

What Are the Basic Functions of Microfilaments? Insights from Studies in Budding Yeast

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TWENTY-FIVE years ago, F-actin was discovered to be the major component of microfilaments in animal cells (36). Since then, actin has been found in virtually all eukaryotic cells. What functions do microfilaments perform? Studies in animal cells have provided us with a picture of F-actin as the backbone of many structurally and functionally diverse assemblies coexisting within any given cell. Microfilaments are important for cell shape determination, cell motility, and various contractile activities, as well as for participating in aspects of transmembrane signaling, endocytosis, and perhaps secretion. Since some actin-binding proteins are regulated by changes in free Ca^{2+} , phospholipids, and by phosphorylation (75), and microfilament organizations can be modulated by small G-proteins (69, 70), microfilaments seem to be under exquisite control. Moreover, recent discoveries that some actin-binding proteins contain SH2 and SH3 domains (56) suggest that microfilaments are integrated into protein-protein signaling pathways. Given these diverse and sophisticated regulatory systems, the gap in our knowledge of the precise functions performed by microfilaments is all the more distressing.

The budding yeast *Saccharomyces cerevisiae* has recently become very popular for studying the function of microfilaments. Why? Yeast cells are nonmotile and they have a rigid cell wall, do not change shape rapidly, and have no obvious surface structures. Therefore, some of the roles that microfilaments play in vertebrate cells are not seen in yeast, yet microfilaments are vital because yeast contains a single essential actin gene (73). The regulation of F-actin distribution during the cell cycle in yeast was the first indication that microfilaments might play a role in cellular morphogenesis (41); that is, in the targeting of secretory vesicles for the assembly of the daughter cell rather than providing an infrastructure for it. This immediately raised a number of questions: how is the distribution of F-actin determined, what are the components that make up these microfilamentous structures, and what are their functions? The relative ease of genetic approaches for the identification and analysis of functionally related components, together with the near religious belief that what is true for *S. cerevisiae* is also fundamentally true for *Homo sapiens*, has driven this popularity. This review seeks to convey the view that the relative

simplicity of yeast provides an advantage for studying the cell cycle-dependent regulation of microfilaments, as well as their roles in morphogenesis and membrane traffic. These results will complement and contribute to studies of microfilaments in higher eucaryotes.

Regulation of Microfilaments during the Cell Cycle

In the vertebrate cell cycle, microfilaments reorganize as cells round up for mitosis and later they provide the contractile force during cytokinesis. In yeast, we shall restrict our discussion to some of the events and changes in microfilament distribution (Fig. 1) necessary for the assembly of a bud.

Bud site selection is regulated because haploids show axial budding, whereas diploids show bipolar budding (reviewed in references 25, 53). Several nonessential genes (including *BUD1/RSR1*, *BUD2-5*) provide the signal that directs cytoskeletal polarization to the proper site, but they are not required for bud site assembly or bud emergence (15). Bud site selection seems to involve a G-protein signal transduction pathway. *Bud1/Rsr1p* is a ras-like GTPase; *Bud5p* is a GDP exchange factor; and *Bud2p* has GAP activity on *Bud1p* (11, 14, 66). Next, genes involved in bud site assembly and bud emergence are required for normal polarization of the cytoskeleton to the prebud site. Mutations in these genes (including *CDC24*, *CDC42*, *CDC43*, and *BEM1-3*) result in failure of the cells to bud and they arrest as large unbudded cells with a randomized actin distribution (5). This assembly process also involves a GTPase cycle because *Cdc42p* is a ras-like GTPase and *Cdc24p* encodes a protein similar to GDP exchange factors. Moreover, *Bem3p* is related to mammalian rho-GAP and may be specific for *Cdc42p* (82). *Cdc43p* is a subunit of a protein geranylgeranyl transferase likely to be required for modification, membrane localization, and function of *Cdc42p* (57). Thus, *CDC42p* seems to be a key regulator that controls bud site assembly and microfilament polarization. There may be some interplay between bud site selection, assembly, and actin localization because overexpression of *Cdc42p*, *Bud5p*, or certain actin binding proteins, such as *Abp1p*, can alter bud site selection.

The ras-related genes *RHO1*, *RHO3*, and *RHO4* have also been implicated in governing cytoskeletal polarity. Conditional *rho1* mutants arrest as small-budded cells under restrictive conditions. *Rho1p* colocalizes with actin cortical patches during bud formation and growth; this distribution

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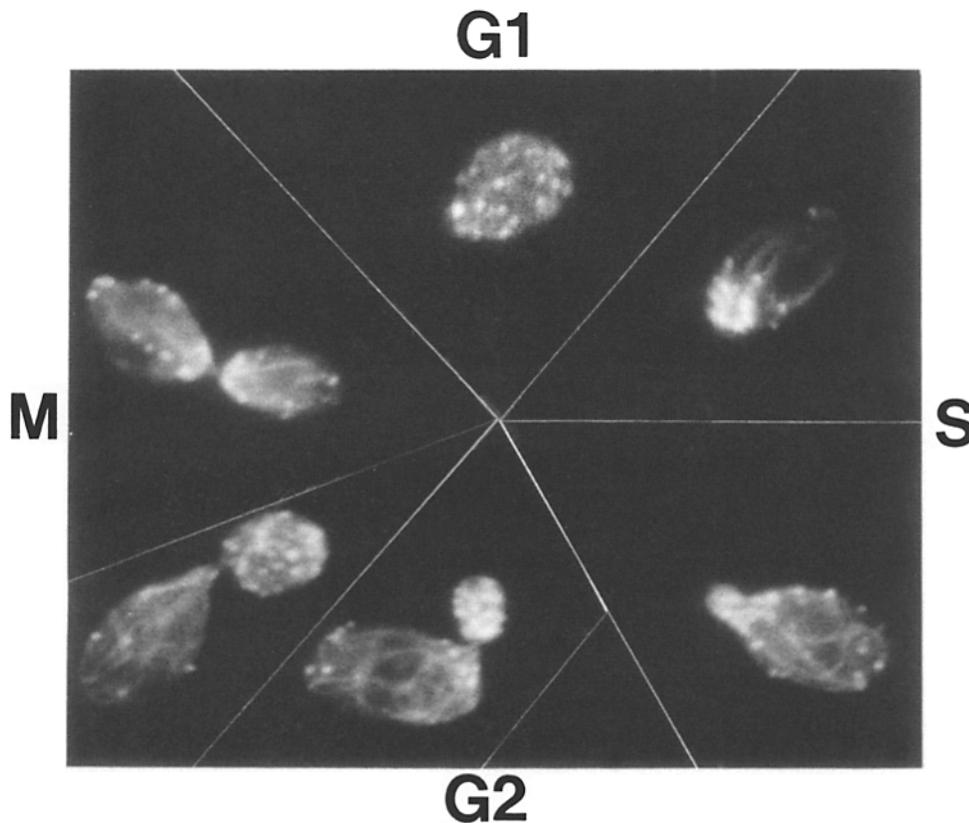


Figure 1. Localization of actin by immunofluorescence microscopy through the cell cycle in wild-type diploid yeast. In G1, the unbudded cell selects a specific prebud site, and then a ring of actin patches forms at this site. Secretion is directed toward the prebud site and bud emergence begins. Cortical actin patches associated with plasma membrane invaginations (61) redistribute to sites of new cell wall growth with bundles of actin filaments ("cables") extending from them into the mother cell. During S and G2, actin cables are aligned toward the bud where patches are localized (41); secretion remains directed toward the bud. Growth at the bud tip predominates in the early budded phase, whereas isotropic growth occurs later as the bud enlarges. During mitosis, actin patches redistribute to the surface of the mother and bud. Before cytokinesis, patches relocate to the mother-bud neck and secretion is targeted to this region for formation of the chitinous septum, which will ultimately remain as a bud scar on the mother and daughter cells.

is lost in *cdc42* mutants (83). Loss of both *RHO3* and *RHO4* products generates cells with randomized actin and delocalized chitin. Since these defects are suppressed by overexpression of the *CDC42* and *BEM1* genes (55), the combined results suggest that the *RHO* gene products act after the initiation of bud formation and determination of cell polarity specified by the Cdc42p pathway.

Changes in microfilament distribution are correlated to Cdc28p kinase activation by distinct cyclins at specific times during the cycle (46). Altering Cdc28p activity affects morphogenesis and the distribution of microfilaments during the yeast cell cycle at three specific stages (47). Activation of Cdc28p by the G1 cyclins (*CLN1*, 2, or 3) in unbudded G1 cells is required for polarization of the cortical actin cytoskeleton to the specified prebud site. This occurs in the absence of de novo protein synthesis and may be mediated by direct protein phosphorylation. One candidate substrate is the newly identified MAP kinase homologue, Slk2/Mpk1p, because defects in this protein enhance the phenotype of *cdc28* mutants. Moreover, *slk2* mutants display an altered actin distribution and accumulate secretory vesicles and membranes, indicating that the kinase is important for establishing cell polarity (58). Activation of Cdc28p by the mitotic cyclins (*CLB1*, 2) in G2 cells is required for the depolarization of the cortical actin cytoskeleton that results in the shift from apical to isotropic bud growth. Cdc28p inactivation by

cyclin destruction in mitosis appears to be necessary for redistribution of cortical actin to the mother-bud neck region and assembly of the actin structures required for cytokinesis.

There are parallels between the findings in yeast and studies in higher cells. Since small G-proteins also modulate growth factor-induced microfilament rearrangements in animal cells (69, 70), the regulation of cytoskeletal remodeling might be quite similar in yeast and animal cells. Also, Cdc28p-related kinases and cyclins control both the yeast and animal cell cycles, but how they regulate microfilament organizations is not yet clear; the emerging studies in yeast should be helpful.

Actins, Myosins, and Microfilament-associated Proteins

Saccharomyces has a single essential conventional actin gene, *ACT1*, that encodes a protein 88% identical to rabbit α -actin. Act1p is biochemically and functionally similar to conventional vertebrate actins (42, 62). An unconventional actin that is 47% identical to α -actin is encoded by another essential gene, *ACT2* (72). Little is known about the function of Act2p. Since unconventional actins have been found associated with microtubule-based functions in higher cells (19, 45), an analysis of Act2p function should be very interesting.

The distribution of actin points to a role for microfilaments in polarized secretion of cell wall components, and the phenotypes of *act1* mutants support this. *act1* mutants, many of which have conditional lethal phenotypes, have been made by random (74) or directed mutagenesis (16, 21, 22, 38, 81). Mutant phenotypes provide a direct test for functions of actin in yeast. Various actin mutations disrupt cell shape, cell polarity, secretion, endocytosis (43), spindle orientation (65), nuclear migration, cytokinesis, and mitochondrial distribution (26).

Yeast contains at least three myosins. The *MYO1* product is a conventional myosin II that is found at the bud neck, suggesting a role in cytokinesis (78). Cells lacking Myo1p form chains or clusters of cells because they are defective in septum formation. In addition, loss of the Myo1p results in diffuse chitin deposition, enlarged cell size, a subtle bud site selection defect, accumulation of membranes, and a tendency to lyse, perhaps suggesting additional functions for this myosin (71). The essential putative two-headed non-filamentous myosin, encoded by *MYO2* (39), is related to the mouse *dilute* locus and brain myosin V. Members of this family differ from myosin II in that the head has a slightly different sequence, the neck region binds several calmodulin molecules followed by a short α -helical coiled-coil region, and the tail terminates in a large globular domain (28). The *MYO2* gene was uncovered in a screen for conditional mutants that produced large cells; possible functions of Myo2p are discussed below. A second, nonessential, *dilute*-like myosin encoded by *MYO4* also exists in yeast (33).

A screen for multicopy suppressors of the *myo2* temperature sensitivity identified *SMY1*, an unusual kinesin-like gene (48). *SMY1* is not essential for yeast growth, but disruption of *SMY1* in the *myo2* conditional mutant is lethal. Smy1p, like Myo2p (12), concentrates at sites of cell growth in wild-type cells (49). As microtubules are not known to be involved in directed growth in yeast, it is an intriguing finding that a protein related to microtubule-based motors appears to perform a function related to Myo2p.

A full repertoire of proteins, many related to those of vertebrate cells, that bind to and modulate actin filaments has been discovered in yeast. A full discussion of these proteins and the genes that encode them has recently appeared (79), and so only a brief overview will be given here. These proteins include actin-binding protein (*ABPI*: 23, 24), tropomyosin, (*TPMI*: 50–52), fimbrin (*SAC6*: 3, 4, 6, 23), cofilin (*COFI*: 37, 60), capping protein (*CAP1*, *CAP2*: 8–10), and profilin (*PFY1*: 31, 32, 54, 77). Genetic approaches have also uncovered several genes (*SAC1-7*, *RAHI-3*, *SLAI*, *SLA2*, *SLC1*, *SLC2*, and *ANCI-4*: 18, 20, 27, 35, 40, 64, 76, 80) encoding novel proteins potentially important for microfilament function. Some of these have domains that are related to proteins of higher cells, such as *SLAI*, which encodes a protein having three SH3 domains, and *SLA2*, which shows homology to the COOH terminus of talin. As in vertebrate cells, these proteins associate with specific microfilamentous structures. For example, tropomyosin is a component of the actin cables; Abp1, cofilin, and capping protein are found in cortical patches; and fimbrin associates with both cables and patches.

Considerable effort has been devoted to studying the phenotypes of strains having mutations in these genes. In some cases, disruption of the gene shows no phenotype (e.g., *ABPI*), whereas other disruptions are either lethal (e.g.,

COFI) or show reduced growth rate, an altered actin cytoskeleton, and aberrant cell morphologies (e.g., *TPMI*, *SAC6*, *CAP1*, *CAP2*, *PFY1*, *ANCI*, *SLAI*, *SLA2*, *SLC1*, *SLC2*), suggestive of abnormal cell growth. How many different processes are these microfilament-associated proteins involved in? Since most of the cytoskeletal proteins are encoded by single nonessential genes, it is informative to ask if combinations of mutations in different genes are lethal, which could indicate that they participate in an essential process (7, 76). By this accounting, more than half a dozen distinct essential functions are indicated; a major challenge is to determine precisely what these are.

Membrane Trafficking and Targeting

Correct targeting of secretory vesicles that carry plasma membrane proteins, periplasmic enzymes, and materials needed to build the cell wall is required for the growth of buds in growing cells and the development of “shmoo” projections in mating cells. The large, round morphology of conditional-lethal *act1* mutants suggests that inappropriate growth occurs in the mother cell rather than being targeted to the bud. Whereas most of the chitin in wild-type cells is concentrated at the bud neck and remains as a “bud scar” after cell division (13, 34), the chitin in the *act1-1* mutant is distributed randomly on the cell surface when the cells are grown at a restrictive temperature (63), again suggesting a defect in targeting of secretory vesicles. As may be expected, actin cables are not detected in *act1-1* mutants at the restrictive temperature, and cortical patches are randomly distributed throughout both mother and bud (63). The distribution of cortical patches in both wild-type cells and *act1* mutants is consistent with the belief that it is the location of these structures that determines the sites of secretion and polarized growth (1, 47). As actin cables frequently appear to terminate on cortical patches, they may guide secretory vesicles to their site of fusion with the plasma membrane. Furthermore, *act1* mutants have a partial defect in the secretion of the periplasmic enzyme invertase, and they accumulate abundant vesicles that resemble secretory vesicles accumulated by mutants having a block late (post-Golgi) in the secretory pathway (63).

The conditional *myo2-66* mutation confers a phenotype similar to that of *act1* mutants and indicates a role for this myosin in secretion and polarized cell growth (39). At the restrictive temperature, large round cells with vesicles are formed. Interestingly, the *myo2-66* mutant is lethal in combination (synthetically lethal) with mutations in many of the *SEC* genes that function late in the secretory pathway, but not with those *SEC* genes that function at earlier steps, which implies an involvement in the late part of the pathway (30). Myo2p localizes mostly to regions of active surface growth, further implicating it in directed growth (12, 49). The morphological defects of the *myo2-66* mutant (39) are very similar to those in *tpml* mutants (52). Both strains accumulate vesicles, but surprisingly, no significant accumulation of the secretory protein invertase is found. Epistasis results suggest that the vesicles in the *tpml* mutant are derived from the normal secretory pathway. It is likely that the accumulated vesicles in the *tpml* and *myo2-66* strains are the same because these two mutations show synthetic lethality (52). One way to reconcile the lack of accumulation of invertase with a defect in secretion is to suggest that two parallel pathways exist

between the Golgi apparatus and the plasma membrane. Both pathways would depend on the late *SEC* gene products, yet one, not carrying invertase, would be facilitated by Tpm1p and Myo2p. It may be that invertase-containing vesicles function in a bulk delivery pathway, whereas vesicles carrying components for cell wall synthesis have to be targeted more precisely. The latter vesicles may fuse inefficiently at inappropriate locations on the cell surface and therefore accumulate. Since different components are required at different stages of the cell cycle, it is possible that the composition of these vesicles varies as the actin cytoskeleton reorganizes during the cell cycle. It is not yet known if defects in other cytoskeletal proteins result in the accumulation of vesicles, but mutations in many of these genes give rise to characteristically large round cells, suggestive of mistargeting. Purification of the vesicles accumulated in cytoskeletal mutants and an analysis of their cargo should help to elucidate their role in cellular morphogenesis.

The concept that myosins directly transport vesicles (or organelles) was originally suggested by cytoplasmic streaming in plant cells and, more recently, by the movement of vesicles from *Acanthamoeba* or from extruded giant axons along actin cables (2, 44). Lately, much attention has focused on Myo2p-related proteins. The mouse *dilute* mutations affect a myosin in this class (59). Severe alleles result in neurological seizures and death, while milder alleles are characterized by light-coat color resulting from a defect in transport of melanin-containing vesicles. Chicken brain myosin V, a homologue of the mouse *dilute* protein, is also proposed to function in vesicle transport (17, 28).

Recently, microfilaments have been implicated in the first step in receptor-mediated and fluid-phase endocytosis in yeast. Certain conditional *act1* alleles are conditional for receptor-mediated α -factor uptake, and cells lacking fimbrin are completely defective (43). Additionally, a mutation isolated on the basis of a defect in endocytosis defines *END4* and turns out to be identical to *SLA2* (Riezman, H., personal communication), a gene found independently as being essential in the absence of Abp1 (35). The requirement of microfilaments for endocytosis from the apical aspect of polarized epithelial cells (29) and in yeast is striking.

Perspectives

During the last few years, a large number of genes encoding proteins important for microfilament organization and function, many related to those in animal cells, have been identified in yeast. Analysis of the functions of these proteins is still in its infancy, but roles for microfilaments in vesicular trafficking and targeting, as well as in endocytosis are beginning to emerge. Moreover, the ability to introduce specific mutations into any desired gene allows one to explore the consequences of precise alterations in vivo and in vitro. The use of genetics will allow suspected relationships to be tested and unexpected ones to be revealed. Together with biochemical and cell biological approaches, this will surely lead to an understanding of the roles of microfilaments in yeast and suggest important avenues for furthering our knowledge about microfilament function in animal cells.

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References

- 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.
- Adams, R. J., and T. D. Pollard. 1986. Propulsion of organelles isolated from *Acanthamoeba* along actin filaments by myosin-1. *Nature (Lond.)*. 322:754-756.
- Adams, A. E. M., and D. Botstein. 1989. Dominant suppressors of yeast actin mutations that are reciprocally suppressed. *Genetics*. 121:675-683.
- Adams, A. E. M., D. Bostein, and D. G. Drubin. 1989. A yeast actin-binding protein is encoded by *SAC6*, a gene found by suppression of an actin mutation. *Science (Wash. DC)*. 243:231-233.
- Adams, A. E., D. I. Johnson, R. M. Longnecker, B. F. Sloat, and J. R. Pringle. 1990. *CDC42* and *CDC43*, two additional genes involved in budding and the establishment of cell polarity in the yeast *Saccharomyces cerevisiae*. *J. Cell Biol.* 111:131-142.
- Adams, A. E. M., D. Bostein, and D. G. Drubin. 1991. Requirement of yeast fimbrin for actin organization and morphogenesis in vivo. *Nature (Lond.)*. 354:404-408.
- Adams, A. E. M., J. A. Cooper, and D. G. Drubin. 1993. Unexpected combinations of null mutations in genes encoding the actin cytoskeleton are lethal in yeast. *Mol. Biol. Cell*. 4:459-468.
- Amatruda, J. F., J. F. Cannon, K. Tatchell, C. Hug, and J. A. Cooper. 1990. Disruption of the actin cytoskeleton in yeast capping protein mutants. *Nature (Lond.)*. 344:352-354.
- Amatruda, J. F., and J. A. Cooper. 1992. Purification, characterization and immunofluorescence localization of *Saccharomyces cerevisiae* capping protein. *J. Cell Biol.* 117:1067-1076.
- Amatruda, J. F., D. G. Gattermeir, T. S. Karpova, and J. A. Cooper. 1992. Effects of null mutations and overexpression of capping protein on morphogenesis, actin distribution, and polarized secretion in yeast. *J. Cell Biol.* 119:1151-1162.
- Bender, A. 1993. Genetic evidence for the roles of the bud-site-selection genes *BUD5* and *BUD2* in control of the Rsr1p (Bud1p) GTPase in yeast. *Proc. Natl. Acad. Sci. USA*. 90:9926-9929.
- Brocknerhoff, S. E., R. C. Stevens, and T. N. Davis. 1994. The unconventional myosin, Myo2p, is a calmodulin target at sites of cell growth in *Saccharomyces cerevisiae*. *J. Cell Biol.* 124:315-323.
- Cabib, E., and B. Bowers. 1975. Timing and function of chitin synthesis in yeast. *J. Bacteriol.* 124:1586-1593.
- Chant, J., K. Corrado, J. R. Pringle, and I. Herskowitz. 1991. Yeast *BUD5* encoding a GDP-GTP exchange factor is necessary for bud site selection and interacts with bud formatin gene *BEM1*. *Cell*. 65:1213-1224.
- Chant, J., and I. Herskowitz. 1991. Genetic control of bud site selection in yeast by a set of gene products that constitute a morphogenetic pathway. *Cell*. 65:1203-1212.
- Chen, X., R. K. Cook, and P. A. Rubenstein. 1993. Yeast actin with a mutation in the "hydrophobic plug" between subdomains 3 and 4 (*L₂₆₆D*) displays a cold-sensitive polymerization defect. *J. Cell Biol.* 123:1185-1195.
- Cheney, R. E.; M. K. O'Shea, J. E. Heuser, M. V. Coelho, J. S. Wolenski, E. M. Espreafico, P. Forscher, R. E. Larson, and M. S. Mooseker. 1993. Brain myosin-V is a two-headed unconventional myosin with motor activity. *Cell*. 75:13-23.
- Chowdhury, S., K. W. Smith, and M. C. Gustin. 1992. Osmotic stress and the yeast cytoskeleton: phenotype-specific suppression of an actin mutation. *J. Cell Biol.* 118:561-571.
- Clark, S. W., and D. I. Meyer. 1992. Centractin is an actin homologue associated with the centrosome. *Nature (Lond.)*. 359:246-250.
- Cleves, A. E., P. J. Novick, and V. A. Bankaitis. 1989. Mutations in the *SAC1* gene suppress defects in yeast Golgi and yeast actin function. *J. Cell Biol.* 109:2939-2950.
- Cook, R. K., W. T. Blake, and P. A. Rubenstein. 1992. Removal of the amino-terminal acidic residues of yeast actin. Studies in vitro and in vivo. *J. Biol. Chem.* 267:9430-9436.
- Cook, R. K., D. Root, C. Miller, E. Reisler, and P. A. Rubenstein. 1993. Enhanced stimulation of myosin subfragment 1 ATPase activity by addition of negatively charged residues to the yeast actin NH₂ terminus. *J. Biol. Chem.* 268:2410-2415.
- Drubin, D. G., K. G. Miller, and D. Botstein. 1988. Yeast actin-binding proteins: evidence for a role in morphogenesis. *J. Cell Biol.* 107:2551-2561.
- Drubin, D. G., J. Mulholland, Z. M. Zhu, and D. Botstein. 1990. Homology of a yeast actin-binding protein to signal transduction proteins and myosin-1. *Nature (Lond.)*. 343:288-290.
- Drubin, D. 1991. Development of cell polarity in budding yeast. *Cell*. 65:1093-1096.
- Drubin, D. G., H. D. Jones, and K. F. Wertman. 1993. Actin structure

- and function: roles in mitochondrial organization and morphogenesis in budding yeast and identification of the phalloidin-binding site. *Mol. Cell Biol.* 4:1277-1294.
27. Dunn, T. M., and D. Shortle. 1990. Null alleles of *SAC7* suppress temperature-sensitive actin mutations in *Saccharomyces cerevisiae*. *Mol. Cell Biol.* 10:2308-2314.
 28. Espreafico, E. M., R. E. Chaney, M. Matteoli, A. A. C. Nascimento, P. V. Camilli, R. E. Larson, and M. S. Mooseker. 1992. Primary structure and cellular localization of chicken brain myosin-V, an unconventional myosin with calmodulin light chains. *J. Cell Biol.* 119:1541-1557.
 29. Gortlieb, T. A., I. E. Ivanov, M. Adesnick, and D. D. Sabatini. 1993. Actin microfilaments play a critical role in endocytosis at the apical but not the basolateral surface of polarized epithelial cells. *J. Cell Biol.* 120:695-710.
 30. Govindan, B., R. Bowser, and P. Novick. 1991. Role of the unconventional myosin gene *MYO2* in the yeast secretory pathway. *J. Cell Biol.* 115:18a.
 31. Haarer, B. K., S. H. Lillie, A. E. M. Adams, V. Magdolen, W. Bandlow, and S. S. Brown. 1990. Purification of profilin from *Saccharomyces cerevisiae* and analysis of profilin-deficient cells. *J. Cell Biol.* 110:105-114.
 32. Haarer, B. K., A. S. Petzold, and S. S. Brown. 1993. Mutational analysis of yeast profilin. *Mol. Cell Biol.* 13:7864-7873.
 33. Haarer, B. K., A. Petzold, S. H. Lillie, and S. S. Brown. 1994. Identification of *MYO4*, a second class V myosin gene in yeast. *J. Cell Sci.* 107:1055-1064.
 34. Hayashibe, M., and S. Katohda. 1973. Initiation of budding and chitin ring. *J. Gen. Appl. Microbiol.* 19:23-39.
 35. Holtzman, D. A., S. Yang, and D. G. Drubin. 1993. Synthetic-lethal interactions identify two novel genes, *SLA1* and *SLA2*, that control membrane cytoskeleton assembly in *Saccharomyces cerevisiae*. *J. Cell Biol.* 122:635-644.
 36. Ishikawa, H., R. Bischoff, and H. Holtzer. 1969. The formation of arrow-head complexes with heavy meromyosin in a variety of cell types. *J. Cell Biol.* 43:312-328.
 37. Iida, K., K. Moriyama, S. Matsumoto, H. Kawasaki, E. Nishida, and I. Yahara. 1993. Isolation of a yeast essential gene, *COF1*, that encodes a homologue of mammalian cofilin, low-M_w actin-binding and depolymerizing protein. *Gene.* 124:155-120.
 38. Johannes, F. J., and D. Gallwitz. 1991. Site-directed mutagenesis of the yeast actin gene: a test for actin function in vivo. *EMBO (Eur. Mol. Biol. Organ.) J.* 10:3951-3958.
 39. Johnston, G. C., J. A. Prendergast, and R. A. Singer. 1991. The *Saccharomyces cerevisiae* *MYO2* gene encodes an essential myosin for vectorial transport of vesicles. *J. Cell Biol.* 113:539-551.
 40. Karpova, T. S., M. M. Lepetit, and J. A. Cooper. 1993. Mutations that enhance the *cap2* null mutant phenotype in *Saccharomyces cerevisiae* affect the actin cytoskeleton, morphogenesis, and pattern of growth. *Genetics.* 135:693-709.
 41. Kilmartin, J. V., and A. E. M. Adams. 1984. Structural rearrangements of tubulin and actin during the cell cycle of the yeast *Saccharomyces cerevisiae*. *J. Cell Biol.* 98:922-933.
 42. Kron, S. J., D. G. Drubin, D. Botstein, and J. A. Spudich. 1992. Yeast actin filaments display ATP-dependent sliding movement over surface coated with rabbit muscle myosin. *Proc. Natl. Acad. Sci. USA.* 89:4466-4470.
 43. Kubler, E., and H. Riezman. 1993. Actin and fimbrin are required for the internalization step of endocytosis in yeast. *EMBO (Eur. Mol. Biol. Organ.) J.* 12:2855-2862.
 44. Kuznetsov, S. A., G. M. Langford, and D. M. Weiss. 1992. Actin-dependent organelle movement in squid axoplasm. *Nature (Lond.)* 356:722-725.
 45. Lees-Miller, J. P., D. M. Helfman, and T. A. Schroer. 1992. A vertebrate actin-related protein is a component of a multisubunit complex involved in microtubule-based vesicle motility. *Nature (Lond.)* 359:244-246.
 46. Lew, D. J., and S. I. Reed. 1992. A proliferation of cyclins. *Trends Cell Biol.* 2:77-81.
 47. 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.
 48. Lillie, S. H., and S. S. Brown. 1992. Suppression of a myosin defect by a kinesin-related gene. *Nature (Lond.)* 356:358-361.
 49. Lillie, S. H., and S. S. Brown. 1994. Immunofluorescence localization of the unconventional myosin, Myo2p, and the putative kinesin-related protein, Smy1p, to regions of growth in *Saccharomyces cerevisiae*. *J. Cell Biol.* 125:825-842.
 50. Liu, H., and A. Bretscher. 1989a. Purification of tropomyosin from *Saccharomyces cerevisiae* and identification of related proteins in *Schizosaccharomyces* and *Physarum*. *Proc. Natl. Acad. Sci. USA.* 86:90-93.
 51. Liu, H., and A. Bretscher. 1989b. Disruption of the single tropomyosin gene in yeast results in the disappearance of actin cables from the cytoskeleton. *Cell.* 57:233-242.
 52. Liu, H., and A. Bretscher. 1992. Characterization of *TMP1* disrupted yeast cells indicates an involvement of tropomyosin in directed vesicular transport. *J. Cell Biol.* 118:285-299.
 53. Madden, K., C. Costigan, and M. Snyder. 1992. Cell polarity and morphogenesis in *Saccharomyces cerevisiae*. *Trends Cell Biol.* 2:22-29.
 54. Magdolen, V., D. G. Drubin, G. Mages and W. Bandlow. 1993. High levels of profilin suppress the lethality caused by overexpression of actin in yeast cells. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 316:41-47.
 55. Matsui, Y., and A. Toh-e. 1992. Yeast *RHO3* and *RHO4* ras super-family genes are necessary for bud growth, and their defect is suppressed by a high dose of bud formation genes *CDC42* and *BEM1*. *Mol. Cell Biol.* 12:5690-5699.
 56. Mayer, B. J., and D. Baltimore. 1993. Signaling through SH2 and SH3 domains. *Trends Cell Biol.* 3:8-13.
 57. Mayer, M. L., B. E. Caplin, and M. S. Marshall. 1992. *CDC43* and *RAM2* encode the polypeptide subunits of a yeast type 1 protein geranylgeranyl transferase. *J. Biol. Chem.* 267:20589-20593.
 58. Mazzoni, C., P. Zarzov, A. Rambourg, and C. Mann. 1993. The *SLT2(MPK1)* MAP kinase homologue is involved in polarized cell growth in *Saccharomyces cerevisiae*. *J. Cell Biol.* 6:1821-1833.
 59. Mercer, J. A., P. K. Seperack, M. C. Strobel, N. G. Copeland, and N. A. Jenkins. 1991. Novel myosin heavy chain encoded by murine dilute coat colour locus. *Nature (Lond.)* 349:709-712.
 60. Moon, A. L., P. A. Janmey, and D. G. Drubin. 1993. Cofilin is an essential component of the yeast cortical cytoskeleton. *J. Cell Biol.* 120:421-435.
 61. 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.
 62. Nefsky, B., and A. Bretscher. 1992. Yeast actin is relatively well behaved. *Eur. J. Biochem.* 206:949-955.
 63. Novick, P., and D. Botstein. 1985. Phenotypic characterization of temperature-sensitive yeast actin mutants. *Cell.* 40:405-416.
 64. Novick, P., B. C. Osmond, and D. Botstein. 1989. Suppressors of yeast actin mutations. *Genetics.* 121:659-674.
 65. Palmer, R. E., D. S. Sullivan, T. Huffaker, and D. Koshland. 1992. Role of astral microtubules and actin in spindle orientation and migration in the budding yeast *Saccharomyces cerevisiae*. *J. Cell Biol.* 119:583-593.
 66. Park, H. O., J. Chant, and I. Herskowitz. 1993. *BUD2* encodes a GTPase-activating protein for *BUD1-RSR1* necessary for proper bud site selection in yeast. *Nature (Lond.)* 365:269-274.
 67. Rath, S., J. Rohrer, F. Crausaz, and H. Riezman. 1993. End3 and end4: two mutants defective in receptor-mediated and fluid-phase endocytosis in *Saccharomyces cerevisiae*. *J. Cell Biol.* 120:55-65.
 68. Read, E. B., H. H. Okamura, and D. G. Drubin. 1992. Actin- and tubulin-dependent functions during *Saccharomyces cerevisiae* mating projection formation. *Mol. Biol. Cell.* 3:429-444.
 69. Ridley, A. J., and A. Hall. 1992. The small GTP-binding protein rho regulates the assembly of focal adhesions and actin stress fibers in response to growth factors. *Cell.* 70:389-399.
 70. Ridley, A. J., H. F. Paterson, C. L. Johnson, D. Diekmann, and A. Hall. 1992. The small GTP-binding protein rac regulates growth-factor-induced membrane ruffling. *Cell.* 70:401-410.
 71. 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. Cytoskel.* 17:301-308.
 72. Schwob, E., and R. P. Martin. 1992. New yeast actin-like gene required late in the cell cycle. *Nature (Lond.)* 355:179-182.
 73. Shortle, D., J. E. Haber, and D. Botstein. 1982. Lethal disruption of the yeast actin gene by integrative DNA transformation. *Science (Wash. DC)* 217:371-373.
 74. Shortle, D., P. Novick and D. Botstein. 1984. Construction and genetic characterization of temperature-sensitive mutant alleles of the yeast actin gene. *Proc. Natl. Acad. Sci. USA.* 81:4889-4893.
 75. Stossel, T. P. 1993. On the crawling of animal cells. *Science (Wash. DC)* 260:1086-1094.
 76. Vinh, D. B. N., M. D. Welch, A. K. Corst, K. F. Wertman, and D. G. Drubin. 1993. Genetic evidence for functional interactions between actin noncomplementing (Anc) gene products and actin cytoskeletal proteins in *Saccharomyces cerevisiae*. *Genetics.* 135:275-286.
 77. Vojtek, A., B. Haarer, J. Field, J. Gerst, T. D. Pollard, S. Brown, and M. Wigler. 1991. Evidence for a functional link between profilin and CAP in the yeast *S. cerevisiae*. *Cell.* 66:497-505.
 78. 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.
 79. Welch, M. D., D. A. Holtzman, and D. G. Drubin. 1994. The yeast actin cytoskeleton. *Curr. Opin. Cell Biol.* 6:110-119.
 80. Welch, M. D., D. B. N. Vinh, H. H. Okamura, and D. G. Drubin. 1993. Screens for extragenic mutations that fail to complement *act1* alleles identify genes that are important for actin function in *Saccharomyces cerevisiae*. *Genetics.* 135:265-274.
 81. Wertman, K. F., D. G. Drubin, and D. Botstein. 1992. Systematic mutational analysis of the yeast *ACT1* gene. *Genetics.* 132:337-350.
 82. Whitters, E. A., A. E. Cleves, T. P. McGee, H. B. Skinner, and V. A. Bankaitis. 1993. SAC1p is an integral membrane protein that influences the cellular requirement for phospholipid transfer protein function and inositol in yeast. *J. Cell Biol.* 122:79-94.
 83. Yamochi, W., K. Tanaka, H. Nonaka, A. Maeda, T. Musha and Y. Takai. 1994. Growth site localization of Rho1 small GTP-binding protein and its involvement in bud formation in *Saccharomyces cerevisiae*. *J. Cell Biol.* 125:1077-1093.
 84. Zheng, Y., M. J. Hart, K. Shinjo, T. Evans, A. Bender, and R. A. Cerione. 1993. Biochemical comparisons of the *Saccharomyces cerevisiae* Bem2 and Bem3 proteins: delineation of a limited Cdc42 GTPase-activating protein domain. *J. Biol. Chem.* 268:24629-24634.