Journal of Electron Microscopy 60(6): 379–391 (2011)
doi: 10.1093/jmicro/dfr073

Biological: Full-length

Ultrastructural disorder of actin mutant suggests uncoupling of actin-dependent pathway from microtubule-dependent pathway in budding yeast†

Marie Kopecká[1,*] and Masashi Yamaguchi[2,*]

[1]Department of Biology, Faculty of Medicine, Masaryk University, Kamenice 5, A6, 62500 Brno, Czech Republic and [2]Medical Mycology Research Centre, Chiba University, 1-8-1 Inohana, Chuo-ku, Chiba 260-8673, Japan

*To whom correspondence should be addressed. E-mail: mkopecka@med.muni.cz; http://www.med.muni.cz/~mkopecka/ (M.K.); E-mail: yama@faculty.chiba-u.jp (M.Y.)

†The paper is dedicated to the memory of our friend and co-worker Miroslav Gabriel, Associate Professor of the Faculty of Medicine Masaryk University, Brno, who died suddenly on 7 June 2008 during our combined work on the first version of this paper.

Abstract Temperature-sensitive actin mutant of *Saccharomyces cerevisiae act1-1* was studied at a permissive temperature of 23°C by light, fluorescent and electron microscopy to elucidate the roles of actin cytoskeleton in the cycling eukaryotic cells. Mutant cells that grew slowly at the permissive temperature showed aberrations in the cytoskeleton and cell cycle. Mutant cells contained aberrant 'faint actin cables,' that failed in directing of mitochondria, vacuoles and secretory vesicles to the bud and the stray vesicles delivered their content to the mother wall instead of the bud. Bud growth was delayed. Spindle pole bodies and cytoplasmic microtubules did not direct to the bud, and nucleus failed to migrate to the bud. Repeated nuclear divisions produced multinucleated cells, indicating continued cycling of actin mutant cells that failed in the morphogenetic checkpoint, the spindle position checkpoint and cytokinesis. Thus, a single actin mutation appears to indicate uncoupling in space and time of the 'actin cytoskeleton-dependent cytoplasmic pathway of bud development and organelle positioning and inheritance' from the 'microtubule-dependent nuclear division pathway' in a budding yeast cell cycle.

Keywords actin, nuclear position, organelle inheritance, microtubules

Received 25 May 2011, accepted 27 August 2011; online 15 October 2011

Introduction

The yeast *S. cerevisiae* possesses *ACT1*, a single essential gene that encodes actin. A null mutation is lethal [1]. Latrunculin A that disrupts all actin structures [2] induces spherical cells incapable of reproducing [3,4]. The roles of yeast actin have been suggested by studies on actin organization [5,6], while experimental analysis was initiated using the temperature-sensitive mutation in the actin gene. This actin mutation is suggested to cause 'faint actin cables' that are not visible; however, bright actin patches could be observed on the buds and mothers. In contrast to the wild-type mothers showing cables along the long axis of cells, the buds exhibit patches of actin. At the restrictive temperature, actin mutation *act1-1* caused disruption of the actin asymmetric staining pattern, delocalized deposition of chitin at the cell surface, intracellular accumulation of secretory vesicles, partial inhibition of secretion of periplasmic protein invertase, osmotic sensitivity

and cell death in the cell-cycle budded portion, implicating roles of actin in yeast cell surface organization and polarized growth [7]. Later studies have identified many other actin functions in endocytosis [8], polarity [9–12], polarized transport of secretory vesicles, mRNA, mitochondria, vacuoles and peroxisomes [10,13–19] and response to extracellular cues [20]. We (and others) studied actin in the formation of the wall [21–27], septum and in cytokinesis [23,28–30], actin cables in correct spatial positioning and orientation of the secretory pathway to the bud and septum by electron microscopy [31] and spindle orientation by fluorescent microscopy [32–39].

Novick and Botstein [7] first identified permissive and restrictive phenotypes of the original actin mutants, *act1-1* and *act1-2*, by fluorescence microscopy; however, they could only identify restrictive phenotypes by electron microscopy. They also noted that mutants exhibited some phenotypic abnormalities even at the permissive temperature, but were unable to demonstrate with electron microscopy. We identified ultrastructural disorder in *act1-1* and *act1-2* actin mutant cells at 23°C in 22 experiments by freeze-fracturing, freeze-substitution and transmission electron microscopy, which was not observed in the wild-type cells used as controls.

The aim of this study was to understand why the actin mutant cells have an ultrastructural disorder even at the permissive temperature. We found that actin mutation caused the defect in actin cables and delayed polarization of actin patches even at the permissive temperature, which resulted in the delay of actin cytoskeleton-dependent events in the cell cycle, whereas microtubule-dependent localized events continued aberrantly and uncoupled. This indicates that positioning and translocation in space and time and inheritance of nucleus and cell organelles and microtubule-dependent events depend on the three-dimensional architecture of the actin cytoskeleton.

Materials and methods

Yeast strains and cell culture

The *S. cerevisiae* strains used were temperature-sensitive actin mutants, kindly provided by Prof. David Botstein and Dr Paula Grisafi (MIT, Cambridge, MA, USA). Diploid strains wild-type DBY 1690 ($ACT1^+/ACT1^+$) and actin mutant *act1-1* DBY 1693 (*act1-1/act1-1*) were used in observing actin, microtubules and nuclei by fluorescent microscopy, and in freeze-substitution, freeze-fracturing, transmission and scanning electron microscopy [7]. The diploid cells are larger and more useful for cytological study than the haploid cells. Haploid strains such as DBY 1691 (*act1-1*), DBY 1692 (*act1-1*), DBY 1694 (*act1-2*) and DBY 1695 (*act1-2*) were used only for freeze-fracture, because they could be frozen better. The construction of actin mutation has been reported in detail elsewhere [40].

The cells were inoculated into YEPD medium or malt extract medium of pH 5.5 and cultivated in a water bath at 23°C overnight with shaking. After ~20 h, the cells were used for fluorescent microscopy, freeze-fracturing or freeze-substitution. Cell proliferation was monitored by measuring the absorbance at 550 nm.

To observe the effect of latrunculin A on actin cytoskeleton, 10, 20, 50, 100 and 200 μM of latrunculin A were tested. Samples with and without dimethyl sulfoxide were used for control experiments. After application of the drug, the cultivation was continued in the dark.

Fluorescence microscopy for observation of actin, microtubules and nucleus

After ~20-h cultivation at 23°C, actin was labeled with rhodamine–phalloidin R-415 (Molecular Probes Eugene, Oregon, USA), following fixation with 5% paraformaldehyde and permeabilization with 1% (v/v) Triton X-100 in phosphate buffered saline (PBS) [41]. Microtubules were visualized with anti-α-tubulin monoclonal antibody TAT1 [34], which was kindly provided by Prof. K. Gull (Manchester University, UK) and with monoclonal anti-tubulin antibody TU 01 [21]. Nuclear staining was performed by adding 1 mg of 4,6-diamidino-2-phenyl-indol dihydrochloride (DAPI) per milliliter (Serva Feinbiochemica) into the mounting medium, or mounting medium containing DAPI was used.

Electron microscopy

For freeze-fracturing, the cells were collected by centrifugation after cultivation for ~20 h, placed on copper grids, frozen in Freon 22 and stored in liquid nitrogen until use. Replicas were prepared in

a Blazers apparatus (BA 360) [42] and photographed in a Tesla BS 500 electron microscope (Brno, Czech Republic).

For ultrathin sectioning, the cells were collected by centrifugation and sandwiched between two copper discs. They were cryofixed by plunging into melting propane kept in liquid nitrogen. The specimens were freeze-substituted in acetone containing 2% osmium tetroxide at –80°C for 2 days and embedded in epoxy resin [43]. Ultrathin sections were stained with uranyl acetate and lead citrate [44], covered by Super support film (Nisshin EM Co., Ltd, Tokyo) and observed with a JEM-1200EX electron microscope (JEOL, Tokyo, Japan) [45].

For scanning electron microscopy, the cells were fixed with 5% paraformaldehyde [4,41], dehydrated with a graded alcohol series, dried using CPD 030 Critical Point Dryer (BAL-TEC) and coated with gold in metal shadowing apparatus BALZERS SCD040 (5 min; 30 mA). The specimens were observed in Vega TS5136 XM (TESCAN) using digital microscopy imaging scanning equipment (Brno, Czech Republic).

Results

Slow growth and bud development and cytoplasmic organelles in actin mutant *act1-1* at 23°C

When compared with the wild-type cells, *ACT1*, actin mutant *act1-1* cells grew slowly at 23°C (data not shown), as first shown by Novick and Botstein [7]. To find out the cause of slow growth of mutant cells at 23°C, we examined the morphology of actin mutant and wild-type cells, the F-actin cytoskeleton differences and the ultrastructural differences by fluorescent and electron microscopy.

Scanning electron microscopy

While the wild-type cells had ovoid mother cells and buds (Fig. 1a), majority of *act1-1* actin mutant cells had spherical mother cells and buds (Fig. 1b). We were interested to know whether disruption of actin cytoskeleton results in the formation of spherical cells.

Effect of latrunculin A on wild-type cells. At a concentration of 100 μM, latrunculin A suppressed growth of wild-type cells, inhibited reproduction by budding, and made wild-type cells spherical at 22 h (Fig. 1c) due to complete disruption of the actin cytoskeleton (Fig. 1d), in contrast to the control (Fig. 1e). We therefore investigated how actin cytoskeleton is structurally affected in actin mutants that have spherical shapes at the permissive temperature, but still reproduce by budding.

Fluorescence microscopy of actin cytoskeleton in actin mutants

We repeated the investigation of actin cytoskeleton by rhodamine–phalloidin staining. The wild-type mother cells had thick actin cables directing to the bud (Figs. 1e and 2b, g and l) and to septum during cytokinesis, and actin patches accumulated in the buds (Figs. 1e and 2g and l) and septum regions [5–7].

Actin mutant cells had actin patches not only in the buds, but also in the mother cells (Figs. 1f and 3f, j, l, q and u), as shown first by Novick and Botstein [7]. Although actin cables were hardly visible, even at 23°C, we identified faint actin cables in the mutant cells by fluorescent microscopy for the first time. They were seen only in the mother cells (Figs. 1f and 3j and q) that had less actin patches than the other cells (Fig. 3f, l and u). Actin cables in the mutant cells were faint and formed fine irregular cytoplasmic network proceeding in all directions (Fig. 1f), in contrast to wild-type cables directed to buds (Fig. 1e). Actin patches accumulating in the buds confirmed the establishment of cell polarity in these cells (Figs. 1f and 3f, j, l and q). An actin cytokinetic ring was detected in actin mutant at the cytokinesis stage (Figs. 1f and 3u), similar to the wild-type cells (Fig. 2p), and at this stage, the actin patches were evenly distributed in the buds and mothers of wild-type (Fig. 2p) and mutant cells (Fig. 3u).

Further difference concerning actin patches in actin mutant cells was delayed cell polarization, as evidenced by the high proportion of randomly distributed actin patches in G1 cells without buds, in contrast to the wild-type cells having high proportion of polarized actin patch distribution (Table 1).

Looking for actin cables by transmission electron microscopy

In the cytoplasm of wild-type cells (Fig. 2), the wide straight actin cables consisted of characteristic actin filaments, regularly arranged in a parallel way (Fig. 2e) pointing to the future bud (Fig. 2b) and to

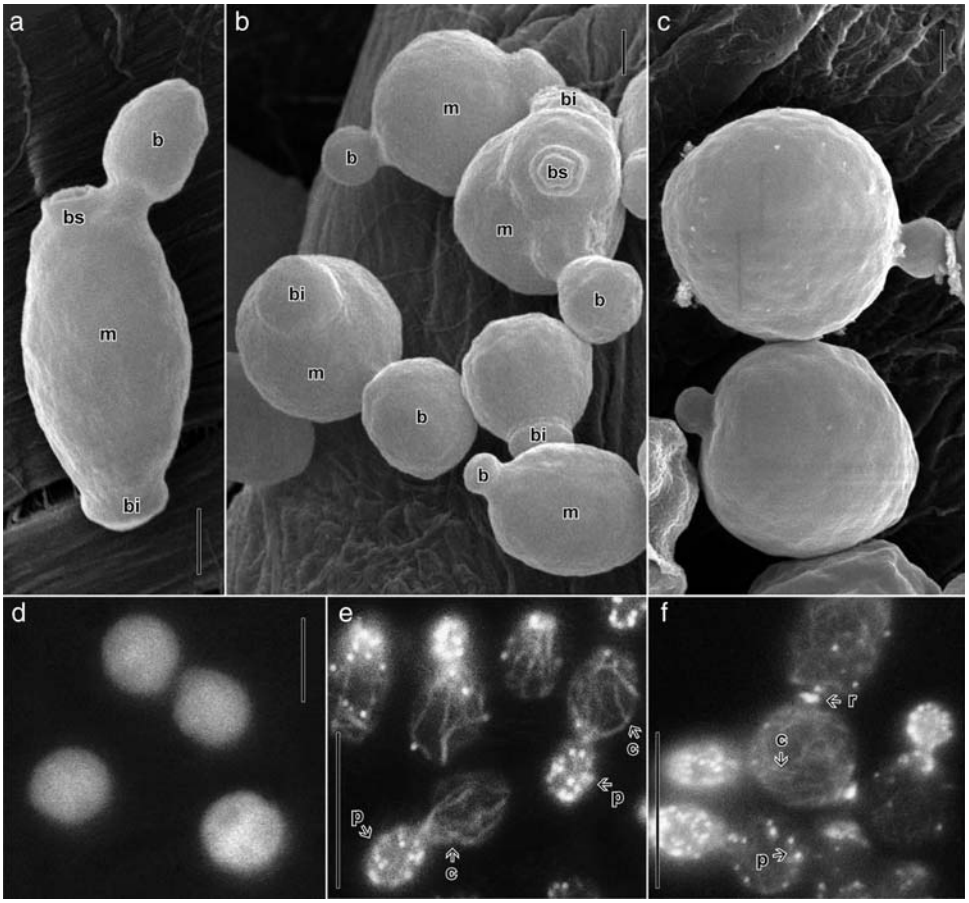

Fig. 1. Scanning electron (a–c) and fluorescent micrographs (d–f) of *S. cerevisiae*. The wild-type DBY 1690 (a) and the actin mutant DBY 1693 (b) at 23°C. The wild-type cells DBY 1690 treated with latrunculin A 100 μM for 22 h (c). Scale bar, 1 μm. m, mother; b, bud; bs, bud scar; bi, birth scar. Fluorescent micrographs of *S. cerevisiae* F-actin after staining with rhodamine–phalloidin (d–f). The wild-type DBY 1690 after treatment with latrunculin A 100 μM for 22 h (d). The wild-type DBY 1690 at 23°C (e). Actin mutant DBY 1693 at 23°C (f). Scale bar, 10 μm. p, actin patches; c, actin cables; r, actin cytokinetic ring.

the growing bud (Fig. 2g and l), where actin patches had accumulated. In more than 30 000 wild-type cells, we observed 12 actin cables by transmission electron microscopy.

When more than 30 000 actin mutant cells were examined at 10 000–40 000 magnification, only one thinner, ragged spindle-shaped filamentous bundle of irregular width (10–100 nm; Fig. 3i) was observed that strikingly differed from the straight thick actin cables of the wild-type (200 nm wide; Fig. 2e). Colocalization with a large amount of secretory vesicles could testify to a 'faint cable' of mutated actin protein Act-1p (Fig. 3i).

Freeze-fracture and freeze-substitution electron microscopy of bud development and cytoplasmic organelles in actin mutant

All the wild-type cells had a normal ultrastructure (Figs. 1 and 2). Cells had few endoplasmic reticulums and secretory vesicles near the area of bud formation (Fig. 2a) and had vacuoles and mitochondria inside the bud (Fig. 2f).

In contrast, actin mutant cells revealed a striking ultrastructural disorder, with many membrane organelles either randomly dispersed or aggregated in groups (Fig. 3). The cells had many small vesicles (100 nm in diameter) randomly scattered in the cytoplasm (Fig. 3a, e, k and o), resembling the *sec1* mutant phenotype blocked at the last stage of the secretory pathway at a restrictive temperature [46]. However, the actin mutant had random distribution of single vesicles (Fig. 3a) or their larger aggregates (Fig. 3o) even at a permissive temperature.

One striking difference observed was in the thickness of the wall of the actin mutant mothers and buds at 23°C (Fig. 3). While the wild-type cells had the same wall width of the mother and of the bud (Fig. 2) (~140 nm, $n = 30$) as that of actin mutant

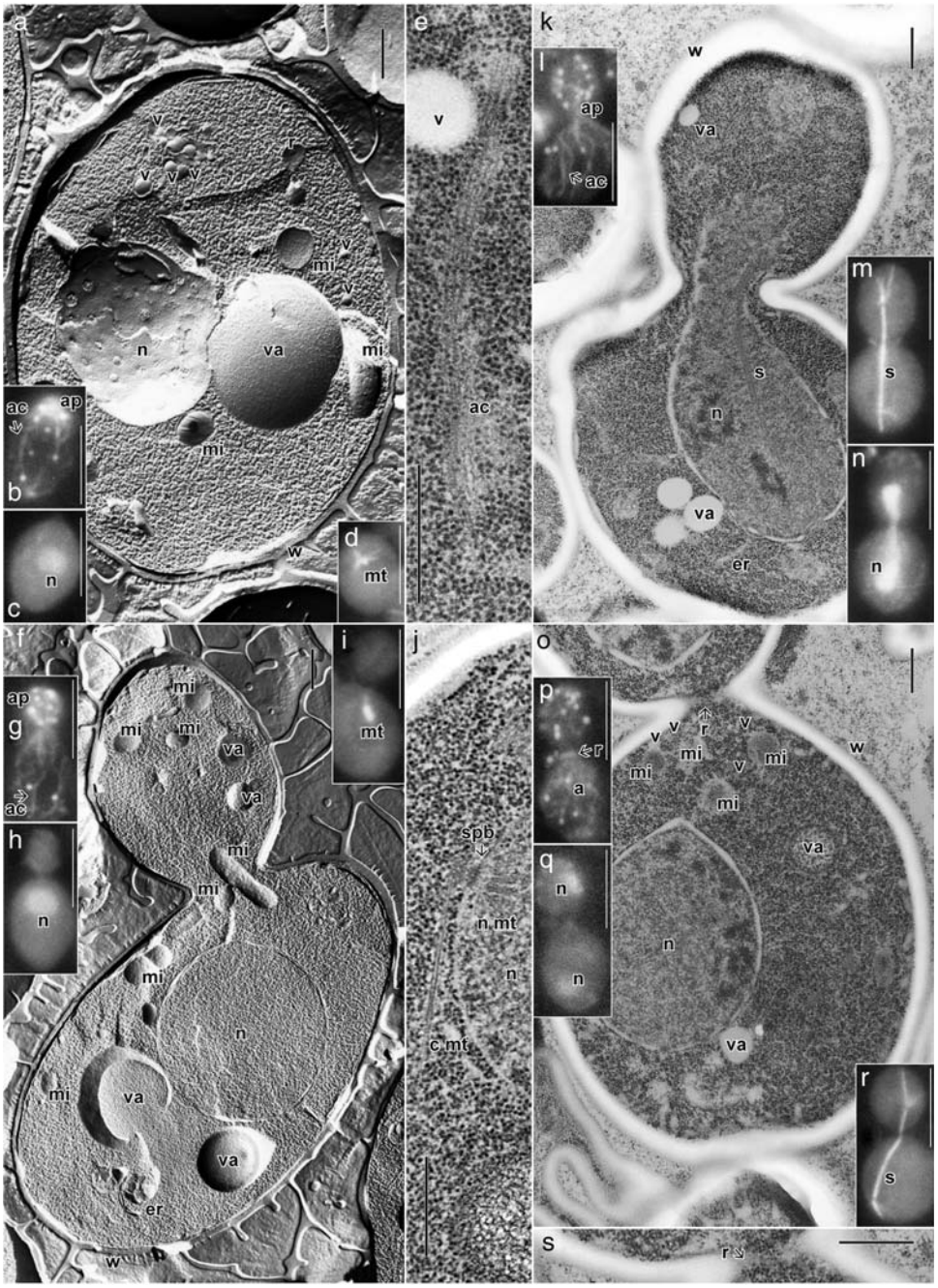

Fig. 2. Electron micrographs (a, e, f, j, k, o and s) and fluorescent micrographs (b, g, l, p, actin staining; c, h, n, q, nuclear staining; d, i, m, r, microtubule staining) of the wild-type DBY 1690 cells at 23°C. b and c, g and h, m and n, and p and q are the same cells. (a) Freeze-fractured interphase cell. (e) Ultrathin section of a freeze-substituted cell showing actin cable (ac). (f) Freeze-fractured budding cell. (j) Interphase cell with SPB and microtubules. (k) Mitotic cell, (l) actin cytoskeleton in M-phase cell, (m) mitotic spindle in M-phase cell (n). (o, s) Cells at cytokinesis. Fluorescent micrographs showing actin cytoskeleton (p), nuclei after mitosis (q), spindle (r). Scale bar, 500 nm (a, e, f, j, k, o, s); Scale bar, 5 µm (b, c, d, g, h, i, l, m, n, p, q, r). n, nucleus; mi, mitochondria; va, vacuoles; v, vesicles; er, endoplasmic reticulum; w, wall; mt, microtubules; ac, actin cables; ap, actin patches; ar, actin cytokinetic ring; s, spindle; bw, bud wall; mw, mother wall; g, Golgi apparatus; cmt, cytoplasmic microtubules; nmt, nuclear microtubules; SPB, spindle pole body (also for Fig. 3).

buds (width of ~140 nm, $n = 30$) (Fig. 3e, k and o), actin mutant mothers had thicker cell walls (Fig. 3e, k and o) reaching up to 280 nm ($n = 20$).

Mitochondria and vacuoles (Fig. 3a, e, k and o) were randomly distributed or aggregated in groups distant from the developing bud, in contrast to the wild-type cells (Fig. 2) that had mitochondria near the bud formation and near the developing septum (not shown), as well as mitochondria and vacuoles in the developing bud (Fig. 2f).

Table 1. Fluorescent microscopy of actin patches in the exponential culture of wild-type and actin mutant cells at 23°C after rhodamine–phalloidin staining in cells without buds (data from three experiments)

Strain	% of cells with random distribution of actin patches	% of cells with polarized actin patches	Total number of cells observed
Wild-type			
DBY 1690	12 ± 6	88 ± 6*	1092
Actin mutant			
DBY 1693	78 ± 10	22 ± 10*	1014

*$t = 9.558$, $P < 0.01$.

These ultrastructures indicate that positioning of secretory vesicles, mitochondria and vacuoles, and their transport to the bud are observed to depend on actin cables.

Nuclear positioning in actin mutant cells, nuclear migration to the bud neck, and nuclear division and cytokinesis

The wild-type cells (Fig. 2) had nucleus in the cell center. Cytoplasmic microtubules radiating from the spindle pole body (SPB) at the nuclear membrane were directed to the future bud and the bud discussed in this study (Fig. 2d, i and j) [47,48]. In contrast, nuclei in mutant cells along with SPBs were not in the cell center, but eccentrically positioned or had more than one nucleus (Fig. 3). Cytoplasmic microtubules radiating from the SPBs had random position and orientation (Fig. 3d, g, n, r, t and x).

Before mitosis, the wild-type nucleus migrated to the bud neck, where mitosis had started (Fig. 2). The nucleus elongated along a longitudinal cell axis (Fig. 2k and n) by the mitotic spindle (Fig. 2k, m and r) and mitosis was followed by cytokinesis. The cytokinetic ring at the bud neck (Fig. 2p) consisted of a narrow bundle of parallel filaments (Fig. 2o and s) that differed from a wide, highly ordered ring of membrane-associated filaments [49]. The actin cytokinetic ring [23,28–30] constricted the cytoplasm between the mother and the bud, followed by formation of septum. The formation of the bud always preceded nuclear migration to the bud neck, where mitosis proceeded along a longitudinal cell axis (Fig. 2k and n) resulting in the inheritance of one nucleus by each cell (Fig. 2o and q).

In contrast, in mutant cells, the nuclei that had an eccentric position in the cytoplasm of the mother' (Fig. 3a, c, e and t) failed to migrate to the bud neck (Fig. 3k) and failed to direct to bud nuclear divisions that started in these abnormal positions, and also had a transversal alignment (Fig. 3k and m). Mitotic spindles elongated randomly and were also transversally oriented (Fig. 3n). Nucleus in some cells could reach to the bud, while in other cells, both the nuclei remained in the cytoplasm of the mother' with anucleated buds (Fig. 3o, p and r) and cytokinesis did not occur in these cells.

Random positioning of the nucleus, SPB and cytoplasmic microtubules in the mutant cells that did not direct to the bud neck and to the bud tip indicates that the positioning of nuclei, SPB and cytoplasmic microtubules require actin cables. In addition, nuclear migration to the bud neck and nuclear division along the longitudinal cell axis also require normal actin cables.

Uncoupling of the nuclear division from bud development and nuclear migration in actin mutants

It is known that passing Start in *S. cerevisiae* triggers multiple cell-cycle events, including: (i) initiation of DNA replication, (ii) bud emergence and (iii) duplication of SPB by Cln/Cdc28. Normally, bipolar spindle formed in wild-type cells, when the bud diameter reached about one-fourth of the mother-cell diameter [48,50]. In contrast, in some actin mutant cells, the nuclear microtubules (Fig. 3s and t) separated after SPB duplication, and formation of short bipolar spindles (Fig. 3d) occurred before the emergence of bud. This indicates delay of bud emergence in some actin mutant cells in relation to SPB duplication, separation, spindle formation and anaphase elongation, sometimes in an eccentric position (Fig. 3a, c and d). One cause for this delay of bud emergence can be delayed polarization in the actin mutant cells, as evidenced by the high proportion of randomly distributed actin patches in G1 actin mutant cells, in contrast to the wild-type cells having the high proportion of polarized actin patch distribution (Table 1). Delayed cell polarization can cause delayed bud initiation, and faint actin cables can delay bud growth. Mutant cells with a small bud

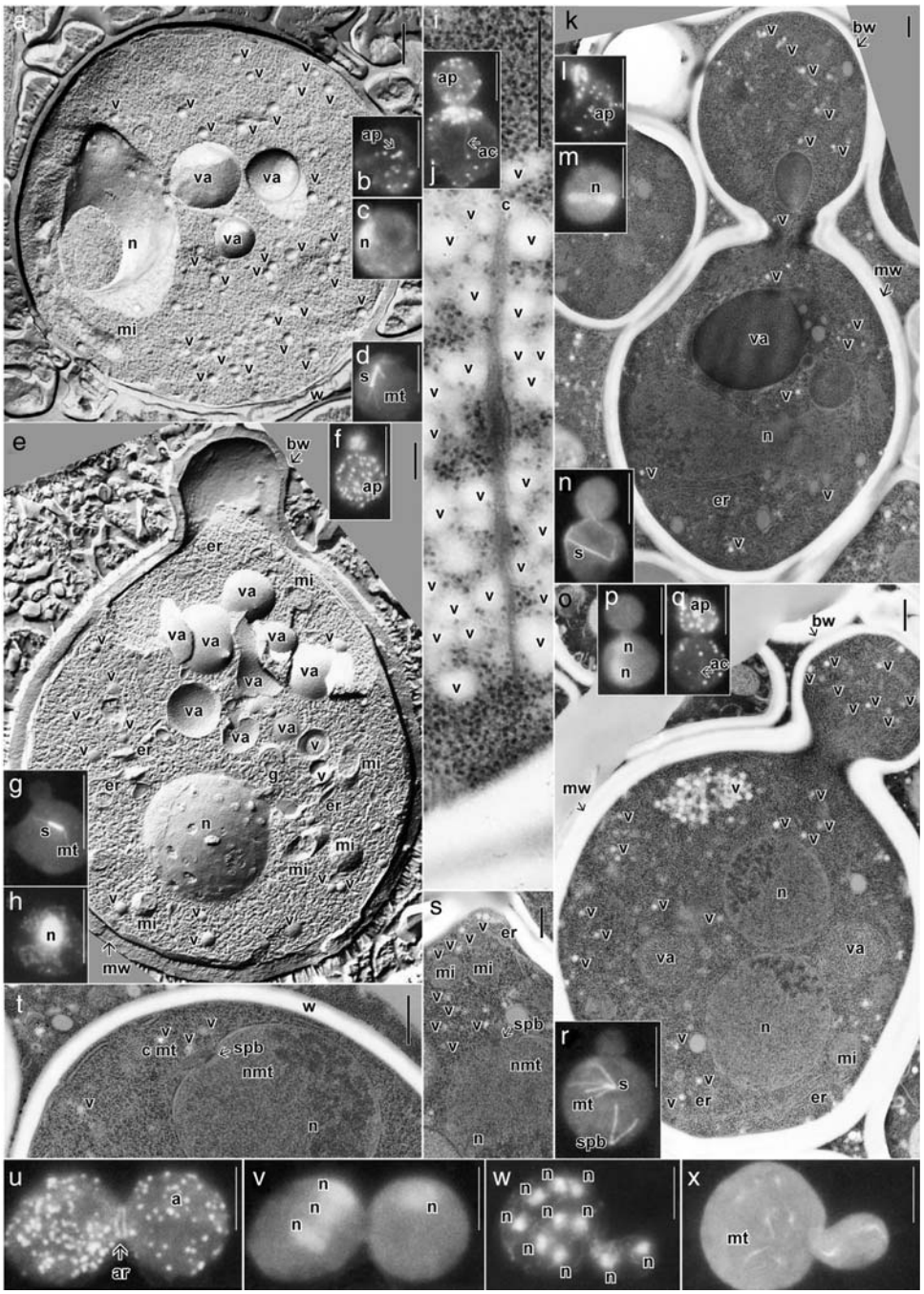

Fig. 3. Transmission electron micrographs (a, e, i, k, o, s, t) and fluorescent micrographs (b, f, j, l, q, u, actin staining with rh-ph; c, h, m, p, v, w, nuclear staining with DAPI; d, g, n, r, x, microtubule staining with TAT1 antitubulin antibody) of actin mutant DBY 1693 cells at 23°C. (a) Freeze-fractured interphase cell with eccentrically located nucleus (n), many small vesicles (v), vacuoles (va), mitochondria (mi). (b) Actin patches (ap), (c) nucleus (n), (d) spindle (s) and microtubules (mt). (e) Freeze-fractured cell with small bud, nucleus (n), vesicles (v), mitochondria (mi), vacuoles (va); Golgi apparatus (g); endoplasmic reticulum (er). (f) Actin patches (ap), (g) spindle (s) and microtubules (mt), (h) nucleus (n). (i) Ultrathin section showing one 'faint' cable (c) associated with many vesicles (v). (j) 'Faint' actin cable (ac) in mother (arrow), and actin patches (ap) in the bud and the neck. (k) Ultrathin section at M-phase cell with transversally elongated mitotic nucleus (n) in mother cell. (l) Polarized actin patches (ap); (m) transversally elongated mitotic nucleus (n); (n) transverse mitotic spindle (s). (o) Actin mutant cell with two-nuclei (n) and small anucleated bud. (p) Two nuclei in mother; (q) polarized actin patches with one 'faint' actin cable (ac). (r) Two groups of microtubules (mt). (s) Interphase cell initiating bud formation. SPB at nuclear membrane and microtubules. (t) Eccentric nucleus and cytoplasmic (c mt) and nuclear (n mt) microtubules and SPB. Scale bar, 500 nm (a, e, i, k, o, s, t). Scale bar, 5 µm (b, c, d, f, g, h, j, l, m, n, p, q, r, u, v, w, x). (u) Actin patches and two actin cytokinetic rings, (v) the same cell has four nuclei; (w) eight nuclei in the mother, and two nuclei in the bud, (x) 10 SPB in the same cell as w.

can contain already elongating mitotic nucleus, which can have an oblique or transversal alignment (Fig. 3m and k), instead of that along a polarity axis, similar to the wild-type. This can explain our electron microscopic photographs of two-nucleated mutant mother cells (Fig. 3o).

Other cells also exhibited delay of bud emergence in relation to the nuclear cycle in the mutant population: (i) two-nucleated mother cells with one small bud (Fig. 3p, o and r); (ii) three-nucleated mother cells and one-nucleated buds (Fig. 3v); or (iii) further uncoupling of nuclear cycles from bud formation, leading to 6–10 visible nuclei in cells having one bud only (Fig. 3w). Fluorescence microscopy thus showed 2–10 nuclei in the mother cell with one bud (Fig. 3p and w). To determine the frequency of multinucleated actin mutant cells at 23°C, we counted the nuclei after DAPI staining by fluorescent microscopy in *act1-1* population. We found about 25% of multinucleated cells in actin mutant after 20 h of cultivation ($n = 300$), while wild-type had <1% of multinucleated cells ($n = 300$). This indicates delay in budding with respect to nuclear division in about one-fourth of the mutant cells that become multinucleated.

Discussion

Actin mutant cells have aberrant faint actin cables at a permissive temperature that affect actin-cable functions in budding yeast

Our electron microscopy of the wild-type cells showed actin cables with a width of 200 nm, which were also clearly visible under a fluorescent microscope. In contrast, the actin mutant cells contained faint actin cables hardly visible via fluorescent microscopy (Figs. 1f and 3j and q). Under electron microscopy, 10–100-nm wide bundles could be found in actin mutant *act1-1* (Fig. 3i), which might be the 'faint actin cable': its thinner width can explain the difficulty in observing faint actin cables by fluorescence microscopy. However, how to explain the differences in thickness and appearance? After F-actin production by formins Bni1p and Bnr1p at the bud and neck [51], it was observed that mutated Act1-1p cannot interact with the bundling protein fimbrin [52,53] that normally stabilizes actin filaments into regular, tightly packed bundles that protect F-actin filaments from depolymerization [54]. Such cables should be present in the wild-type, whereas mutated Act1-1p filaments, probably only randomly associated with the aberrant loose filamentous bundles, can be unstable and depolymerized. This is also in accordance with the biochemical study of fimbrin mutants that had unstable actin filaments in the absence of fimbrin [55]. Using electron microscopy, we found only one 'faint bundle' in about 30 000 actin mutant cells, whereas 12 normal actin cables were in the same number of wild-type cells. This indicates that mutant cells may have 10 times less number of 'actin filament bundles'.

Many researchers (e.g. [56,57]) have studied actin cables. In this study, we have shown that 'faint actin bundles' in actin mutant *act1-1* cause randomly located nuclei, SPB, microtubules, spindles, delayed bud growth, lack of nuclear migration to the bud neck and cytoplasmic chaos in cell organelles at a permissive temperature (Table 2).

Previously, we proved that the vesicle vectorial movement from the mother to the bud requires actin cables, and the correct spatial positioning and orientation of the complete secretory pathway to the bud proceeds along the actin cables [31]. In contrast, 'faint mutant actin bundles' failed in directing the vesicles to the emerging bud and the stray vesicles delivered their content to the mother wall, which became thicker, instead to the bud. This delayed bud initiation and bud growth in relation to nuclear division initiation (see later).

Table 2. A variety of defects in *ts* actin mutant cells *act1–1* identified by light, fluorescent, transmission and scanning electron microscopy after 20 h cultivation at 23°C

1	Aberrant 'faint actin bundles'
2	Delay of polarization of actin patches (see Table 1)
3	Delay of bud growth
4	Aberrant positioning of nuclei
5	Aberrant localization of spindle pole bodies
6	Aberrant location of cytoplasmic microtubules
7	Aberrant arrangement of mitotic spindles
8	Failure of nuclear migration to the bud neck
9	Cytoplasmic chaos in cell organelles (disordered localizations of mitochondria, vacuoles, secretory vesicles, cisternae of endoplasmic reticulum, Golgi apparatuses)
10	Thickening of mother cell walls than bud walls
11	Abnormal spherical shape of mutant cells
12	Multinucleation of mother cells with one bud

It is known that ellipsoidal shape of *S. cerevisiae* cells is the result of combined polarized apical growth and non-polarized isotropic growth. Owing to 'faint mutant actin cables', polarized apical growth in the mutant is deficient, whereas isotropic growth predominates and makes actin mutant cells spherical.

Fluorescent microscopy of other actin mutants proved actin cables are necessary for the movement of mitochondria and vacuoles [14,16,17]. Mitochondria – the main producers of adenosine tri-phosphate – are apparently required for the formation of bud and for the development of septum; their absence here can delay bud emergence, bud growth and septation. This can also contribute to delayed bud formation and growth in *act1-1* cells.

Our ultrastructural data confirm that the correct spatial positioning and movement of membrane organelles from the mother to the bud require actin cables. In actin mutants having 'faint actin bundles', these events are chaotic, delayed and mislocalized, similar to the situation when a highway is blocked and only collateral transport along narrow ways can proceed slowly.

Actin-dependent nuclear positioning in budding yeast

Nuclear positioning mechanism based on actin filaments was recently suggested in plant and animal cells [58]. We presume that such mechanism should also function in budding yeast, because actin mutants with faint actin bundles have seriously affected nuclear positioning – random and eccentric interphase nuclear position, as well as random position and orientation of mitotic nucleus leading to multinucleated cell (Fig. 3). We suggest that nuclear positioning in budding yeast is based on actin cables that are required for the correct central positioning of nucleus before mitosis, during mitosis and after mitosis for nuclear genome inheritance by the dividing cells.

Actin cables and spindle positioning and movement to the bud neck in budding yeast

Fluorescent microscopy showed the principal role of astral microtubules in yeast mitosis: they are required to establish the position of spindles and orientate the spindles to the bud neck before anaphase for subsequent separation of chromosomes [59–61]. The lack of astral microtubules caused by β-tubulin mutation led to block of spindle movement to the bud neck [59–61]. However, other fluorescence microscopic studies [32–34] observed incorrectly positioned spindles at improper actin function, followed by many others reporting, using fluorescence microscopy that pre-anaphase spindle position and orientation requires microtubule translocation along actin cables by Myo2, in addition to cortically located dynein-dependent sliding that directs microtubule ends to the bud. Bud–tip attachment of the microtubules radiated from old SPB at G1 ensures later proper spindle positioning that is necessary for equal segregation of the genome to mother and daughter cells. Dynein at the bud cortex provides vector for spindle orientation via cortical anchors to generate pulling forces for spindle movement in nuclear division [18,37,38,62]. Spindle movement to the bud neck is known to use two pathways: (i) microtubules-Bim1-Kar9-Myo2 pre-anaphase movement along the actin cables to the bud neck, and (ii) microtubule-dynein-dynactin movement in anaphase and after it (see [35,37,38,62–64] and many others). These two pathways normally cooperate in nuclear migration; however, in actin mutants, the microtubule – 'faint actin cable' – cooperation in nuclear migration to the bud neck could be affected, as actin pathway is deficient.

Our ultrastructural study confirms that spindle movement to the bud neck requires actin cables. What can be the primary defect in actin mutant cells at the permissive temperature? The primary defect can be delayed mutant cell polarization attributable to random distribution and poor organization of actin. Insufficient polarization might then corrupt bud-site selection, preventing or delaying bud initiation in relation to mitosis initiation. If the budding site is not defined in time, then it cannot orient spindles in time. In such an 'apolar' cell, the spindle can elongate in any possible direction. The evidence for delayed mutant cell polarization is the high proportion of randomly distributed actin patches in G1 cells, in contrast to the wild-type having high proportion of asymmetric actin patch distribution (Table I).

Can cell-cycle checkpoints function normally in actin mutant cells?

The 'morphogenetic checkpoint'. Actin mutant bud emergence delayed in relation to mitosis suggests that the actin mutant cannot have normally functioning 'morphogenetic checkpoint' that should maintain coordination between the bud formation and nuclear event [65,66], because the actin mutant cells have two-nucleated mothers and anucleated buds.

The 'mitotic actin checkpoint'. This blocks mitosis when an incorrectly oriented spindle occurs in the fission yeast [67–69] and cannot function in the actin mutants of budding yeast cells, in which incorrectly oriented mitoses continue in cycling cells (Fig. 3k, m and n) that become multinucleated (Fig. 3p, o, v and w).

The 'spindle position/orientation checkpoint' is normally linked to the 'spindle assembly checkpoint' [67–70] and must be disrupted in the actin mutants, because mitosis proceeds, but with incorrectly oriented nuclei and spindles (Fig. 3k, m and n).

Mitotic exit network. In the wild-type cells, the nucleus migrates before mitosis to the bud neck, and SPB containing Tem1p-GDP enters the bud body, where the protein Lte1p activates Tem1p-GDP to Tem1p-GTP and triggers the pathway of mitotic exit and release of Cdc14 phosphatase from the nucleolus after mitosis, which enables the inactivation of mitotic kinase and the start of cytokinesis [36]. When the mitotic spindle in actin mutant is not positioned at the bud neck and mitosis proceeds in the mother's cytoplasm (Fig. 3k, m and n), SPB cannot enter the bud, the mitotic exit network is kept inactive, the mitotic kinase cannot be inactivated, the actin ring cannot be formed and cytokinesis cannot proceed.

If all of these checkpoints actually exist in the budding yeast cell division cycle, but do not function in the actin mutants, they need actin cables for their normal function.

Actin-dependent pathway and microtubule-dependent pathway that uncouple in actin mutant *act1-1* could correspond to two dependent pathways in the cell division cycle of budding yeast observed in studies of *cdc* mutants by Hartwell *et al.* [71] (Fig. 4). Aberrant 'faint actin cables' in actin mutant *act1-1* enabled us to identify 'actin-dependent cytoplasmic pathway of bud development and organelle positioning and inheritance' and 'microtubule-dependent pathway' of nuclear genome and SPB segregation and inheritance. We hypothesize that our 'actin-dependent pathway of bud development and organelle positioning and inheritance' correspond to the external dependent pathway in the cell division cycle of budding yeast (Fig. 4), which includes bud emergence (BE) and nuclear migration (NM), and converging at cytokinesis (CK) with inner dependent pathway that includes initiation of DNA synthesis (iDS), DNA synthesis (DS), nuclear division at the middle stage (mND) and at the late stage (lND) [71], i.e. our microtubule-dependent nuclear division pathway of genome and SPB segregation and inheritance or at least its nuclear division part. Nuclear migration to the bud neck depends on combined cooperation of 'actin pathway' and 'microtubule pathway'; however, these pathways uncouple in ~25% of mutant cells that become multinucleated.

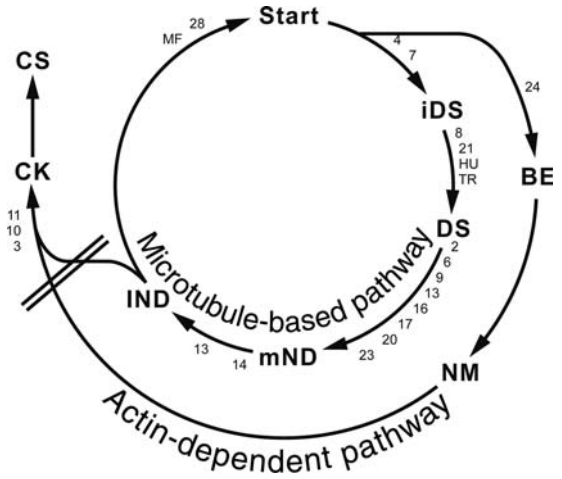

Fig. 4. Diagram of two Hartwell's pathways in the cell division cycle of budding yeast [71], and our two cytoskeleton pathways functioning in the cell cycle and dissociated in actin mutant, as discussed in the text. The 'actin-dependent pathway' and 'microtubule-based pathway' demonstrated here should correspond to two dependent pathways in the cell division cycle of budding yeast discovered by Hartwell *et al.* [71]. The first pathway (outer circle) that includes bud emergence (BE) and nuclear migration (NM) correspond to our 'actin-dependent pathway', whereas the second Hartwell pathway including DNA replication (iDS, DS) and nuclear division (mND, lND) (inner circle) corresponds to our 'microtubule-dependent nuclear pathway'.

In summary, cell division pathway of budding yeast includes actin-dependent pathway proceeding from the mother to the bud, which is necessary for: (i) cell polarity establishment; (ii) bud development; (iii) central positioning of nucleus and cytoplasmic microtubules radiating from SPB to join future bud

site; (iv) correct positioning and movement of membrane organelles (secretory vesicles, mitochondria and vacuoles) to the bud site assembly and to emerging bud for inheritance of corresponding part of cytoplasm and cell organelles; (v) nuclear migration to the bud neck (in cooperation with microtubules), correct SPB and cytoplasmic and spindle microtubule position, alignment, orientation and nuclear division along longitudinal cell axis and distribution of one nucleus and SPB to each cell; (vi) functioning of cell-cycle checkpoints; (vii) cytokinesis and septum formation; and (viii) coordination and integration of cell-cycle events in time and space to ensure cell integrity.

Concluding remarks

Our ultrastructural study identified 'actin-dependent pathway of bud development and organelle positioning and inheritance', and 'microtubule-dependent nuclear division pathway of nuclear genome and SPB segregation and inheritance'. Actin and microtubule pathways cooperate during nuclear migration to the bud neck, but this cooperation is disrupted in space and time in about one-fourth of 'seriously ill' mutant cells that become multinucleated even at 23°C – apparently –23°C is not fully 'permissive' for these cells. These two cytoskeleton-dependent pathways can correspond to two dependent pathways in the cell division cycle of budding yeast, which were observed 37 years before in studies of cdc mutants [70].

Funding

This work was financially supported by the Japanese Ministry of Education, Science and Culture to M.K. while she was a Guest Professor at Chiba University, Research Centre for Pathogenic Fungi and Microbial Toxicoses, Japan in 1998–1999, where part of this work was done and also supported by Grant Agency of the Czech Republic grant number 310/06/0605.

Acknowledgements

We thank Prof. David Botstein and Dr Paula Grisafi for kindly providing yeast strains and Prof. Keith Gull for providing the TAT1 antibody. We also thank Dr Ladislav Ilkovics, Vladimíra Ramíková, Věra, Trávníčková, Věra Vinařická, Jan Krobauer and, especially, Jan Šlancar for their excellent technical help.

References

1 Botstein D, Amberg D, Huffaker T, Mulholand J, Adams A, Drubin D, and Stearns T (1997) The yeast cytoskeleton. In: Broach J R, Pringle J R, and Jones E W (eds.), *The Molecular and Cellular Biology of the Yeast* Saccharomyces, pp 1–90 (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, USA).

2 Ayscough K R, Stryker J, Pokala N, Sanders M, Crews P, and Drubin D G (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 inhbitor Latrunculin-A. *J. Cell Biol.* **137**: 399–416.

3 Kopecká M and Gabriel M (2009) Microtubules and actin cytoskeleton of potentially pathogenic basidiomycetous yeast as targets for antifungals. *Chemotherapy* **55**: 278–286.doi:10.1159/000224655.

4 Kopecká M, Ilkovics L, Ramíková V, and Yamaguchi M (2010) Effect of cytoskeleton inhibitors on conidiogenesis and capsule in the long neck yeast Fellomyces examined by scanning electron microscopy. *Chemotherapy* **56**: 197–202. doi:10.1159/000316330.

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

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

7 Novick P and Botstein D (1985) Phenotypic analysis of temperature-sensitive yeast actin mutants. *Cell* **40**: 405–406.

8 Kaksonen M, Toret C P, and Drubin D G (2006) Harnessing actin dynamics for clathrin-mediated endocytosis. *Nat. Rev. Mol. Cell Biol.* **7**: 404–414.

9 Drubin D G (1991) Development of cell polarity in budding yeast. *Cell* **65**: 1093–1096.

10 Pruyne D, Legesse-Miller A, Gao L, Dong Y, and Bretscher A (2004) Mechanisms of polarized growth and organelle segregation in yeast. *Ann. Rev. Cell Dev. Biol.* **20**: 559–591.

11 Irazoqui J E and Lew D J (2004) Polarity establishment in yeast. *J. Cell Sci.* **117**: 2169–2171.

12 Slaughter B D, Smith S E, and Li R (2009) Symmetry breaking in the life cycle of the budding yeast. *Cold Spring Harb. Perspect Biol.* **1**: a003384.

13 Johnston G C, Prendergast J A, and Singer R A (1991) The *Saccharomyces cerevisiae MYO2* gene encodes an essential myosin for vectorial transport of vesicles. *J. Cell Biol.* **113**: 539–551.

14 Drubin D G, Jones H D, and Wertman K F (1993) Actin structure and function: roles in mitochondrial organization and morphogenesis in budding yeast and identification of the phalloidin-binding site. *Mol. Biol. Cell* **4**: 1277–1294.

15 Lazzarino D, Boldogh I, Smith M G, Rosand J, and Pon L A (1994) Yeast mitochondria contain ATP-sensitive, reversible actin-binding activity. *Mol. Biol. Cell* **5**: 807–818.

16 Simon V R, Swayne T C, and Pon L A (1995) Actin-dependent mitochondrial motility in mitotic yeast and cell-free systems: identification of a motor activity on the mitochondrial surface. *J. Cell Biol.* **130**: 345–354.

17 Hill K L, Catlet N L, and Weisman L S (1996) Actin and myosin function in directed vacuole movement during cell division in *Saccharomyces cerevisiae*. *J. Cell Biol.* **135**: 1535–1549.

18 Bretscher A (2003) Polarized growth and organelle segregation in yeast: the tracks, motors and receptors. *J. Cell Biol.* **160**: 811–816.

19 Moseley J B and Goode B L (2006) The yeast actin cytoskeleton: from cellular function to biochemical mechanism. *Microbiol. Mol. Biol. Rev.* **70**: 605–645.

20 Park O and Bi E (2007) Central roles of small GTPases in the development of cell polarity in yeast and beyond. *Microbiol. Mol. Biol. Rev.* **71**: 48–96. doi:10.1128/MMBR.00028-06.

21 Gabriel M and Kopecká M (1995) Disruption of the actin cytoskeleton in budding yeast results in formation of an aberrant cell wall. *Microbiol. SGM* **141**: 891–899.

22 Gabriel M, Svoboda A, and Kopecká M (1992) Structural rearrangement of the actin cytoskeleton in regenerating protoplasts of budding yeast. *J. Gen. Microbiol. SGM* **138**: 2229–2234.

23 Gabriel M, Horký D, Svoboda A, and Kopecká M (1998) Cytochalasin D interferes with contractile actin ring and septum formation in *Schizosaccharomyces japonicus* var. *versatilis*. *Microbiol. SGM* **144**: 2331–2344.

24 Kobori H, Yamada N, Takai A, and Osumi M (1989) Actin is associated with the formation of the cell wall in reverting protoplasts of the fission yeast *Schizosaccharomyces pombe*. *J. Cell Sci.* **94**: 635–646.

25 Kopecká M and Gabriel M (1995) Actin cortical cytoskeleton and cell wall synthesis in regenerating protoplasts of the *Saccharomyces cerevisiae* actin mutant DBY 1693. *Microbiol. SGM* **141**: 1289–1299.

26 Qadota H, Python C P, Innoue S B, Arisawa M, Anraku Y, Zheng Y, Watanabe T, Levin D E, and Ohya Y (1996) Identification of yeast Rho1p GTPase as a regulatory subunit of 1,3-β-glucan synthase. *Science* **272**: 279–281.

27 Utsugi T, Minemura M, Hirata A, Abe M, Watanabe D, and Ohya Y (2002) Movement of yeast 1,3-beta-glucan synthase is essential for uniform wall synthesis. *Genes Cells* **1**: 1–9.

28 Bi E, Maddox P, Lew D J, Salmon E D, McMillan J N, Yeh E, and Pringle J R (1998) Involvement of an actomyosin contractile ring in *Saccharomyces cerevisiae* cytokinesis. *J. Cell Biol.* **142**: 1301–1312.

29 Lippincott J and Li R (1998) Dual function of Cyk2, a cdc15/PSTPIP family protein, in regulating actomyosin ring dynamics and septin distribution. *J. Cell Biol.* **143**: 1947–1960.

30 Balasubramanian M K, Bi E, and Glotzer M (2004) Comparative analysis of cytokinesis in budding yeast, fission yeast and animal cells. *Curr. Biol.* **14**: R806–R818.

31 Yamaguchi M and Kopecká M (2010) Ultrastructural disorder of the secretory pathway in temperature-sensitive actin mutants of *Saccharomyces cerevisiae*. *J. Electron Microsc.* **59**: 141–152. doi:10.1093/jmicro/dfp050

32 Gabriel M and Kopecká M (1992) A single mutation in the actin gene can dissociate nuclear division from the cytokinesis in the cell cycle of budding yeasts. *Yeast* **8**: S357.

33 Palmer R E, Sullivan D S, Huffaker T, and Koshland D (1992) Role of astral microtubules and actin in spindle orientation and migration in budding yeast, *Saccharomyces cerevisiae*. *J. Cell Biol.* **119**: 583–593.

34 Kopecká M and Gabriel M (1998) Aberrant positioning of nuclei and the microtubular cytoskeleton in *Saccharomyces cerevisiae* due to improper actin function. *Microbiol. SGM* **144**: 1783–1797.

35 Winsor B and Schiebel E (1997) Review: an overview of the *Saccharomyces cerevisiae* microtubule and microfilament cytoskeleton. *Yeast* **13**: 399–434.

36 Pereira G and Schiebel E (2001) The role of the yeast spindle pole body and the mammalian centrosome in regulating late mitotic events. *Curr. Opin. Cell Biol.* **13**: 762–769.

37 Kusch J, Liakopoulos D, and Barral Y (2003) Spindle asymmetry: a compass for the cell. *Trends Cell Biol.* **13**: 562–569.

38 Pearson C G and Bloom K (2004) Dynamic microtubules lead the way for spindle positioning. *Nat. Rev. Mol. Cell Biol.* **5**: 481–492.

39 Fagarasanu A and Rachubinski R (2007) Orchestrating organelle inheritance in *Saccharomyces cerevisiae*. *Curr. Opin. Microbiol.* **10**: 528–538.

40 Shortle D, Novick P, and Botstein D (1984) Construction and genetic characterization of temperature-sensitive alleles of the yeast actin gene. *Proc. Natl Acad. Sci. USA* **81**: 4889–4893.

41 Gabriel M, Kopecká M, Yamaguchi M, Svoboda A, Takeo K, Yoshida S, Ohkusu M, Sugita T, and Nakase T (2006) Cytoskeleton in the unique cell reproduction by conidiogenesis of the long neck yeast *Fellomyces (Sterigmatomyces) fuzhouensis*. *Protoplasma* **229**: 33–44.

42 Moor H and Mühlethaler K (1963) Fine structure in frozen-etched yeast cells. *J. Cell Biol.* **17**: 609–628.

43 Yamaguchi M, Okada H, and Namiki Y (2009) Smart specimen preparation for freeze-substitution and serial ultrathin sectioning of yeast cells. *J. Electron Microsc.* **58**: 261–266.

44 Yamaguchi M, Shimizu M, Yamaguchi T, Ohkusu M, and Kawamoto S (2005) Repeated use of uranyl acetate solution for section staining in transmission electron microscopy. *Plant Morphol.* **17**: 57–59.

45 Yamaguchi M, Namiki Y, Okada H, Mori Y, Furukawa H, Wang J, Ohkusu M, and Kawamoto S (2011) Structome of *Saccharomyces cerevisiae* determined by freeze-substitution and serial ultrathin sectioning electron microscopy. *J. Electron Microsc.*, doi: 10.1093/jmicro/dfr052.

46 Schekman R and Novick P (1982) The secretory process and yeast cell-surface assembly. In: Strathern J N, Jones E W, and Broach J E (eds.), *The Molecular Biology of the Yeast* Saccharomyces cerevisiae*: Metabolism and Gene Expression*, **Vol. II**, pp 361–398 (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, USA).

47 Byers B (1981) Cytology of the yeast life cycle. In: *Molecular Biology of the Yeast* Saccharomyces*: Life Cycle and Inheritance*, pp 59–96 (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, USA).

48 Pringle J R and Hartwell L H (1981) The *Saccharomyces cerevisiae* cell cycle. In: *Molecular Biology of the Yeast* Saccharomyces*: Life Cycle and Inheritance*, pp 97–142 (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, USA).

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

50 Lew D J, Weinert T, and Pringle J R (1997) Cell cycle control in *Saccharomyces cerevisiae*. In: Broach J R, Pringle J R, and Jones E W (eds.), *The Molecular and Cellular Biology of the Yeast* Saccharomyces, pp 607–695 (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, USA).

51 Watanabe N and Higashida C H (2004) Formins: processive cappers of growing actin filaments. *Exp. Cell Res.* **301**: 16–22.

52 Adams A E, Botstein D, and Drubin D G (1989) A yeast actin-binding protein is encoded by *SAC6*, a gene found by suppression of an actin mutation. *Science* **243**: 231–233.

53 Adams A E, Botstein D, and Drubin D G (1991) Requirement of yeast fimbrin for actin organization and morphogenesis *in vivo*. *Nature* **354**: 404–408.

54 McCurdy D W and Staiger Ch (2000) Fimbrin. In: Staiger C J, Baluška F, Volkmann D, and Barlow P W (eds.), *Actin: A Dynamic Framework for Multiple Plant Cell Functions*, **Vol. 49**, pp 87–102 (Kluwer Academic Publishers, Dordrecht, Boston, London).

55 Karpova T S, Tatchel K, and Cooper J A (1995) Actin filaments in yeast are unstable in the absence of capping protein and fimbrin. *J. Cell Biol.* **131**: 1483–1493.

56 Yang H C H and Pon L A (2002) Actin cable dynamics in budding yeast. *Proc. Natl Acad. Sci. USA* **99**: 751–756.

57 Sagot I, Klee S K, and Pellman D (2002) Yeast formins regulate cell polarity by controlling the assembly of actin cables. *Nat. Cell Biol.* **4**: 42–50.

58 Starr D A and Han M (2003) ANChors away: an actin based mechanism of nuclear positioning. *J. Cell Sci.* **116**: 211–21664.

59 Huffaker T C, Thomas J H, and Botstein D (1988) Diverse effects of β-tubulin mutations in microtubule formation and function. *J. Cell Biol.* **106**: 1997–2010.

60 Jacobs C H W, Adams A E M, Szanislo P J, and Pringle J R (1988) Functions of microtubules in *Saccharomyces cerevisiae*. *J. Cell Biol.* **107**: 1409–1426.

61 Sullivan D S and Huffaker T C (1992) Astral microtubules are not required for anaphase B in *Saccharomyces cerevisiae*. *J. Cell Biol.* **119**: 379–388.

62 Hwang E, Kusch J, Barral Y, and Huffaker T C (2003) Spindle orientation in *Saccharomyces cerevisiae* depends on the transport of microtubule ends along polarized actin cables. *J. Cell Biol.* **16**: 483–488.

63 Huisman S M and Segal M (2005) Cortical capture of microtubules and spindle polarity in budding yeast – where's the catch? *J. Cell Sci.* **118**: 463–471.

64 Segal M and Bloom K (2001) Control of spindle polarity and orientation in *Saccharomyces cerevisiae*. *Trends Cell Biol.* **11**: 160–166.

65 McMillan J N, Sia R A L, and Lew D J (1998) A morphogenetic checkpoint monitors the actin cytoskeleton in yeast. *J. Cell Biol.* **142**: 1487–1499.

66 Keaton M A and Lew D J (2006) Eavesdropping on the cytoskeleton: progress and controversy in the yeast morphogenesis checkpoint. *Curr. Opin. Microbiol.* **9**: 540–546.

67 Nakaseko Y and Yanagida M (2001) Cell biology: cytoskeleton in the cell cycle. *Nature* **412**: 291–292.

68 Gachet Y, Tournier S, Millar J B A, and Hyams J (2001) A MAP kinase-dependent actin checkpoint ensures proper spindle orientation in fission yeast. *Nature* **412**: 352–355.

69 Gachet Y, Reyes C, Gildstone S, and Tournier S (2006) The fission yeast spindle orientation checkpoint: a model that generates tension? *Yeast* **23**: 1015–1029.

70 Hoyt M A (2000) Exit from mitosis: spindle pole power. *Cell* **102**: 267–270.

71 Hartwell L H, Culotii J, Pringle J R, and Reid B J (1974) Genetic control of the cell division cycle in yeast. *Science* **183**: 46–51.