

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

Anthony Bretscher, Beth Drees, Edina Harsay, Daniel Schott, and Tongtong Wang

Section of Biochemistry, Molecular and Cell Biology, Biotechnology Building, Cornell University, Ithaca, New York 14853

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

Address all correspondence to Anthony Bretscher, Section of Biochemistry, Molecular and Cell Biology, Biotechnology Building, Cornell University, Ithaca, NY 14853. Phone: (607) 255-5713; fax: (607) 255-2428.

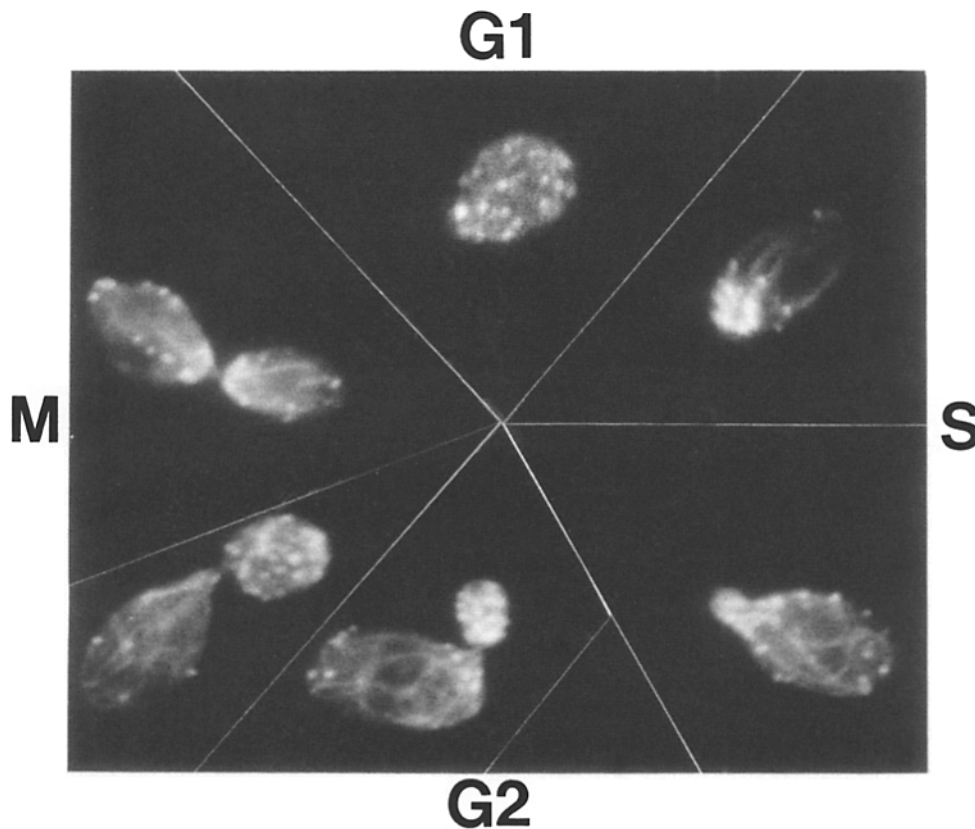


Figure 1. Localization of actin by immunofluorescence microscopy through the cell cycle in wild-type diploid yeast. In G1, the unbudded cell selects a specific prebud site, and then a ring of actin patches forms at this site. Secretion is directed toward the prebud site and bud emergence begins. Cortical 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*, *RAH1-3*, *SLA1*, *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 *SLA1*, 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*, *SLA1*, *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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