: roles in cytokinesis and other processes. *Curr. Opin. Cell Biol*. 8:106–119.

Longtine, M.S., A. McKenzie III, D.J. DeMarini, N.G. Shah, A. Wach, A. Brachat, P. Philippsen, and J.R. Pringle. 1998. Additional modules for versatile and economical PCR-based gene deletion and modification in *Saccharomyces cerevisiae. Yeast.* 14:953–961.

Marks, J., and J.S. Hyams. 1985. Localization of F-actin through the cell division cycle of *Schizosaccharomyces pombe*. *Eur. J. Cell Biol*. 39:27–32.

May, K.M., F.Z. Watts, N. Jones, and J.S. Hyams. 1997. Type II myosin involved in cytokinesis in the fission yeast, *Schizosaccharomyces pombe*. *Cell Motil. Cytoskeleton*. 38:385–396.

Miller, K.G., and D.P. Kiehart. 1995. Fly division. *J. Cell Biol*. 131:1–5.

Momany, M., and J.E. Hamer. 1997. Relationship of actin, microtubules, and crosswall synthesis during septation in *Aspergillus nidulans*. *Cell Motil. Cytoskeleton.* 38:373–384.

Mulholland, J., D. Preuss, A. Moon, A. Wong, D. Drubin, and D. Botstein. 1994. Ultrastructure of the yeast actin cytoskeleton and its association with the plasma membrane. *J. Cell Biol.* 125:381–391.

Mulholland, J., A. Wesp, H. Riezman, and D. Botstein. 1997. Yeast actin cytoskeleton mutants accumulate a new class of Golgi-derived secretory vesicle. *Mol. Biol. Cell.* 8:1481–1499.

Mullins, J.M., and J.J. Biesele. 1973. Cytokinetic activities in a human cell line: the midbody and the intercellular bridge. *Tissue Cell.* 5:47–61.

Mullins, J.M., and J.J. Biesele. 1977. Terminal phase of cytokinesis in D-985 cells. *J. Cell Biol*. 73:672–684.

Mullins, J.M., and J.R. McIntosh. 1982. Isolation and initial characterization of the mammalian midbody. *J. Cell Biol*. 94:654–661.

Neufeld, T.P., and G.M. Rubin. 1994. The *Drosophila peanut* gene is required for cytokinesis and encodes a protein similar to yeast putative bud neck filament proteins. *Cell*. 77:371–379.

Neujahr, R., C. Heizer, and G. Gerisch. 1997. Myosin II-independent processes in mitotic cells of *Dictyostelium discoideum*: redistribution of the nuclei, rearrangement of the actin system and formation of the cleavage furrow. *J. Cell Sci*. 110:123–137.

Pringle, J.R., and J.-R. Mor. 1975. Methods for monitoring the growth of yeast cultures and for dealing with the clumping problem. *Methods Cell Biol*. 11: 131–168.

Pringle, J.R., and L.H. Hartwell. 1981. The *Saccharomyces cerevisiae* cell cycle. *In* The Molecular Biology of the Yeast *Saccharomyces*: Life Cycle and Inheritance. J.N. Strathern, E.W. Jones, and J.R. Broach, editors. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. 97–143.

Pringle, J.R., A.E.M. Adams, D.G. Drubin, and B.K. Haarer. 1991. Immunofluorescence methods for yeast. *Methods Enzymol*. 194:565–602.

Pringle, J.R., E. Bi, H.A. Harkins, J.E. Zahner, C. De Virgilio, J. Chant, K. Corrado, and H. Fares. 1995. Establishment of cell polarity in yeast. *Cold Spr. Harb. Symp. Quant. Biol.* 60:729–744.

Rappaport, R. 1996. Cytokinesis in Animal Cells. Cambridge University Press, New York. 386 pp.

Roberson, R.W. 1992. The actin cytoskeleton in hyphal cells of *Sclerotium rolfsii. Mycologia.* 84:41–51.

Rodriguez, J.R., and B.M. Paterson. 1990. Yeast myosin heavy chain mutant: maintenance of the cell type specific budding pattern and the normal deposition of chitin and cell wall components requires an intact myosin heavy chain gene. *Cell Motil. Cytoskeleton*. 17:301–308.

Sabry, J.H., S.L. Moores, S. Ryan, J.-H. Zang, and J.A. Spudich. 1997. Myosin heavy chain phosphorylation sites regulate myosin localization during cytokinesis in live cells. *Mol. Biol. Cell*. 8:2605–2615.

Sambrook, J., E.F. Fritsch, and T. Maniatis. 1989. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Sanders, S.L., and C.M. Field. 1994. Septins in common? *Curr. Biol.* 4:907–910.

Satterwhite, L.L., and T.D. Pollard. 1992. Cytokinesis. *Curr. Opin. Cell Biol*. 4:43–52.

Schroeder, T.E. 1972. The contractile ring. II. determining its brief existence, volumetric changes, and vital role in cleaving *Arbacia* eggs. *J. Cell Biol*. 53: 419–434.

Schroeder, T.E., and J.J. Otto. 1988. Immunofluorescent analysis of actin and myosin in isolated contractile rings of sea urchin eggs. *Zool. Sci*. 5:713–725.

Shaw, S.L., E. Yeh, K. Bloom, and E.D. Salmon. 1997*a*. Imaging green fluorescent protein fusion proteins in *Saccharomyces cerevisiae*. *Curr. Biol*. 7:701–704.

Shaw, S.L., E. Yeh, P. Maddox, E.D. Salmon, and K. Bloom. 1997*b*. Astral microtubule dynamics in yeast: a microtubule-based searching mechanism for spindle orientation and nuclear migration into the bud. *J. Cell Biol*. 139:985–994.

Sikorski, R.S., and P. Hieter. 1989. A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. *Genetics*. 122:19–27.

Spudich, J.A. 1989. In pursuit of myosin function. *Cell Regul*. 1:1–11.

Staehelin, L.A., and P.K. Hepler. 1996. Cytokinesis in higher plants. *Cell*. 84: 821–824.

Streiblová, E., J. Hašek, and E. Jelke. 1984. Septum pattern in *ts* mutants of *Schizosaccharomyces pombe* defective in genes *cdc3*, *cdc4*, *cdc8* and *cdc12*. *J. Cell Sci*. 69:47–65.

Wach, A., A. Brachat, C. Alberti-Segui, C. Rebischung, and P. Philippsen. 1997. Heterologous *HIS3* marker and GFP reporter modules for PCR-targeting in *Saccharomyces cerevisiae*. *Yeast*. 13:1065–1075.

Waddle, J.A., T.S. Karpova, R.H. Waterston, and J.A. Cooper. 1996. Movement of cortical actin patches in yeast. *J. Cell Biol.* 132:861–870.

Watts, F.Z., D.M. Miller, and E. Orr. 1985. Identification of myosin heavy chain in *Saccharomyces cerevisiae*. *Nature*. 316:83–85.

Watts, F.Z., G. Shiels, and E. Orr. 1987. The yeast *MYO1* gene encoding a myosin-like protein required for cell division. *EMBO (Eur. Mol. Biol. Organ.) J*. 6:3499–3505.

Wick, S.M. 1991. Spatial aspects of cytokinesis in plant cells. *Curr. Opin. Cell Biol*. 3:253–260.

Yeh, E., R.V. Skibbens, J.W. Cheng, E.D. Salmon, and K. Bloom. 1995. Spindle dynamics and cell cycle regulation of dynein in the budding yeast, *Saccharomyces cerevisiae*. *J. Cell Biol*. 130:687–700.

Zang, J.-H., G. Cavet, J.H. Sabry, P. Wagner, S.L. Moores, and J.A. Spudich. 1997. On the role of myosin-II in cytokinesis: division of *Dictyostelium* cells under adhesive and nonadhesive conditions. *Mol. Biol. Cell*. 8:2617–2629.