

Central Roles of Small GTPases in the Development of Cell Polarity in Yeast and Beyond

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INTRODUCTION

Cell polarity is central to the development of most eukaryotes. It is also critical for the function of many cell types involved in vectored processes such as nutrient transport, neuronal signaling, and cell motility. Cell polarization in response to extracellular or intracellular cues appears to follow a common plan (128). First, a spatial cue marks the site of polarized growth. Signaling molecules relay the spatial information to the downstream components of polarity establishment, leading to asymmetric organization of the cytoskeleton. Polarity is then reinforced with targeted secretion that leads to the deposition of molecules needed for growth at the chosen site. It is apparent from a large number of studies of diverse organisms that the small-molecular-weight GTPases function as key signaling molecules in polarity development and that there is a remarkable conservation of these GTPases from yeast to humans at both structural and functional levels (61, 62, 137, 203).

The budding yeast *Saccharomyces cerevisiae* is a particularly attractive model organism because it displays pronounced cell polarity in response to intracellular and extracellular cues. Cells of budding yeast undergo polarized growth during various phases of their life cycle, such as budding during vegetative growth, mating between haploid cells of opposite mating types, and filamentous growth (FG) upon deprivation of nutrients such as nitrogen (Fig. 1). There are three cell types in yeast (here we sometimes refer to *S. cerevisiae* as “yeast,” although we realize that other yeasts such as *Schizosaccharomyces pombe* are somewhat different from *S. cerevisiae*): \mathbf{a} and α cells (such as normal haploids) and \mathbf{a}/α cells (such as normal diploids), which are determined by their mating-type loci (220). Both haploid and diploid cells initiate budding when they reach the critical cell size in the late G_1 phase of the cell cycle. Bud growth is initially targeted to the bud tip (apical growth) and then throughout the bud (isotropic growth) until nuclear

division and cytokinesis take place (Fig. 1A). The second form of polarized growth occurs when haploid \mathbf{a} and α cells encounter each other and undergo mating to form diploid \mathbf{a}/α cells (Fig. 1B). During both budding and mating, the overall cellular organization is similar, although budding cells have a constriction between mother and bud called the bud neck (Fig. 1A). A specific site for cell polarization during budding is determined by the cell type, whereas cell polarization during mating is chemotropic; i.e., a cell of one mating type responds to a gradient of a peptide mating pheromone secreted by a cell of the opposite mating type (221). The most prominent feature in both processes is the organization of the polarized actin cytoskeleton, which guides secretion towards the bud site or the tip of a mating projection, resulting in polarized cell growth (126, 457).

The process of budding is controlled both spatially and temporally: bud emergence occurs at a particular site in the cell cortex and at a particular time in the cell cycle. The early stage of budding can be viewed as a number of sequential, coordinated events orchestrated by a cascade of small GTPase modules (Fig. 2) (85, 126, 221, 454). First, a specific site for polarized growth is determined in the bud site selection step. The Ras family GTPase Rsr1/Bud1 (hereafter called Rsr1) and its regulators are known to play a key role in this step. Second, the assembly of components required for bud formation takes place at the chosen site to restrict cell growth to that position. Unlike the bud site selection step, this bud site assembly is an essential step for growth, which requires a group of proteins including the Rho family GTPase Cdc42 and its regulators. Cdc42 interacts with several proteins to trigger downstream processes, including polarization of the actin cytoskeleton and secretion towards the sites of cell growth. Rho GTPases are also involved in the polarized organization of the actin cytoskeleton and cell wall biogenesis. Finally, according to the

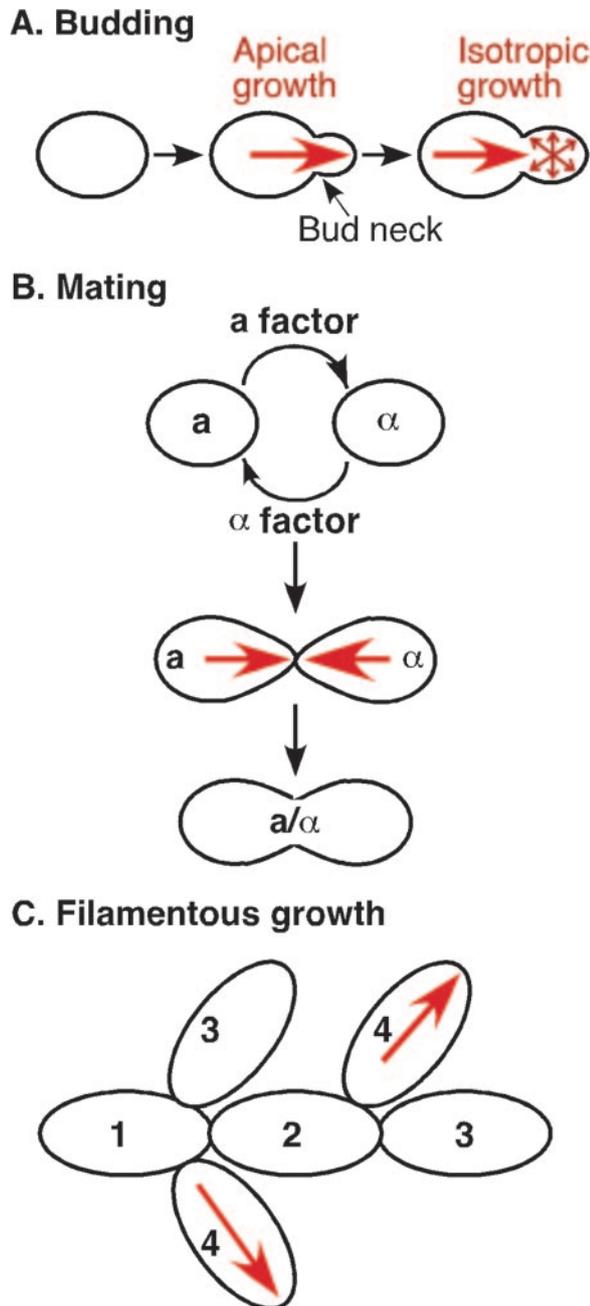


FIG. 1. Polarized growth in the life cycle of the budding yeast *S. cerevisiae*. Polarized growth occurs during budding, mating, and nutritional starvation. Nutritional starvation triggers filamentous growth, which can be divided into invasive growth (exhibited by haploid cells) and pseudohyphal growth (exhibited by diploid cells). Numbers in panel C indicate the order of each budding event. The direction of growth is indicated by red arrows.

polarization cue directed by Cdc42 and Rho GTPases, the Rab family GTPase Sec4 regulates secretion or exocytosis from the Golgi apparatus to the plasma membrane, resulting in the emergence and growth of the bud. The purpose of this article is to review the molecular mechanisms underlying the development of cell polarity in budding yeast, with a particular focus on the roles of small GTPases. We will discuss GTPase signal-

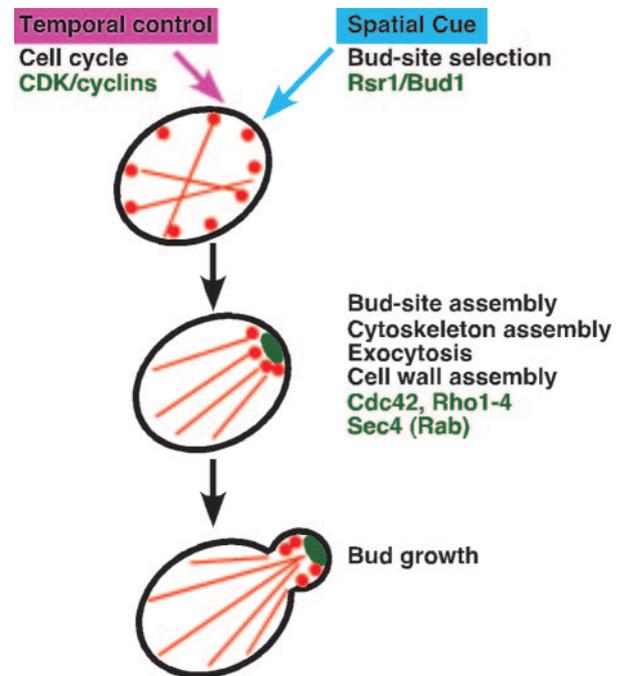


FIG. 2. Small GTPases and the major events in the early stage of budding in *S. cerevisiae*. Two major structures of actin filaments, actin cables and actin patches, are indicated with red lines and red dots, respectively, to highlight the polarized organization of the actin cytoskeleton.

ing pathways and their coordination mechanisms that are central to polarized growth. We will also discuss the regulatory mechanisms involved in the spatial and temporal control of cell polarity. Finally, we will discuss a few key concepts that may define unified intellectual frameworks for studying cell polarizations in diverse organisms.

ESTABLISHMENT AND MAINTENANCE OF Cdc42 POLARIZATION

Yeast cells organize the polarity establishment machinery in response to the spatial cues from budding landmarks or mating pheromones. The key player in polarity establishment is Cdc42 GTPase, which is involved in actin organization, septin organization, and exocytosis (8, 259, 335) (Fig. 3A). In this section, we will first describe the components of the Cdc42 GTPase module. We will then discuss how the establishment and maintenance of Cdc42 polarization at the sites of polarized growth are regulated. This part includes a discussion of the concept of “symmetry breaking,” which may explain the development of cell polarity in the absence of spatial cues. In wild-type cells, the establishment of Cdc42 polarization occurs at a specific site in response to a spatial cue, which is discussed in depth below (see “Coupling of the Rsr1 GTPase Module to the Cdc42 GTPase Module”).

The Rho Family GTPase Cdc42 and Its Regulators

Cdc42 GTPase. After being identified first in *S. cerevisiae*, Cdc42 and its homologs have been found in various other

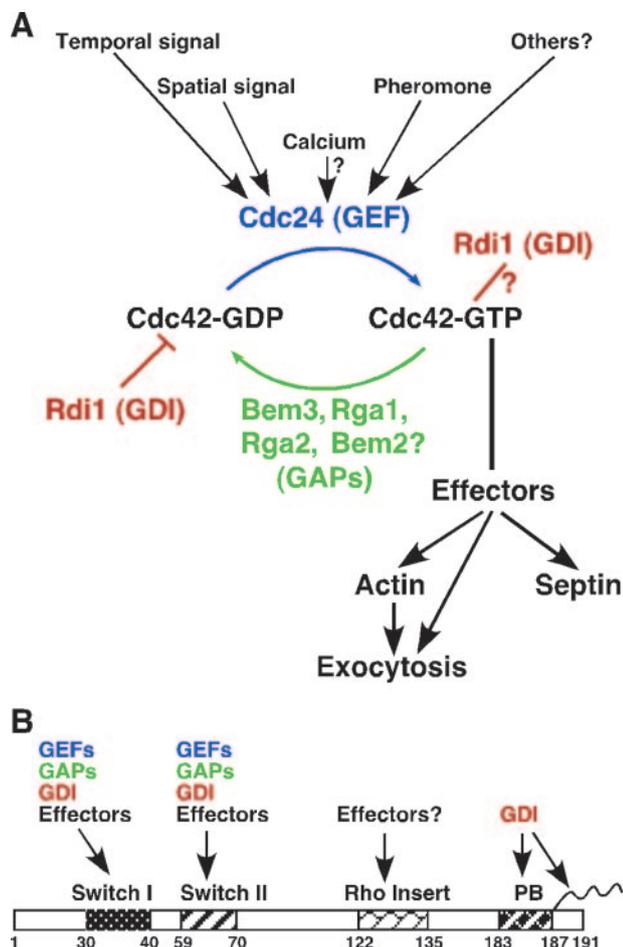


FIG. 3. Regulation of Cdc42 in *S. cerevisiae*. (A) Cdc42 activity is regulated by at least three types of regulators: the GEF Cdc24; the GAPs Bem3, Rga1, Rga2, and Bem2; and the GDI Rdi1. The GEF and perhaps the GAPs are likely to mediate the regulation of Cdc42 by most internal and external signals. Once activated, Cdc42 regulates the organization of the actin cytoskeleton and the septins and interacts with components of the exocytic machinery. The polarized actin cytoskeleton guides exocytosis, leading to polarized cell growth. (B) Structure motifs of *S. cerevisiae* Cdc42 and their potential binding partners based on studies of Cdc42 from yeast to mammals. The switch I region (which is also known as the Ras-like effector region), the switch II region, the Rho insert domain (which is present only in Rho-type GTPases), the polybasic (PB) region (¹⁸³KKSKK¹⁸⁷), the CAAAX box (¹⁸⁸CAIL¹⁹¹), and their binding partners are indicated. The cysteine residue in the CAAAX box is modified by prenylation (wavy line), and the last three amino acids are cleaved off, followed by carboxymethylation (see the text for details).

eukaryotes including *Caenorhabditis elegans*, *Drosophila melanogaster*, and humans (for a review, see reference 259). Cdc42, which belongs to the Rho subfamily of the Ras superfamily GTPases, is highly conserved from yeast to humans at both the sequence (80 to 95% identity in the predicted amino acid sequence) and functional (259) levels. Like all GTPases in the Ras superfamily, Cdc42 contains the putative Ras-like effector motif (also known as “switch I”) near the N terminus (Fig. 3B). The binding partners of Cdc42 through this effector domain are proteins containing the CRIB (for “Cdc42/Rac interactive binding”) domain (also known as the p21-binding domain or

the GTPase-binding domain [GBD]). The CRIB domain is found in many Cdc42 downstream effectors, including the p21-activated kinase (PAK) family of protein kinases, which preferentially interact with GTP-bound Cdc42 (515). Mutational studies of *CDC42* in *S. cerevisiae* indicate that the effector domain may differentially interact with multiple CRIB domain-containing effectors (see below).

Cdc42 also contains the “Rho insert domain” of ~13 amino acid residues, which is unique to Rho-type GTPases (Fig. 3B). This domain has been implicated in interactions with some of its downstream effectors such as IQGAPs and guanine nucleotide dissociation inhibitors (GDIs) for human Cdc42 (375, 621). However, the crystallographic structure of the Cdc42/Rho GDI complex reveals that the switch I and switch II domains and the geranylgeranyl moiety of Cdc42 interact with Rho GDI (229). Cdc42 also contains the C-terminal CAAAX (A is aliphatic; X is any amino acid) box, CTIL, for prenylation with the geranylgeranyl isoprene group at the Cys residue (651). Geranylgeranylation has been found in Cdc42s from all other organisms examined. This posttranslational modification is important for the membrane attachment and function of Cdc42. Cell fractionation experiments indicate that Cdc42 is found in both soluble and particulate pools, with most in the latter fraction, which includes the plasma membrane, secretory vesicles, and other dense materials (651). The particulate pool of Cdc42 is eliminated by mutagenizing the Cys188 in the C-terminal ¹⁸⁸CTIL and is decreased in the geranylgeranyl-transferase mutant *cdc43* (651). Lipid modification of Cdc42 is thus required for its targeting to the particulate pool. Indeed, Cdc42^{C188S} fused to green fluorescent protein (GFP) fails to localize to any internal and plasma membranes (466). A GFP fused to the CAAAX motif alone (GFP-¹⁸⁸CTIL) localizes to the internal membranes but not to the plasma membranes, while GFP-KKSKKCTIL (the polybasic motif plus the CAAAX box) is sufficient for targeting to both membranes. However, GFP-KKSKKCTIL still fails to cluster at sites of polarized growth (466), supporting the idea that Cdc42 clustering may require guanine nucleotide binding (468). Thus, it appears that the CAAAX box and its immediate upstream polybasic domain are sufficient for the targeting of Cdc42 to the plasma membrane, while other regions of Cdc42 are likely to be involved in its clustering at sites of polarized growth.

Cdc42 localizes to the plasma membrane and to sites of polarized growth, first to the incipient bud site, the tips of growing buds, and then the mother-bud neck in large-budded cells (466, 651). It is noteworthy that Cdc42 localization to the incipient bud site is not disrupted by incubation with latrunculin A, which sequesters G actin and thus causes a rapid depolymerization of the actin filaments (30). Thus, Cdc42 is likely to arrive to the presumptive bud site independently of the localization or integrity of the actin cytoskeleton during budding (see below for a further discussion of Cdc42 polarization).

The phenotype of a temperature-sensitive (Ts) *cdc42* mutant, *cdc42-1*, has provided the first clue for the function of Cdc42 (8, 217). This mutant fails to form a bud at 37°C but continues DNA replication, nuclear division, and the increase in cell mass and volume, resulting in an enlarged unbudded cell. The actin cytoskeleton is haphazardly distributed in the mutant cells, indicating that Cdc42 is required for the polar-

ized organization of the actin cytoskeleton (8). Mutations of *CDC42* or *CDC24*, which encodes a GDP-GTP exchange factor (GEF) for Cdc42, also prevent targeted secretion so that new cell surface growth is not limited to the bud or mating projection (8, 535). Null mutants of *CDC42* or *CDC24* are not viable, as expected from their essential role in budding (260). Analyses of many *cdc42* mutants have provided much information about the functional domains of Cdc42 and have uncovered a variety of processes in which Cdc42 is involved (84, 183, 259, 293, 394, 465) (see below).

Cdc24, a GEF for Cdc42. As discussed above, *CDC24* is also required for bud emergence or the establishment of cell polarity (215, 216). Temperature-sensitive mutations in *CDC24* result in the formation of large, round, unbudded cells at the restrictive temperature (215, 216, 534, 535), as is the case with some of the *cdc42*-Ts mutants, suggesting that *CDC42* and *CDC24* are likely to function in the same process, i.e., bud emergence (8, 260). These *cdc24* mutants exhibit an overall defect in cell polarity resulting in delocalized chitin and mannan deposition throughout the cell surface (473, 534, 535).

Cdc24 contains a Dbl homology (DH) domain (388), which is generally associated with its GEF activity (228). Indeed, Cdc24 displays GEF activity towards Cdc42 (563, 645). Because a hyperactive mutation in *CDC42* (*cdc42^{G60D}*) can bypass the requirement for Cdc24, it is likely that the GEF activity towards Cdc42 is the sole essential function for Cdc24 in polarized growth (84). Cdc24 also contains other highly conserved domains including a calponin homology (CH) domain, which has been implicated in binding to actin in some proteins (551), and the pleckstrin homology (PH) domain, which is thought to bind phosphoinositides (310). A recent genome-wide characterization of all potential PH domains in yeast indicates that the PH domain of Cdc24, like most of the other yeast PH domains, binds phosphoinositides but with no headgroup specificity and with low binding affinity (635). In addition, the PH domain of Cdc24 alone fails to localize to the membrane (635). Thus, the functional significance of the PH domain of Cdc24 is not clear. Cdc24 also has two calcium binding domains (residues 649 to 658 and 820 to 831). It has been reported previously that Bem1, another protein important for polarity establishment, binds directly to Cdc24 and that this interaction is inhibited by Ca^{2+} in vitro (644), which may explain why *CDC24* was also identified from a screen for mutants sensitive to a high level of exogenous calcium (421, 422).

Despite no obvious transmembrane domain or hydrophobic stretches, Cdc24 fractionates to a particulate pool (383, 388, 454). It is possible that Cdc24 associates with membrane through its PH domain and/or through interactions with other proteins on the plasma membrane. Analyses of deletion and point mutations of *CDC24* have identified a 56-amino-acid domain (amino acids 647 to 703) as being necessary and sufficient for its localization to sites of polarized growth (572). This domain alone, however, is unable to anchor Cdc24 at these sites or is unable to support a tight association of Cdc24 with the plasma membrane. Anchoring of Cdc24 requires the Cdc24 carboxyl-terminal PC (phox and Cdc) domain that interacts with Bem1 and also requires Bem1, Rsr1, or the potential transmembrane protein Tos2/Ygr221C (572). Not much is known about Tos2 except that it exhibits a two-hybrid interaction with Cdc24 and also localizes to sites of polarized

growth (123). Together, these data suggest that Cdc24 localization requires both membrane-specific targeting and subsequent anchoring within a multiprotein complex.

Cdc24 interacts with a number of proteins including Rsr1, Cdc42, Bem1, and Far1 (47, 70, 434, 644). Domains of Cdc24 that interact with some of its binding partners have been mapped. The conserved PB1 (phox and Bem) domain of Bem1 interacts with the PC motif-containing region at the C terminus of Cdc24 (47, 69, 249, 565). The mating-specific alleles of *cdc24*, which fail to interact with Far1 but are not defective in budding, have been mapped in the CH domain, suggesting that Far1 interacts with the CH domain of Cdc24 (70, 408, 409). Which domain of Cdc24 interacts with Rsr1 is less clear. Rsr1 in its GTP-bound state can interact with the C-terminal half of Cdc24 in vitro (434), and this interaction is necessary for the localization of Cdc24 to the presumptive bud site (436). Consistent with these data, the C-terminal half of Cdc24 is important for its localization to the site of polarized growth, whereas the N-terminal region is required for its localization to the nucleus (571, 572). On the other hand, it has been shown that Rsr1 exhibits a two-hybrid interaction with the CH domain of Cdc24 (523), which overlaps with the Far1 binding domain located near the N terminus. It is possible that more than one domain of Cdc24 interacts with Rsr1 and that each study may have overlooked the other binding site. Several questions remain. For example, does the CH domain interact directly with Rsr1 in a GTP-dependent manner? Does Rsr1 interact with more than one domain of Cdc24 at the same time in vivo? Is the Rsr1-Cdc24 interaction important only for guiding Cdc24 to the proper bud site, or does it also affect the GEF activity of Cdc24? It has been reported that Cdc24 inhibits both the intrinsic and the GTPase-activating protein-stimulated GTPase activity of Rsr1, suggesting that Cdc24 acts as a GTPase inhibitor protein for Rsr1 (644). However, this notion is somewhat surprising given that Rsr1 does not exhibit a high intrinsic GTPase activity in vitro (even in the absence of Cdc24) (435), and thus, the physiological significance of this observation is unclear.

Cdc24 localizes to the presumptive bud site in the late G_1 phase and to sites of polarized growth during the cell cycle (408, 522, 571). In wild-type cells, the localization of Cdc24 to the presumptive bud site is likely to occur through the interaction with Rsr1-GTP (434, 436, 644). However, Cdc24 still localizes to a single site, although at a random location, in the absence of Rsr1 in the late G_1 phase, indicating that other mechanisms operate in the Cdc24 clustering in cells lacking Rsr1. In the late M and early G_1 phases, Cdc24 localizes to the nucleus through the interaction with Far1 (411, 522). The export of Cdc24 from the nucleus is triggered either by entry into the cell cycle or by mating pheromones. Activation of the cyclin-dependent kinase (CDK) Cdc28 by G_1 cyclin Cln2 triggers the degradation of Far1, and as a result, Cdc24 is relocated from the nucleus to the presumptive bud site (196). This Cdc28/Cln2-triggered relocation of Cdc24 defines one step for the temporal regulation of polarity establishment (see Temporal Control of Polarity Establishment during Yeast Budding for further discussion).

Isolation of the mating-specific alleles of *CDC24* uncovers important roles of Cdc24 in polarity establishment as well as cell fusion during mating (see below [*Cell Polarization during*

Mating”]). Certain alleles of *CDC24* also exhibit the bud site selection defect (534, 535), while others display sensitivity to high-calcium growth media (421, 422). In addition, certain *cdc24* alleles are also sensitive to high- Na^+ growth media and exhibit synthetic lethality with a vacuolar ATPase subunit mutant, *vma5* (611), suggesting that Cdc24 might be involved in Na^+ tolerance and in vacuole function. However, the role of Cdc24 in calcium-mediated regulation or in vacuole function is not well established. The genetic interaction between *CDC24* and the genes involved in vacuole morphology or function is likely to be related to its role as a GEF for Cdc42 since Cdc42 is implicated in vacuole membrane fusion (402). Cdc42 promotes the assembly of the actin cytoskeleton (see below), which is also involved in vacuole inheritance or movement (224).

Cdc42 GAPs. There are four potential GTPase-activating proteins (GAPs) for Cdc42: Bem2 (46, 282), Bem3 (645), Rga1/Dbm1 (91, 550), and Rga2 (181, 536). In vitro GAP assays indicate that Bem2 acts on Cdc42 (357) and Rho1 (446); Bem3 acts mainly on Cdc42 and, to a lesser extent, on Rho1 (645, 646); and both Rga1 and Rga2 act on Cdc42 (181, 536). These in vitro assays, together with two-hybrid interaction data (536, 550), suggest that Bem3, Rga1, and Rga2 are more specific to Cdc42, whereas Bem2 is a GAP for both Cdc42 and Rho1 (Fig. 3A) (see the section on Rho1 GAPs below for more discussion on Bem2).

Why does Cdc42 need multiple GAPs? Are the GAPs involved in distinct functions, or do they share a redundant role? Can they also carry out a specific biological function as a part of the Cdc42 effector? Answers to these questions are not yet clear. In contrast to *CDC42*, none of the genes encoding the putative Cdc42 GAPs is essential. *BEM3* was originally isolated as a multicopy suppressor of a *bem2*-Ts mutant, which is defective in bud emergence (645). *RGAI/DBM1* was identified in a genetic screen designed to isolate mutants that activate the pheromone response pathway in the absence of the Ste4 G β subunit (550) and also as a dominant suppressor of a *bem2*-Ts mutant (91). Deletion of *RGAI* results in increased expression of a *FUS1::lacZ* reporter gene. Another potential Cdc42 GAP, Rga2, was identified through its homology to Rga1 (536, 550). The *rga1 Δ bem3 Δ* mutant in some strain backgrounds produces a high percentage of elongated cells, while the single mutant of either *rga1 Δ* or *rga2 Δ* does not produce such abnormally shaped cells (83, 181, 536). A *bem3 Δ* mutant also produces a low percentage of abnormally shaped cells, such as cells that are peanut or finger shaped, depending on strain backgrounds. The *rga1 Δ bem3 Δ* double mutant and the *rga1 Δ rga2 Δ bem3 Δ* triple mutant show more misshapen cells than the *bem3 Δ* single mutant. Deletion of *RGAI* also leads to an increase in the bipolar budding pattern in haploids instead of the axial budding pattern (91, 536), suggesting that Rga1 has a distinct function in bud site selection that is not shared by Rga2 or Bem3. It is not clear why a mutation of a Cdc42 GAP leads to a defect specifically in the axial budding pattern. Distinct phenotypes of strains lacking *RGAI*, *RG2*, or *BEM3* led to a suggestion that each GAP may regulate different functions of Cdc42, although quantitative differences in the GAP activities of the mutants may contribute to the overall phenotype (536). Importantly, the strain lacking all three GAPs, *rga1 Δ rga2 Δ bem3 Δ* , displays a much more elongated bud morphology and

increased induction of *FUS1-LacZ* than any single or double GAP mutants (although the extent of these defects seems to vary depending on strain backgrounds) (83, 181, 536), both of which are consistent with more activation of Cdc42. Rga1, Rga2, and Bem3 may thus play some overlapping roles in regulating morphogenesis and mitogen-activated protein (MAP) kinase (MAPK) activation during the mating response, presumably via their GAP activity towards Cdc42.

The localization of Bem3 and Rga2 is similar to that of Cdc42: both Bem3 and Rga2 were observed at the presumptive bud site, the tips of small buds, and the mother-bud necks of cells late in the cell cycle, although no distinct signal was detected at intermediate stages (83). Rga1 exhibits a distinct localization pattern. It localizes to the presumptive bud site and to the cortex of a tiny bud rather than only at the bud tip. Unlike Bem3 and Rga2, Rga1 localizes as a ring to the bud site of the neck in cells with a medium or large bud. Later in the cell cycle, Rga1 localizes to the neck as a double ring, with approximately equal intensities for both rings. Localization of these Cdc42 GAPs to the neck, but not to the presumptive bud site or bud tip, depends on septins (83). A large-scale localization study indicated that Bem2 is found at the bud neck and the cytoplasm (237). It remains to be determined whether the localization pattern of each GAP contributes to the functional differences in Cdc42 GAPs. In summary, Cdc42 GAPs are likely to play differential and overlapping roles in regulating polarized cell growth via their GAP activity. An important question is how Cdc42 GAPs are temporally and spatially regulated in the cell cycle so that they can coordinately regulate Cdc42 activity.

Rho GDI. Rho GDI (GDP dissociation inhibitor) is known to display three biochemical activities: inhibiting the dissociation of GDP from Cdc42, Rho, and Rac (95, 165, 312); inhibiting the intrinsic and GAP-stimulated GTPase activity of Cdc42, Rho, and Rac (95, 208, 214); and extracting Cdc42, Rac, and Rho from cellular membranes into cytosol (232, 416). The first and third activities inhibit GDP/GTP exchange and decrease the membrane pool of small GTPases, respectively, leading to the perception that Rho GDIs act as negative regulators of these GTPases. In contrast, the second activity maintains Cdc42, Rho, and Rac in their GTP-bound form, suggesting that Rho GDI may also play a positive role in the functions of these GTPases. The C-terminal lipid modification of Rho GTPases is essential for their binding to the GDIs (208, 229, 232) (Fig. 3B). In addition, the switch I and switch II regions of Rho GTPases, which bind to their GEFs, GAPs, and effectors, and the polybasic region, which is required for their targeting to the plasma membrane, are also involved in their interactions with the GDIs (110, 229, 259) (Fig. 3B). Unlike Rab GDIs, which bind preferentially to the GDP-bound form of Rab GTPases (17, 448), Rho GDIs bind equally well to both the GTP- and the GDP-bound forms of Cdc42, Rho, and Rac (208, 416). Rab GDIs also play an important role in delivering and/or loading Rab GTPases to target membranes (177, 447, 448), whereas such a role may not exist for Rho GDIs (120), as Cdc42 and Rac mutants deficient in their interactions with Rho GDIs appear to target to plasma membranes and elicit normal biological responses (167, 174, 175).

Rdi1, the only known Rho GDI in *S. cerevisiae* (365), can efficiently extract Cdc42 and Rho1 from the vacuolar membrane

(133) and can also extract Cdc42 from other membranes including the plasma membrane (468, 563). Deletion of *RDI1* does not produce any detectable phenotypes (365) and does not affect the clustering of Cdc42 at the sites of polarized growth (468). Rho1 and Cdc42 were found in the cytosol of the *rdi1*Δ cells to a similar extent as in wild-type cells, suggesting that other mechanisms or other GDI-like activities are responsible for the cycling of these GTPases between membranes and the cytosol (288). Overexpression of Rdi1 causes a slightly rounder cell morphology in some strain backgrounds (563) but causes lethality in other strain backgrounds (365) for reasons that are not known. In a *cdc24*-Ts mutant where the activation of Cdc42 is compromised, overexpression of Rdi1 causes lethality with cells arrested as large, round, unbudded cells, which is indicative of a loss of cell polarity (563). These overexpression phenotypes are consistent with Rdi1 being a negative regulator of Cdc42.

Rdi1 localizes to the sites of polarized growth in the cell cycle, the tip of a small bud and the mother-bud neck during cytokinesis (468). Unlike Cdc42 (466), Rdi1 does not appear to localize to the presumptive bud site and internal membranes (468). The Rdi1 localization pattern is consistent with the possibility that there might be a pool of Cdc42-GDP at sites of polarized growth. This notion is supported by the observation that the GDP-locked form of Cdc42, Cdc42^{D57Y}, clusters at the presumptive bud site as efficiently as the wild type and the active form of Cdc42 (605).

Cycling of Cdc42 between the GDP- and GTP-bound states. *CDC24*, which encodes the only known GEF for Cdc42 (563, 645), is an essential gene (215, 388, 534), suggesting that the exchange of GTP for GDP is essential for Cdc42 function. Three *cdc42* alleles, *cdc42*^{G12V}, *cdc42*^{Q61L}, and *cdc42*^{D118A}, which contain mutations in the putative GTP-binding and hydrolysis domains, cause dominant dosage-dependent lethality, suggesting that GTP hydrolysis by Cdc42 is essential for its normal function (650). Consistent with this interpretation, the triple mutant of Cdc42 GAPs, *rga1*Δ *rga2*Δ *bem3*Δ, is defective in bud morphogenesis and septin organization (see below for details) (83, 181, 536). Cdc42 still possesses GTPase activity to some extent in the *rga1*Δ *rga2*Δ *bem3*Δ mutant (645), consistent with the fact that the phenotype of the GAP triple mutant is less severe than that of the mutants expressing the GTP-locked form of Cdc42. It is possible that there is an additional GAP (such as Bem2) for Cdc42 and/or that the intrinsic GTPase activity of Cdc42 is sufficient for cell survival.

Cdc42 GTPase appears to function in two different modes: one, like Ras, to turn on signaling pathways in its GTP-bound state and another, like the translation elongation factor EF-Tu (EF-1 in eukaryotes), to assemble a macromolecular structure such as the septin ring (181). The cycle of nucleotide binding and GTP hydrolysis is critical for the latter mode of action, as was also proposed for Sec4 (601) and Rsr1 (434, 435, 484). The cycling feature of Cdc42 can best explain the paradoxical phenotypes of the *cdc42*^{G60D} mutation, which makes Cdc42 hyperactive and causes multiple buddings per cell cycle yet is completely recessive in terms of the budding phenotype (84). Perhaps the Cdc42^{G60D} mutant protein may cycle more slowly than wild-type Cdc42. If rapid cycling of Cdc42 is required for establishing and maintaining a single site for budding in each cell, wild-type Cdc42 should outcompete the mutant Cdc42 for recruiting effectors and other downstream components to a

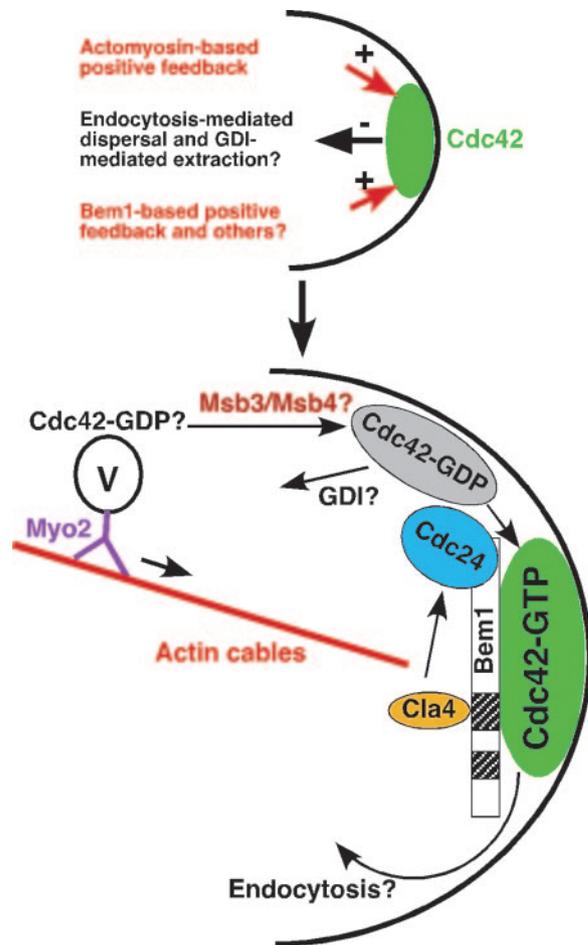


FIG. 4. Model for the establishment and maintenance of Cdc42 polarization in the absence of a spatial cue. (Top panel) It is thought that activated Cdc42 clusters spontaneously and transiently and becomes stabilized by interactions among its effectors and/or regulators. To enhance and/or maintain the initial Cdc42 polarization at a growth site, at least two positive feedback mechanisms are operating: an actomyosin-based delivery of Cdc42 and a Bem1-based activation of Cdc42. These positive actions are counteracted by endocytosis-mediated dispersal and GDI-mediated extraction of Cdc42 away from the plasma membrane. (Bottom panel) A molecular model for Cdc42 polarization at a growth site. Cdc42 in its GDP-bound state is carried on secretory vesicles, which are transported along actin cables to the growth site by the type V myosin Myo2. The “Cdc42-GDP” cargo is captured by Msb3 and Msb4 or other factors capable of binding to Cdc42-GDP, resulting in the local accumulation of Cdc42-GDP, poised for activation by the GEF Cdc24. The Bem1-based complex functions to bring Cdc42, Cdc24, and the PAK Cla4 together, resulting in the phosphorylation of Cdc24 by Cla4 and in the further accumulation of Cdc42-GTP at the growth site. How endocytosis mediates the dispersal of Cdc42 from the polarization site remains unknown.

single site, thus suppressing the mutant phenotype (84). This hypothesis may explain, at least in part, the recessiveness of the *cdc42*^{G60D} allele. This hypothesis is also supported by a recent observation: fluorescence recovery after photobleaching (FRAP) analysis indicates that wild-type Cdc42 at the presumptive bud site recovers much more quickly after photobleaching than Cdc42^{Q61L} and Cdc42^{D57Y}, which are expected to be the GTP- or GDP-locked Cdc42, respectively (605). However, it is likely that there are some intrinsic differences

between the *cdc42^{G60D}* and *cdc42^{Q61L}* mutants, since the former is recessive, whereas the latter is dominant.

Regulation of Cdc42 Clustering at Sites of Polarized Growth

One of the key issues concerning the role of Cdc42 in polarity development is to understand how Cdc42 itself becomes polarized and how its polarization state is maintained during the cell cycle (Fig. 4). Cdc42 localizes to the presumptive bud site, which is determined by the bud site selection machinery. However, Cdc42 localizes to sites of polarized growth even in the absence of spatial cues (245, 466, 604, 605, 651). Although Cdc42 polarization at a random bud site occurs under non-physiological conditions, it has allowed us to decipher the mechanisms of cell polarization and appreciate the concept of “symmetry breaking” in budding yeast. In this section, we will discuss how Cdc42 polarization is established and maintained. Conceptually, it is useful to distinguish the establishment of Cdc42 polarization from the maintenance of its polarization. It is, however, difficult to separate these two processes, because they are occurring almost simultaneously at the same site. For the purpose of discussion, we will separate the two issues here.

Establishment of Cdc42 polarization. Establishment of Cdc42 polarization in wild-type cells occurs at a spatially defined site, which requires the coupling of the Cdc42 module to the Rsr1 GTPase module (see below [“Coupling of the Rsr1 GTPase to the Cdc42 GTPase Module”]). Activation of Cdc42 by its GEF Cdc24 is required for its polarization. When the spatial regulatory mechanism of cell polarity is absent, such as in *rsr1Δ* mutants, the budding process does not seem to be compromised, except that the mutant cells bud at a random site (45, 87). This spontaneous cell polarization process in the apparent absence of spatial cues is called “symmetry breaking.” Cdc42 polarization also occurs normally once per cell cycle at a random location in *rsr1Δ* cells (245, 466, 604, 605, 651). Thus, there is a default pathway leading to Cdc42 polarization in the absence of putative upstream events such as bud site selection. Other mechanisms must operate in *rsr1Δ* (and presumably also in wild-type) cells to establish and maintain Cdc42 polarization. Cdc42 polarization can occur in the *rsr1Δ* and *bem1* single mutants but not in the *rsr1Δ bem1* double mutant (245) or in *bem1Δ* cells in which filamentous actin (F-actin) has been disrupted by latrunculin A (LatA) (605). Cdc42 still polarizes to a single random site when bud site selection, F-actin, and microtubules are simultaneously disrupted (245). Thus, the predetermined spatial cue, F-actin, and the microtubule are not essential for Cdc42 polarization per se, although it does not rule out the possibility that these components act cooperatively to enhance Cdc42 polarization. Taken together, these results indicate that bud site selection proteins, the signaling protein Bem1, which binds to Cdc42-GTP (58), and F-actin all share an essential role in achieving a polarization state of Cdc42 (245, 605). One possibility is that the spontaneous clustering of activated Cdc42 in the absence of a spatial cue initiates Cdc42 polarization, but Bem1 or F-actin plays a crucial role in stabilizing, amplifying, and/or maintaining this initial polarization state.

Various active forms of Cdc42, Cdc42^{G12V} (196, 466) and Cdc42^{Q61L} (604), are able to polarize in G₁-arrested cells in the presence of endogenous Cdc42. Cdc42^{G60D}, as the sole

source of Cdc42 in the cell, can also cluster on the plasma membrane randomly at more than one site, resulting in multiple budding events per nuclear cycle (84). Thus, it has been proposed that the activated Cdc42 becomes clustered through stochastic movement on the plasma membrane (84, 604) and that this initial clustering of Cdc42 could be stabilized by the interactions with Cdc42 effectors in the cell cortex (84).

Maintenance of Cdc42 polarization. Cdc42 is highly dynamic at the sites of polarized growth, as indicated by FRAP analysis (605), suggesting that the Cdc42 concentration at these sites has to be dynamically maintained. Because GTP- or GDP-locked Cdc42 recovers more slowly than wild-type Cdc42 after photobleaching, cycling between the two nucleotide-bound states of Cdc42 is likely to be important for its dynamic accumulation at sites of polarized growth (605).

(i) The central role of Bem1 in maintaining Cdc42 polarization. Bem1 binds to Cdc24, Cdc42-GTP, and Cla4, a PAK known to be an effector of Cdc42 (58, 196, 446, 644). It has been suggested that this protein complex enhances Cdc42 polarization and is thus involved in both the establishment and the maintenance of Cdc42 polarization. Cdc28/G₁ cyclin complexes trigger Cdc42 activation indirectly through Cdc24, a GEF for Cdc42. Activated Cdc42 binds to Bem1, which, in turn, binds to Cdc24 and Cla4, and Cla4 phosphorylates Cdc24 (58, 69, 196, 245). This cascade of events may result in the accumulation of more Cdc24 and Cdc42 at sites of polarized growth. Thus, it has been proposed that the components of the Bem1-mediated protein complex constitute a positive feedback loop to establish and maintain Cdc42 polarization (69, 245) (see below [Temporal Control of Polarity Establishment during Yeast Budding] for further discussion).

(ii) Role of F-actin in maintaining Cdc42 polarization. Two major filamentous actin structures found in yeast are actin cables and actin patches, which are required for polarized exocytosis and endocytosis, respectively (457). Cdc42 polarizes normally in cells treated with latrunculin A (30, 246), which disrupts all F-actin structures, suggesting that Cdc42 polarization can be established and maintained in the absence of F-actin. However, when cells are treated with a less potent drug, latrunculin B (which disrupts all actin cables but not all actin patches), Cdc42 fails to cluster at the sites of polarized growth, suggesting that endocytosis may be involved in Cdc42 dispersal from its polarization site. In addition, in mutants conditionally defective in actin cable formation or in post-Golgi vesicle transport, Cdc42 polarization can occur initially but cannot be maintained (246). Thus, it appears that once Cdc42 polarization is established in an F-actin-independent manner, actin cable-mediated delivery of Cdc42 is required to counteract the actin patch-mediated dispersal of Cdc42 such that a dynamic pool of Cdc42 can be maintained at the sites of polarized growth.

(iii) Role of exocytosis in maintaining Cdc42 polarization. Cdc42 polarization is not maintained in a number of mutants that are defective at various stages of exocytosis. For example, Cdc42 fails to maintain polarization in the tropomyosin mutant *tpm2Δ tpm1-2* (246, 460, 604, 637), the type V myosin mutants *myo2-16* (246) and *myo2-66* (604), and the late *sec* mutants *sec4-8* and *sec5-24* (637). These data indicate that Cdc42 is delivered to sites of polarized growth through exocytosis and that actomyosin-based vesicle transport and the subsequent

vesicle tethering and/or fusion are required for maintaining Cdc42 polarization. It is noteworthy that the establishment of Cdc42 polarization is independent of polarized exocytosis, as Cdc42 polarization can occur in cells treated with LatA (30, 246) as well as in cells carrying tropomyosin and *myo2* mutations (246, 460, 604, 637), all of which block polarized secretion but not secretion per se. In summary, current data support the view that Cdc42 polarization initiates polarized growth, while polarized exocytosis reinforces or maintains polarized growth by delivering more polarity factors, including Cdc42, to sites of polarized growth.

(iv) **A possible mechanism of concentrating Cdc42-GDP at sites of polarized growth.** The Rsr1 GTPase module is likely to be involved in the recruitment of Cdc42 to the proper bud site (292). In *rsr1Δ* cells, however, a mechanism involving Msb3 and Msb4 is likely to operate. Msb3 and Msb4 may also carry out a similar function in wild-type cells as well, but it becomes more apparent in *rsr1Δ* cells. Msb3 and Msb4, a pair of homologous proteins each containing a Rab GAP domain (12, 13, 168), function as dosage-dependent suppressors of *cdc24* and *cdc42* mutants (51, 168, 563). Although a deletion of either *MSB3* or *MSB4* does not produce any obvious defect, the deletion of both genes results in a large, round mother with small buds, and a significant fraction of the double mutant cells has a disorganized actin cytoskeleton (32, 51). Thus, Msb3 and Msb4 may function in the same pathway as Cdc42. Msb3 and Msb4 interact with Spa2, a scaffold protein of the “polarisome” (563) (see below). Spa2 localizes to the presumptive bud site prior to Start in the late G₁ phase, and this localization is dependent upon Cdc42 but not its GEF Cdc24, suggesting that Cdc42-GDP may play some role in bud site assembly (470). Interestingly, Msb3 and Msb4 bind specifically to Cdc42-GDP and Rho1-GDP but not Rho3 and Rho4 (563). Like Cdc42, Msb3 and Msb4 localize to sites of polarized growth (51, 168). Together, these results have led to a hypothesis that Msb3 and Msb4 are involved in recruiting Cdc42 from the cytosol and/or capturing Cdc42 from the secretory pathway, increasing a local pool of Cdc42-GDP at the sites of polarized growth, which is poised for activation by the GEF Cdc24 (563). Other functions of Msb3 and Msb4, including their GAP activity towards Rab GTPases and their functions in the Rho1 pathway, will be discussed below.

DETERMINATION OF THE AXIS FOR CELL POLARIZATION DURING BUDDING

Different cell types in *S. cerevisiae* display distinct patterns in the selection of a cortical site (bud site) for polarized growth. Haploid **a** and α cells bud in the axial pattern in which both mother and daughter cells select a bud site immediately adjacent to their previous division site. In contrast, diploid **a/α** cells bud in the bipolar pattern: mother cells select a bud site adjacent to their daughter or on the opposite end of the cell, whereas daughter cells almost exclusively choose a bud site directed away from their mother (89, 158, 222) (Fig. 5A). These two distinct patterns of budding reflect genetic programming of cell polarization. The choice of a bud site determines the axis of cell polarity and ultimately the cell division plane, which is perpendicular to the axis of cell polarity. These patterns of cell division result in characteristic shapes of micro-

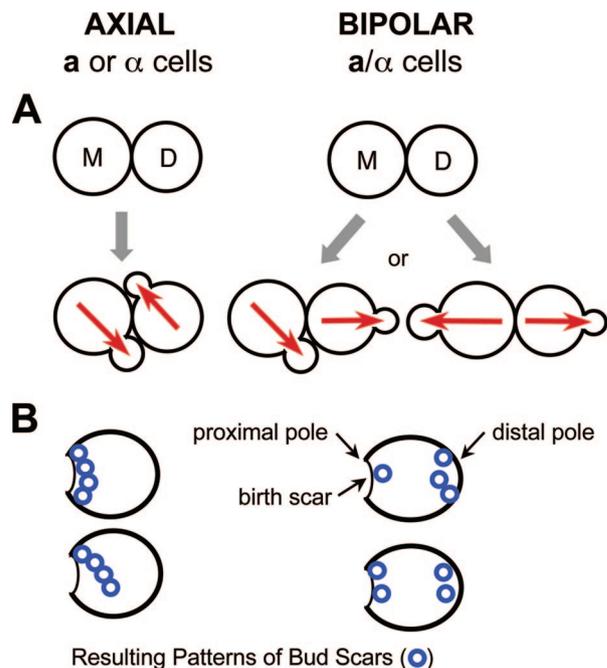


FIG. 5. Cell type-specific budding patterns in *S. cerevisiae*. (A) Axial and bipolar patterns of cell division as observed in cells growing on a solid surface. The axes of cell polarity are indicated with red arrows. (B) Patterns of bud scars on the yeast cell surface resulting from the two modes of budding. On each cell, a single birth scar marks the pole at which the cell was attached to its mother. A bud scar shown as a blue ring marks a division site on the mother cell surface. Bud scars can be visualized by staining with calcofluor dye or by scanning electron microscopy. In the axial pattern, scars form a continuous chain as shown in the two cells on the left. In the bipolar pattern, scars cluster around the poles: the birth pole (proximal pole) and the pole opposite the birth end (distal pole). (Modified from reference 85 with permission. © 1999 by Annual Reviews.)

colonies on a solid surface (Fig. 5A) and distinct patterns of bud scars, which mark the sites of cell division on the mother cell surface (Fig. 5B). In cells undergoing axial budding, the division site is likely marked by a spatial signal(s) that specifies the location of the new bud site (89). Since the starvation and refeeding of axially budding cells result in the formation of a new bud at a nonaxial site, the spatial signal for the axial budding pattern appears to be transient in that it lasts only from one budding event to the next (89). Despite the transient nature of the axial spatial cues, haploid **a** or α cells exhibit the axial pattern with remarkably high fidelity during continuous logarithmic growth. Unlike the transient nature of the axial spatial cue, the bipolar spatial cue(s) appears to consist of persistent cortical markers that are present at both poles of diploid **a/α** cells (89).

The different budding patterns are likely to occur in response to cell type-specific cortical markers, which are associated with the plasma membrane. A large number of genes are required for producing these specific budding patterns (45, 87, 163, 207, 476, 636). These genes can be divided into three groups based on their requirement for each budding pattern. The first group of genes, which includes *RSR1/BUD1*, *BUD2*, and *BUD5*, is required for both budding patterns and thus encodes proteins that constitute the “general site selection

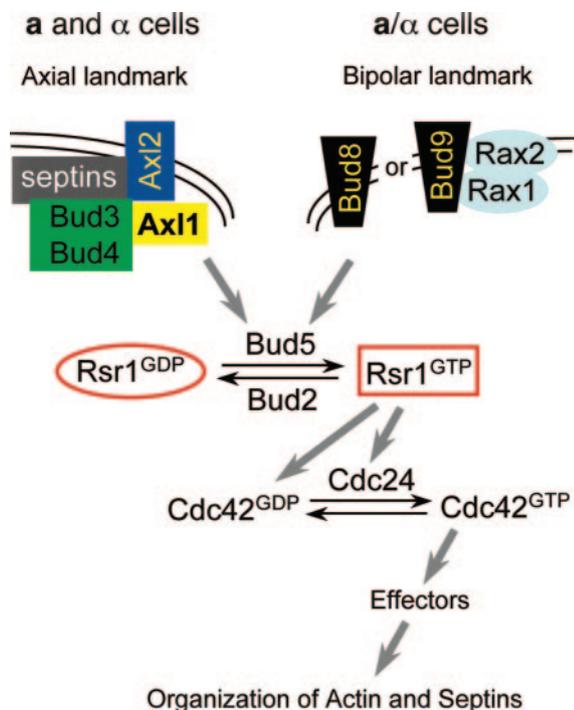


FIG. 6. Model for the molecular pathway governing axial and bipolar budding in haploid *a* or α cells and in diploid *a*/ α cells. Although physical interactions have been demonstrated for some proteins, many of them are postulated based on genetic and localization data. The figure also does not imply that all the proteins shown necessarily interact at the same time (see the text for details and Fig. 11 for Cdc24 effectors and their downstream components). (Modified from the supporting online material from reference 273 with permission of the publisher.)

machinery” (89). The second group, which includes *BUD3*, *BUD4*, *AXL1*, and *AXL2/BUD10*, is required only for the axial pattern. The third group, which includes *BUD7* to *BUD9*, is required only for bipolar budding. The deletion of any of these genes, collectively called “*BUD* genes,” results in a bud site selection defect but no obvious growth defect. Thus, the *BUD* gene products are involved in marking the site for polarized growth or directing growth at a specific location. In this section, we will first discuss the Ras-like GTPase Rsr1 and its regulators Bud2 and Bud5, which play a key role in linking the spatial cues to the downstream polarity machinery. We will then discuss how the Rsr1 GTPase module may be coupled to the Cdc24 module. Finally, we will discuss the molecular nature of the spatial landmarks and models for the cell type-specific budding patterns.

The Rsr1 GTPase Module—Rsr1, Bud2, and Bud5

Each component of the Rsr1 GTPase module, Rsr1, Bud2, and Bud5, belongs to a highly conserved family of proteins (45, 86, 87, 435). Rsr1 belongs to the Ras family of GTPases (45). The putative effector region of Rsr1 is identical to that of Ras (45) and is necessary for the interaction with its downstream target, Cdc24 (434). Bud2 is a large polypeptide of 1,104 amino acid residues containing a domain similar to that of the Ras GAP family of proteins such as NF1 (435). Bud2 activates GTP

hydrolysis by Rsr1, and thus Bud2 acts as a GAP for Rsr1 (44, 435, 438). Bud5 is likely to encode a polypeptide of 608 amino acid residues (272) containing a domain similar to that of the GEFs for the Ras family of GTPases (86, 453). Bud5 acts as a GEF for Rsr1 (44, 644). Thus, Rsr1 GTPase, the GAP Bud2, and the GEF Bud5 constitute a functional GTPase module involved in the selection of a proper site for growth (435) (Fig. 6).

Phenotypes of the *rsr1* mutants provided the first clue for the mechanism by which the Rsr1 GTPase module functions. Expression of *rsr1*^{G12V} or *rsr1*^{K16N}, which is expected to encode Rsr1 constitutively in the GTP-bound state or in the GDP-bound (or nucleotide-empty) state, respectively, leads to random budding (484). Consistent with this observation, the deletion of *BUD2* or *BUD5* results in random budding (86, 435). The cycling of Rsr1 between the GTP- and GDP-bound states is therefore critical for its function in bud site selection. Rsr1 interacts with specific binding partners depending on its GTP- or GDP-bound state (292, 434, 644), as discussed below (see Coupling of the Rsr1 GTPase Module to the Cdc24 GTPase Module).

The localization of Rsr1, Bud2, and Bud5 is consistent with their functions at the presumptive bud site in each cell type. Rsr1 fused to GFP localizes to the plasma membrane and then becomes concentrated at sites of polarized growth, first to the presumptive bud site, the bud tip, and then the mother-bud neck at a later stage of the cell cycle (436). GFP-Rsr1 is also present at the internal organelle membranes, particularly the vacuolar membrane (436). Subcellular fractionation of Rsr1 is also consistent with its localization pattern (434). Localization of Rsr1 to the plasma membrane and to the sites of polarized growth requires both the *CAAX* box and the polylysine residues near the C terminus (436). The replacement of Cys269 with Ser in the *CAAX* box of Rsr1 abolishes all membrane association of Rsr1, whereas the replacement of all Lys residues at positions 260 to 264 with Ser disrupts the localization of Rsr1 to the plasma membrane and to the sites of polarized growth but not to the internal organelle membrane (436). Since both mutations cause random budding (436), localization of Rsr1 to the plasma membrane and to the sites of polarized growth is necessary for its function in bud site selection, whereas its localization to the internal organelle membrane is not sufficient for its function. It is not known whether the localization of Rsr1 to the internal membrane indicates other unknown functions of Rsr1 or reflects intermediate locations during its delivery to the plasma membrane.

Bud2 localizes in a patch at the incipient bud site in the late *G*₁ phase: an axial bud site in haploid *a* or α cells or either the proximal or distal pole of diploid *a*/ α cells (437). Bud2 localizes to the mother-bud neck after bud emergence and then delocalizes around the *G*₂/*M* phase in all cell types (358, 437). In haploid *a* or α cells, Bud5 localizes to the presumptive bud site in *G*₁, to the tip of growing buds after bud emergence, and then to the mother-bud neck around the *G*₂/*M* phase. In the late *M* phase, Bud5-GFP appears as a double ring at the neck, which splits into two single rings upon cell separation, and both mother and daughter cells inherit a single ring. Thus, most of the newly born *G*₁ cells have the Bud5 ring at the division site (273, 358). This localization pattern of Bud5 is similar to that of Ax12, which is required for the axial budding pattern (see below), throughout the cell cycle. Bud5-GFP exhibits distinct

localization patterns in diploid a/α cells, particularly during G_1 and M phases. Before bud emergence, Bud5-GFP is present at both poles: as a ring at one pole, which is the previous division site, and in a patch at the opposite pole, which becomes a new bud site. After bud emergence, Bud5-GFP localizes throughout the periphery of the bud, as seen in haploid cells. At a later stage of the cell cycle, Bud5-GFP localizes to the neck and one pole of the mother cell (and/or bud tip), while a small percentage of cells shows a Bud5-GFP signal only at the neck (273, 358). Taken together, these specific patterns of localization of Bud2 and Bud5 are probably important for proper bud site selection, since the overexpression of Bud2 or Bud5 results in the mislocalization of each protein and causes random budding (273). Consistent with this notion, some alleles of *BUD5* that disrupt the proper localization of Bud5 only in a/α cells are specifically defective in the bipolar budding pattern (273). Mislocalization of Bud5 in other *bud* mutants suggests that Bud5 localizes to specific sites in each cell type through the interaction with the cell type-specific landmark (272, 273) (see below).

Coupling of the Rsr1 GTPase Module to the Cdc42 GTPase Module

Interaction between Rsr1 and the Cdc42 module. Numerous genetic interactions between the *BUD* genes and genes involved in bud site assembly have suggested a functional interaction between the Rsr1 GTPase module and the Cdc42 GTPase module (for reviews, see references 126 and 221) (Fig. 6). *RSR1* was isolated as a multicopy suppressor of a *cdc24-Ts* (*cdc24-4*) mutant (45). Certain alleles of *CDC24*, including *cdc24-4*, exhibit a bud site selection defect (534, 535). *RSR1* also interacts with *CDC42* (292) (see below). A *bud5* mutation exacerbates the phenotype of a *bem1* mutant (*bem1-2*): the *bud5 bem1-2* double mutant fails to undergo bud emergence at the nonpermissive temperature, while the single mutants can (86). Rsr1 also physically interacts with proteins involved in bud site assembly in a guanine nucleotide-dependent manner. Rsr1-GTP interacts with Cdc24 (434, 644) through its putative "Ras-like effector domain" (434) and also with Cdc42 (292), whereas Rsr1-GDP interacts with Bem1 (434).

The interaction between Rsr1 GTPase and Cdc42 GTPase is particularly interesting. *RSR1* was identified as an allele-specific dosage suppressor of a *cdc42* allele (*cdc42-118*) that is defective in polarity establishment (292). This suppression requires the "Ras-like effector domain" of Rsr1 and the cycling of Rsr1 between its GDP- and GTP-bound states (292). In addition, an *rsr1* deletion mutant was found to be synthetic lethal at 30°C with *cdc42-118* (292). Rsr1 also physically interacts with Cdc42, and this interaction appears to be enhanced in the presence of Cdc24 (292). Interestingly, the *rsr1-7* mutant, which carries mutations in the polylysine repeat near the C terminus of Rsr1, suppresses a *cdc24* mutant but fails to suppress a *cdc42* mutant. The mutation may thus disrupt the interaction between Rsr1 and Cdc42 but not the interaction between Rsr1 and Cdc24. These data are consistent with the idea that the interaction between Rsr1 and Cdc42 is likely to be direct, rather than being bridged by Cdc24, a GEF for Cdc42 (292). These findings provide a novel mechanism of action of GTPases controlling polarity establishment. Interestingly,

GTPase heterodimerization as a mechanism for activating GTPase signaling in bacteria (518) and plants (607) has recently been reported.

What would the physiological significance of the interaction between Rsr1 and Cdc42 be? The interaction between Rsr1 and Cdc42 may contribute to the localization of Cdc42 to the proper bud site, although it has not been directly addressed. The genetic interaction between *RSR1* and *CDC42* suggests that Rsr1 functions not only in selection of a growth site but also in polarity establishment. The latter role of Rsr1 becomes phenotypically apparent when polarity establishment is compromised as in a *cdc42-Ts* mutant (292). In addition, *RSR1* is essential in the absence of *GIC1* and *GIC2*, which encode two related targets of Cdc42 that are involved in polarity establishment (278). Cells lacking all three genes, *RSR1*, *GIC1*, and *GIC2*, fail to undergo bud emergence. A detailed analysis of live cells by high-resolution microscopy also indicates that Rsr1 is required for selecting and stabilizing the polarity axis in the G_1 phase of the cell cycle (426). Thus, it is likely that Rsr1 has a role in polarity establishment, although the exact role of Rsr1 in polarity establishment is yet to be determined.

Model for coupling bud site selection to polarity establishment. By analogy to Sec4 guiding vesicle targeting to the plasma membrane (61, 601), a scheme in which the Rsr1 GTPase cycle orchestrates bud site assembly to the proper bud site has been proposed (Fig. 7) (292, 434). The model hypothesizes that the cycle between Rsr1-GTP and Rsr1-GDP coupled with the differential affinities of each of these species for binding partners may trigger the ordered assembly of a complex at the bud site. First, Bud5 catalyzes the conversion of Rsr1 from the GDP-bound state to the GTP-bound state at the site where a cortical cue is located (step 1). Rsr1-GTP associates with Cdc24 and Cdc42 and guides these proteins to the presumptive bud site where Bud2 (and Bud5) localizes (step 2). Bud2 activates GTP hydrolysis by Rsr1, resulting in the dissociation of Cdc24 from Rsr1 (step 3). Cdc24 then catalyzes the conversion of GDP-Cdc42 to GTP-Cdc42 (step 4), which then triggers actin cytoskeleton assembly and targeted secretion. Finally, the action of Bud5 may recycle Rsr1, thus allowing further shuttling of the essential components for bud site assembly. Bem1 might join the complex through an interaction with Cdc24, Cdc42, or Rsr1-GDP (58, 69, 434, 446, 644). Several rounds of this cycle may be necessary to assemble critical levels of Cdc42-GTP and its associated proteins at the proper bud site. The model hypothesizes that the ordered assembly of a complex through multiple protein-protein interactions might ensure the establishment of polarity at a correct location. This model provides a simplified view for linking the bud site selection machinery to the Cdc42 module, but the details remain to be tested.

In the absence of the Rsr1 GTPase module, localization of Cdc24 and Cdc42 to a random bud site may occur through a distinct default pathway yet to be identified or by a "symmetry-breaking" mechanism (245, 604, 605) as discussed above.

Spatial Landmarks That Specify the Site for Polarized Growth

The Rsr1 GTPase module is coupled not only to the key polarity machinery but also to the spatial landmark that spec-

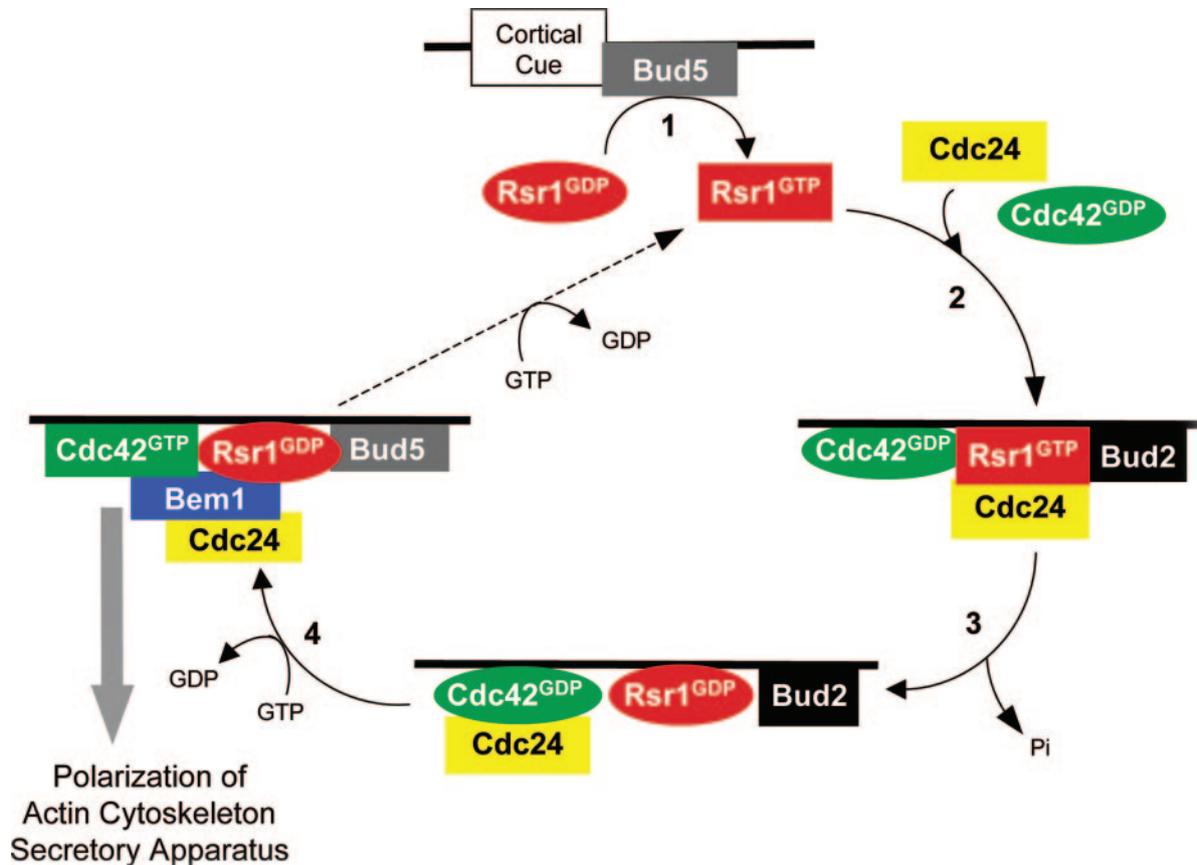


FIG. 7. Model of how the Rsr1/Bud1 GTPase cycle directs polarity establishment to a specific site. In step 1, Bud5 exchanges GDP for GTP on Rsr1. In step 2, Rsr1-GTP associates with Cdc24 and Cdc42 and guides them to the bud site. In step 3, Bud2 activates Rsr1 to hydrolyze the GTP bound to Rsr1. In step 4, Dissociation of GDP-bound Rsr1 from Cdc24 may activate Cdc24, which catalyzes the exchange of GDP for GTP on Cdc42. Cdc42-GTP then triggers actin assembly and exocyst localization to establish an axis of polarity. Bud5 at the bud site may convert Rsr1 to a GTP-bound state (dashed line), allowing for another cycle of signal transduction (see the text for details). (Adapted from reference 292 with permission of the publisher.)

ifies the site for polarized growth in each cell type. In this section, we first will describe proteins that are involved in the determination of the axis for cell polarization in each cell type. We will then discuss how the spatial landmarks are linked to the Rsr1 GTPase module and how the cell type-specific budding patterns might be determined.

Axial landmarks. The axial budding pattern depends on a transient cortical marker that involves a group of proteins such as Bud3, Bud4, Axl1, and Axl2/Bud10 (hereafter called Axl2) (4, 87, 88, 163, 207, 476, 491). Mutations in *BUD3*, *BUD4*, *AXL1*, and *AXL2* result in bipolar budding in haploid *a* or α cells but do not affect normal bipolar budding of diploid *a*/ α cells. Genetic and localization data support the view that the cycle of assembly and disassembly of a protein complex at the mother-bud neck provides a spatial memory of the position from one cell cycle to the next, acting as an inherited landmark for axial budding.

(i) **Bud3, Bud4, and septins.** Bud3 and Bud4 localize to the mother-bud neck at or after the G_2 phase (88, 491). Prior to cytokinesis, Bud3 and Bud4 appear as a double ring encircling the mother-bud neck, which splits into two single rings, one on each progeny, after cell division (88, 491). Septins, a family of related proteins including Cdc3, Cdc10, Cdc11, Cdc12, and

Shs1/Sep7 (335), also play an important role in axial budding. Some alleles of septin genes, such as *cdc10-10* and *cdc11-6*, are defective in axial budding (88, 152). Extra copies of *BUD4* suppress the temperature-sensitive growth of a *cdc12* mutant (491). Localization of Bud3 and Bud4 depends on the integrity of septins (88, 491), which localize as a ring to the incipient bud site and as a collar to the neck of budded cells (153, 202, 279, 336) (see below). Thus, Bud3 and Bud4 are likely to assemble at the mother-bud neck through a direct interaction with septins during the G_2 and M phases, whereas the assembly of septins and the bud site complex at the axial bud site in the subsequent division cycle is likely to occur through the action of the Rsr1 and Cdc42 GTPase modules during the G_1 phase (88, 181, 491). However, molecular details of the assembly of the axial landmark and the mechanisms by which the septins determine the localization of Bud3 and/or Bud4 are not known. Understanding the biochemical properties of Bud3 and Bud4 will shed light on their structural and/or regulatory role in axial budding.

(ii) **Axl1 and Axl2.** Axl1 and Axl2 are also required for the axial pattern but not for the bipolar pattern (163, 207, 476). Interestingly, Axl1 is expressed in *a* and α cells but not in *a*/ α cells, and ectopic expression of *AXL1* increases axial budding

in a/α cells (163). Although Axl1 is likely to be a key component for the cell type-specific budding patterns, how Axl1 functions in axial budding is yet to be established. Axl1 shares homology with the insulin-degrading enzyme family of endoproteases and is also required for processing of the mating pheromone a -factor precursor (4). Amino acid substitutions within the presumptive active site of Axl1 cause defects in the processing of the a -factor precursor but do not perturb bud site selection (4), suggesting that the protease activity of Axl1 is not important for bud site selection.

Axl2 is a transmembrane glycoprotein with an N-terminal signal sequence and a transmembrane domain in the middle. Axl2 is thus predicted to have the type I membrane topology, similar to that of integrin (476). As expected from the predicted structure, Axl2 is heavily glycosylated at the N-terminal half of the protein. Axl2 exhibits no similarity to the ligand-binding or catalytic domains of known transmembrane receptors. It is possible that Axl2 functions in a manner analogous to that of noncatalytic receptors, such as integrins, for which clustering appears to be important for sending a signal to the downstream components (207). However, it remains a possibility that a specific extracellular ligand for Axl2 exists, such as a component of the cell wall. It is also not known whether Axl2 undergoes clustering and the formation of oligomeric complex like the integrin or other extracellular matrix receptors and whether such clustering is required for the axial pattern.

Unlike Bud3 and Bud4, Axl1 and Axl2 are detectable before the G_2 phase: the Axl2 signal is most intense in cells with emerging buds and appears at the periphery of small buds (207, 476). The localized Axl1 signal is absent in late G_1 phase and weak in the S phase but is prominent in newly divided cells in early G_1 phase and in medium- and large-budded cells (341). Both Axl1 and Axl2 localize to the mother-bud neck as a ring in cells with medium- or large-sized buds. Axl1 and Axl2 appear as a double ring encircling the mother-bud neck prior to cytokinesis, and this double ring splits into two single rings after cytokinesis (207, 476). Axl1 localizes normally in the absence of a component of the Rsr1 GTPase module, which is predicted to function downstream of the axial landmark. In contrast, the Axl1-GFP signal is completely lost or diminished in the absence of Bud4 or Bud3, respectively (340). Neither Bud3 nor Axl2 requires the other for localization to the mother-bud neck, although Bud3 seems to be necessary for the efficient assembly of tight double rings of Axl2 at the neck (207). Bud4 seems to localize to the mother-bud neck normally in the absence of Axl2 (476).

Axl2 is delivered to the cell surface via the secretory pathway (452, 490). Since secretion is directed to the incipient bud site, this would suggest that the spatial cue directing targeted secretion is already present at the site before delivery of Axl2 in late G_1 phase. In this view, localization of Axl2 to the mother-bud neck during M phase and its subsequent inheritance at the division site after cytokinesis are likely to be more important for the axial pattern than its localization to the incipient bud site in late G_1 phase. It is noteworthy that Axl2 fails to localize specifically to the bud side of the mother-bud neck in the *pmt4* mutants, which are defective in O-linked glycosylation of some secretory and cell surface proteins, and daughter cells of the *pmt4* mutants exhibit a specific defect in the axial pattern

(490). These data support the notion that the localization of Axl2 to the mother-bud neck is important for proper bud site selection in the subsequent cell division cycle. On the other hand, a series of experiments addressing the significance of cell cycle-dependent expression of *AXL2* indicates that a G_1 pulse of *AXL2* expression plays a direct role in the localization and function of Axl2 (341), suggesting that the localization of Axl2 depends on the timing of its passage through the secretory pathway. It would be important to understand how the axial landmark is assembled and when/how the axial landmark is linked to the downstream component, i.e., the Rsr1 GTPase module (see below).

Bipolar landmarks. The bipolar budding pattern appears to depend on the persistent cortical markers that are present at both poles of diploid a/α cells (89). A group of genes including *BUD7* to *BUD9*, *RAX1*, and *RAX2* is specifically required for bipolar budding (93, 162, 271, 636). A mutation in any of these genes disrupts the bipolar budding pattern of a/α cells but does not affect the axial budding pattern of a or α cells.

(i) **Bud8 and Bud9.** *BUD8* and *BUD9* appear to have highly specific roles in the bipolar pattern. The *bud8* mutants bud almost exclusively at the proximal pole (the birth pole) (Fig. 5B), whereas *bud9* mutants bud predominantly at the distal pole (the pole opposite the birth end) (636). These unipolar patterns are different from the axial pattern, since bud sites do not appear in a sequential chain as viewed by the staining of bud scars. Rather, they occur as a cluster in the vicinity of either pole in no particular order. These distinct phenotypes of *bud8* and *bud9* mutants indicate that *BUD8* and *BUD9* encode the key components that mark the poles distal and proximal to the birth pole of the daughter cell, respectively (636). Indeed, GFP fusions of Bud8 and Bud9 localize to the distal pole and the proximal pole of daughter cells, respectively (93, 210, 271, 495). Although one report suggests that both Bud8 and Bud9 localize to the distal pole of daughter cells (553), this discrepancy is likely due to the overexpression of the proteins or differences in strain backgrounds.

Bud8 is delivered to the presumptive bud site (i.e., the distal pole of a newly born cell) just before bud emergence, while Bud9 is delivered to the bud side of the mother-bud neck (which becomes the proximal pole of the daughter cell) just before cytokinesis (495). However, the levels of *BUD8* mRNA and *BUD9* mRNA peak in late G_2/M and G_1 phase, respectively, suggesting that their translation and/or delivery to the cell surface are delayed and presumably regulated in a cell cycle-dependent manner (495). This timing of transcription of Bud8 and Bud9 is important for their localization, as shown by a promoter swap experiment (495). The delivery of Bud8 and Bud9 is dependent on actin, and the delivery of Bud9, but not that of Bud8, is also dependent on septins (210, 495). Bud8 fails to localize in cells lacking the formin Bni1, which nucleates the formation of actin cables (458), indicating that the bud tip-directed actin cables are necessary for Bud8 localization (210, 415, 563). Bud8 also fails to localize to the bud tip in the absence of *RAX1*, whose localization is also dependent on Bni1 (271) (see below). It is puzzling, however, that daughter a/α cells of actin mutants are not defective in distal-pole budding despite the fact that the localization of Bud8 is dependent on

actin. It is possible that these actin mutants still have enough actin cables and thus Bud8 directed towards the bud tip (see also below [Other proteins necessary for the bipolar budding pattern]).

Both Bud8 and Bud9 contain a predicted N-terminal extracellular domain, which appears to be heavily glycosylated, and a short cytoplasmic domain that is sandwiched by two transmembrane domains. The cytoplasmic domains of Bud8 and Bud9 are very similar to each other, unlike their extracellular domains. It has been postulated that the similar cytoplasmic domains may provide essentially the same signal at both poles of a diploid cell, thus being recognized by a common downstream target such as a component of the Rsr1 GTPase module (Fig. 6) (210) (see below).

(ii) **Rax1 and Rax2.** *RAX1* and *RAX2* were originally identified as extragenic suppressors of the *axl1* mutant (163). Although a functional linkage between *Axl1* and *Rax1* (or *Rax2*) still remains to be examined, recent studies indicate that *Rax1* and *Rax2* are important for bipolar budding (93, 162, 271, 415). The budding patterns of the *rax1* and *rax2* mutants suggest that both proteins are involved in selecting a bud site at the distal pole of daughter cells as well as at the distal or proximal pole of mother cells (271). Both *Rax1* and *Rax2* appear to be integral membrane proteins. *Rax2* has a type I orientation, with its long N-terminal domain in the extracytoplasmic space (271). GFP-tagged *Rax1* and *Rax2* localize to the distal pole as well as to the division site on both mother and daughter cells, and their localization to the division sites is persistent through multiple cell division cycles (93, 162, 271). In fact, *Rax1* and *Rax2* appear to be very stable proteins, unlike *Axl2*, which undergoes a rapid turnover (93, 271). These properties of *Rax1* and *Rax2* fit well with the persistent nature of the bipolar landmark, which has been postulated based on physiological observations (89). Localization and biochemical studies suggest that *Rax1* and *Rax2* interact closely with each other and with Bud8 and Bud9 in the establishment and/or maintenance of the cortical landmarks for bipolar budding (271). *Rax1* may be necessary for the efficient delivery of Bud8 and Bud9 to the proper sites.

Several questions regarding the bipolar budding pattern remain unanswered. In particular, what provides the spatial signal for bipolar budding? Although Bud9 at the proximal pole of daughter cells was detectable in their first budding cycle after cell division, and GFP-Bud8 was sometimes detected on the mother side of the mother-bud neck, Bud8 or Bud9 is rarely present at the division sites on mother cells (210, 495). If *Rax1* and *Rax2* by themselves can provide the spatial signal at these sites as persistent bipolar markers, it is puzzling that they seem unable to do so at the proximal pole of daughter cells.

(iii) **Other proteins necessary for the bipolar budding pattern.** The actin cytoskeleton is likely to play a direct role in the placement of bipolar budding cues, since specific actin mutations that have little or no effect on cell growth, such as *act1-116* and *act1-117*, nevertheless perturb the bipolar budding pattern (629). Actin is required for the proper localization of the potential bipolar landmarks Bud8 and Bud9 (210, 495). Interestingly, in *a/α* cells carrying specific mutations in the *ACT1* gene, daughter cells correctly position their first bud at the distal pole of the cell, unlike the mother cells, which bud randomly, supporting the notion that different rules govern

bud site selection of mother and daughter cells in *a/α* diploids (629). Mutations in several other genes, including those that encode components of the “polarisome,” such as *BN11*, *SPA2*, *PEA2*, and *BUD6/AIP3*, are defective in the bipolar budding pattern, but the wild-type gene products of these mutants are also involved in other cellular processes (415, 521, 538, 588, 636). Mutations in *RVS161*, *RVS167*, *AIP3/BUD6*, *SEC3*, *SEC4*, *SEC9*, *END3*, *VRP1*, *PHO85*, *MSB3*, or *MSB4* also convert the bipolar pattern to a random pattern without affecting the axial pattern (51, 150, 533, 538, 563, 564, 584, 588, 629, 636). In addition, a genome-wide screen for mutants defective in bipolar budding has identified many other genes, including genes involved in protein modification, lipid metabolism, gene transcription, and translation (415). It is unlikely that all of these gene products have a direct role in bipolar budding. Some of these genes are likely to control the actin cytoskeleton or secretory pathway and thus affect the delivery of bipolar landmarks such as Bud8, Bud9, *Rax1*, or *Rax2*. Others may be involved in the expression of a component of the bipolar landmark.

Regulation of Cell Type-Specific Budding Patterns

Coupling of spatial landmarks to the Rsr1 GTPase module.

Bud2 and Bud5 are likely linked to the cell type-specific landmarks. Bud5 mislocalizes in haploid *a* cells carrying a deletion of *AXL2* (*axl2Δ*) but not in diploid *a/α* cells homozygous for *axl2Δ*. Similarly, Bud5 mislocalizes in haploid cells lacking Bud3 and, to a lesser extent, in cells lacking Bud4 (273, 358). Bud5 also fails to localize properly in *a/α* cells homozygous for *bud8Δ* but not in *a bud8Δ* cells (273). These localization studies suggest that the interaction of Bud5 with the axial or bipolar landmark determines its localization in haploid or diploid cells, respectively (Fig. 6). Consistent with this notion, Bud5 copurifies with *Axl2* and Bud8 (272, 273). This idea is further supported by the isolation of bipolar-specific alleles of *BUD5*, which are defective in the bipolar budding pattern but not in the axial pattern (272, 273, 636). These bipolar-specific *bud5* mutant proteins fail to localize in diploid *a/α* cells but not in haploid *a* cells (272, 273).

The distinct localization of Bud2, a GAP for Rsr1, and the isolation of the bipolar-specific alleles of *BUD2* suggest that Bud2 localizes to the presumptive bud site independently of Bud5. Although Bud2 and Bud5 appear to localize to the presumptive bud site independently, localization of each protein to the proper bud site cannot be maintained in the absence of the other protein (273, 437). Mutations in the conserved residues of the putative GAP domain of Bud2 lead to random budding in all cell types (A. Sanson and H.-O. Park, unpublished data), whereas specific *bud2* mutants that are defective in the bipolar pattern but not in the axial pattern have been isolated (636). Thus, the Bud2 GAP activity is necessary for both budding patterns, whereas a distinct domain of Bud2 is likely to be involved in the interaction with the bipolar landmark. It is not known yet whether Bud2 directly interacts with any spatial landmark. Localization studies suggest that Bud2 is likely to arrive at the presumptive bud site after Bud5 during the G₁ phase (437). Thus, Bud5 may be important for initiating the Rsr1 GTPase cycle by activating Rsr1 to the GTP-bound state, whereas Bud2 may be more important for targeting the

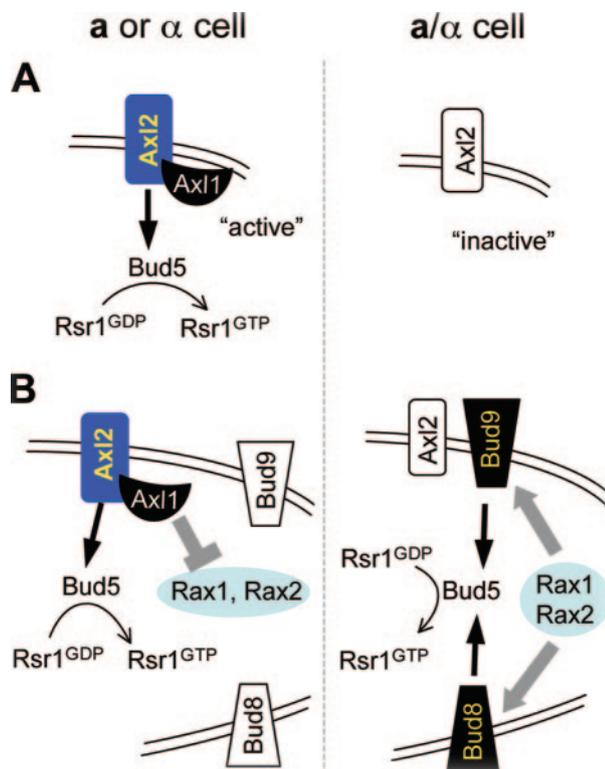


FIG. 8. Models for cell type control of budding patterns. (A) Axl1 may act positively on the axial landmark proteins, and bipolar budding may occur as a default in the absence of Axl1 in *a/α* cells. (B) Axl1 may act indirectly by blocking the function of Rax1 and Rax2, which play a positive role in promoting bipolar budding in the absence of Axl1 in *a/α* cells. For simplicity, only Axl2, which interacts with Bud5, is shown as an axial landmark (see the text for details).

bud site assembly proteins, which are brought to the presumptive bud site through the interaction with Rsr1-GTP, to the proper bud site (see below).

Model for cell type-specific budding patterns. Budding patterns depend on the cell types rather than ploidy (158, 222). Cell types are controlled by transcriptional regulators encoded by the mating loci *MATa* and *MATα* (220). How is the spatial information inherited from one cell division cycle to the next, thus allowing cells to exhibit the specific budding patterns with such high fidelity? Despite our current knowledge of several proteins that control or constitute the spatial landmarks, the mechanism by which the cell type-specific budding pattern is determined is mostly unknown. Here, we consider the two simplest models to explain the budding patterns, although many other models are also possible.

It has been hypothesized that the cell type-specific budding pattern is produced by the transcriptional repression of some genes critical to axial budding by the corepressor *a1-α2* present in diploid *a/α* cells (87). *AXL1* may be such a gene, because Axl1, which is necessary only for axial budding, is expressed in *a* or *α* cells but not in *a/α* cells (163). Furthermore, ectopic expression of *AXL1* in *a/α* cells reverts bipolar budding to axial budding to some extent (163). Thus, Axl1 may be a key component of the axial landmark or may facilitate the usage of the axial landmark in *a* or *α* cells. According to this model, an axial

spatial landmark such as Axl2 would be active only in the presence of Axl1. In *a/α* cells where *AXL1* is not expressed, the axial landmark is no longer active. This model predicts that bipolar budding may occur by default (Fig. 8A). However, some recent data suggest that the determination of the cell type-specific budding pattern is more complex than this model would predict. In the absence of both putative bipolar landmarks, Bud8 and Bud9, diploid *a/α* cells exhibit a partially randomized budding pattern with an increased tendency to bud at the proximal pole rather than at random sites, suggesting that *a/α* cells have some ability to use the axial cues in the absence of both putative bipolar landmarks. In fact, when a potential axial landmark is also absent in the *a/α bud8 bud9* mutant, a more fully randomized budding pattern is observed (210). These observations suggest that bipolar budding does not occur simply by default. The second model hypothesizes that the bipolar budding pattern is established by the concerted action of multiple proteins including Bud8, Bud9, Rax1, and Rax2, which are specifically required for bipolar budding. According to this model, Axl1 may block the function of Rax1 and/or Rax2, thus inhibiting bipolar budding in haploid *a* or *α* cells. In diploid *a/α* cells where *AXL1* is not expressed, Rax1 and Rax2 would be active for the establishment of the bipolar landmark (Fig. 8B). Several aspects of these models remain to be tested. Many factors other than those considered in these models are also likely to contribute to cell type-specific budding patterns.

TEMPORAL CONTROL OF POLARITY ESTABLISHMENT DURING YEAST BUDDING

Control of the cell division cycle and several cell cycle-regulated events such as DNA replication, mitosis, and cellular morphogenesis in budding yeast have been well described elsewhere previously (317). Here, we will limit our discussion to potential regulatory mechanisms for the control of bud emergence. An important, but still poorly understood, aspect of budding is how bud emergence is triggered at the right time in the cell cycle and also only once per cell cycle. Activation of the cyclin-dependent protein kinase Cdc28 complexed with a G_1 cyclin, Cln1 or Cln2 (G_1 CDK), is crucial for the polarization of the actin cytoskeleton in the late G_1 phase (316). Since bud site selection and bud site assembly are likely to precede polarized actin organization (Fig. 2) (126), a protein functioning in these steps may be regulated by G_1 CDKs. Consistent with this notion, recent studies suggest that the localization of Cdc24, a GEF for Cdc42, to the incipient bud site requires the G_1 CDK (196, 390). In addition, Cdc24 is phosphorylated by Cdc28/Cln kinase in vitro (390). However, mutations in six putative CDK phosphorylation sites of Cdc24 do not seem to affect its function in vivo (196). It is thus unlikely that G_1 CDKs directly regulate the GEF activity or localization of Cdc24 by phosphorylating Cdc24 in vivo. Analyses of some of *cdc42* mutants such as *cdc42^{G60D}* and *cdc42^{D38E}* have also suggested that Cdc42 plays a critical role in ensuring that bud emergence occurs once per cell cycle (84, 465). Regulation of Cdc24 GEF activity is thus likely to be crucial for polarity establishment at the right time in the cell cycle. However, how Cdc24 is regulated is still not clear. It has been proposed that Cdc24 is autoinhibited and that the inhibition is released by its interac-

tion with Rsr1 and Bem1 during vegetative growth and with Far1 during mating (523, 614). Although these are intriguing possibilities, whether Rsr1 and/or Far1 activates Cdc24 has not been directly tested.

Although Cdc24 is unlikely to be a direct target of G₁ CDKs, it is phosphorylated by the PAK Cla4, which is a target of Cdc42 (58, 69). There are opposing views about the functional significance of Cdc24 phosphorylation. One study suggests that the phosphorylation of Cdc24 by Cla4 leads to the dissociation of Cdc24 from Bem1, ending polarized bud growth (196). On the other hand, another group found no difference in the association of phosphorylated Cdc24 and Bem1. In addition, overexpression of a B-type cyclin mutant, *clb1Δ152*, which is known to trigger the depolarizing switch effectively, did not alter the association of Cdc24 with Bem1 (58). Instead, that study argued that Cdc24 phosphorylation by Cla4 is likely to be a part of the positive feedback loop to promote polarized growth. Thus, it is not clear whether phosphorylation is involved in the initial activation of Cdc24 at bud emergence or termination of polarized growth.

Bem1 may also play an important role in the temporal regulation of polarity establishment. Bem1 might be a direct target of CDK since recent proteomic data using an analog-sensitive *cdc28* mutant (*cdc28-as*) identified Bem1 as one of many proteins phosphorylated by Cdc28 (580). A positive feedback loop has been proposed in which Cdc42-GTP interacts with Bem1, which localizes to the incipient bud site and stabilizes Cdc24 at the site, and Cdc24 in turn activates Cdc42, leading to apical growth (58, 69). However, the temporal order of these events at the bud site is not completely understood. Several questions need to be addressed in order to assess the role of Bem1 in polarized growth. For example, is Bem1 phosphorylated by CDK *in vivo*? If so, when does it occur in the cell cycle? Is Bem1 phosphorylation important for bud emergence or is it involved in the termination of polarized growth?

Some of the potential landmark proteins for bud site selection may be regulated in a cell cycle-dependent manner. A proteomics study using *cdc28-as* indicates that Bud3, Bud4, and Axl2 are phosphorylated by CDK (580). Although the functional significance of the phosphorylation of these Bud proteins is not known, it is tempting to speculate that a landmark protein whose activity is regulated by CDK triggers the activation of the Rsr1 GTPase module at a specific time. The Rsr1 GTPase module may then promote polarity establishment at the right time and at the right place. Another potential target of cell cycle-dependent regulation is the Rsr1 GAP Bud2, since a *bud2* mutant is lethal in the absence of two G₁ cyclins, *CLN1* and *CLN2* (48, 103). Recent genome-wide two-hybrid screens also identified interactions between *BUD2* and *CLN2* (123, 581). However, whether Bud2 is a target of the G₁ CDK is not known. In summary, despite the existence of many pieces of thought-provoking data, how polarity establishment is regulated in a cell cycle-dependent manner is not well understood.

Cdc42 EFFECTORS AND THEIR ROLES IN POLARITY DEVELOPMENT

Cdc42 is required for the independent polarization of the actin and septin cytoskeletons. Cdc42 is also required for po-

larized exocytosis, mainly through its role in actin organization (66, 247, 259, 454, 456) but also through its direct interactions with the secretory components (5, 641). There are three classes of Cdc42 effectors: the formin Bni1 (138); the PAKs Ste20 (532, 643), Cla4 (102), and Skm1 (361); and the pair of CRIB motif-containing, yeast-specific proteins Gic1 and Gic2 (67, 68, 90). In the following section, we will describe the roles of the actin and septin cytoskeletons in polarized cell growth and how Cdc42 regulates the polarized organization of these cytoskeletons through its effectors. We will also discuss exocytosis and other biological processes in which Cdc42 is involved.

Actin Organization

There are three filamentous actin structures in *S. cerevisiae*: actin patches, actin cables, and actin rings (398) (Fig. 9B). Actin patches are concentrated at sites of polarized growth, and actin cables are polarized towards these sites. The actomyosin-based ring assembles at the mother-bud neck in late anaphase (9, 274). Mutations in *ACT1*, the only gene encoding actin in *S. cerevisiae*, or the disruption of F-actin by latrunculin A causes depolarized growth but does not block secretion *per se* (30, 418). Together, these data firmly establish that the polarized actin cytoskeleton is required for polarized exocytosis. The key question is how Cdc42 regulates the polarized organization of these actin structures.

Actin patches and endocytosis. Signaling pathways critical for cellular processes such as cell growth, cell polarity, cell motility, stress responses to environmental stimuli, nutrient uptake, and ion homeostasis are all initiated at the plasma membrane. Many proteins and lipids involved in these processes are recycled back to the plasma membrane or are targeted for degradation through endocytosis (131, 135). In *S. cerevisiae*, there is receptor-mediated endocytosis as well as fluid-phase endocytosis, both of which require the actin cytoskeleton for the internalization step (135, 403). Recent studies have led to a much clearer picture of the molecular pathways underlying receptor-mediated endocytosis (263, 267, 268, 413), whereas the mechanisms underlying fluid-phase endocytosis remain unclear. The best-studied receptor-mediated endocytosis in *S. cerevisiae* involves the internalization and degradation of Ste2, the receptor for the mating pheromone α -factor (135, 403). Endocytosis also regulates cell wall assembly by controlling the levels of the plasma membrane-associated, cell wall synthetic enzymes such as chitin synthase III (Chs3) (585, 649) and 1,3- β -glucan synthase (Fks1) (135, 583).

Actin patches are motile, short-lived, cortical foci with a diameter of \sim 200 nm (475) and a life span of \sim 10 s (76, 121, 274, 537, 599). The patches are made of short, branched actin filaments (475, 634). These filaments are nucleated by the evolutionarily conserved Arp2/3 complex (451, 615, 616), which is activated by Las17 (also known as Bee1), a yeast homolog of Wiskott-Aldrich syndrome protein (WASP) (319, 350, 615), Pan1 (129, 560), the type I myosins Myo3 and Myo5 (139, 171, 172, 190, 303), Abp1 (127, 189), and F-actin (for a review, see reference 398). The Arp2/3 complex is also required for actin patch motility (267, 616). Accumulating evidence indicates that actin patches are the sites of endocytosis. First, there are approximately 50 genes involved in receptor internalization, and most of these gene products colocalize

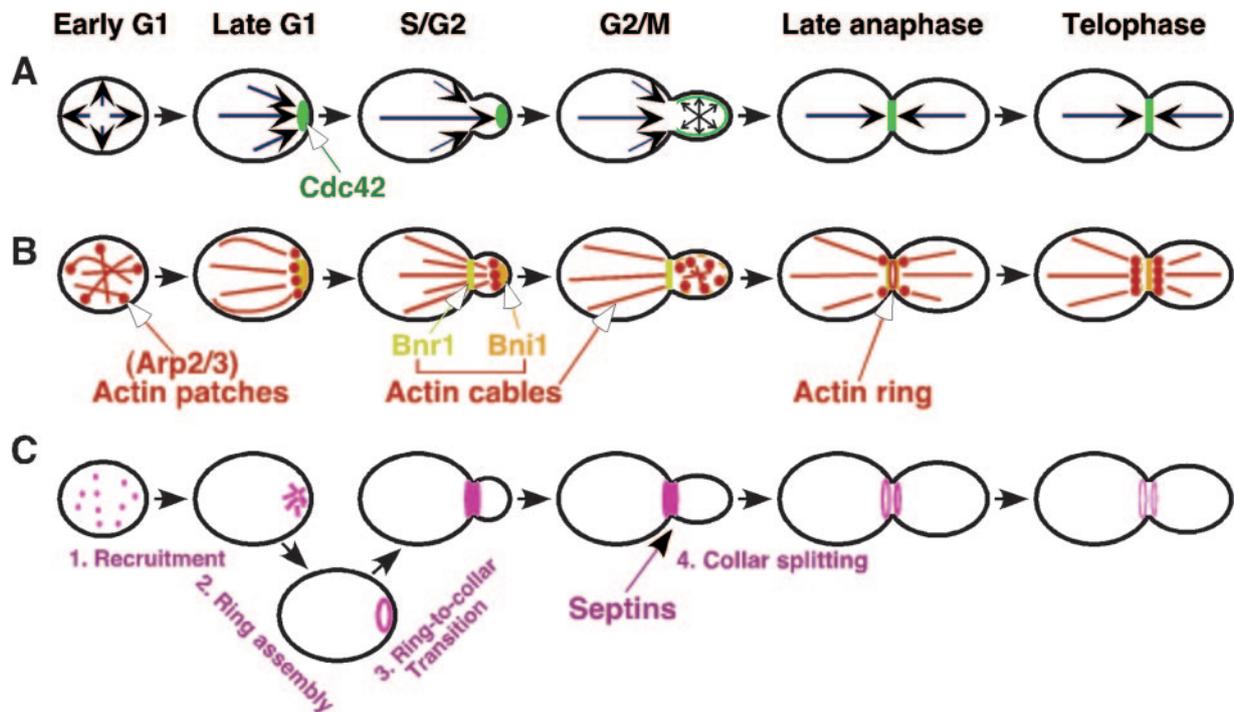


FIG. 9. Localization of Cdc42 and Cdc42-controlled actin and septin organization. (A) Cdc42 localization (green) and the directionality of cell growth (blue arrows) in the cell cycle are indicated. Components of the exocytic machinery such as the exocyst display a similar localization pattern as Cdc42. A cell cycle-triggered switch from apical growth to isotropic growth occurs around the G_2/M transition. (B) Actin organization in the cell cycle. Actin patches (red spots), actin cables (red lines), and the actin ring (red circle) are indicated. The short, branched actin filaments in the patches are nucleated by the Arp2/3 complex and its activators, whereas the linear actin filaments in the cables and the rings are nucleated by the formins Bni1 (brown) and Bnr1 (yellow), whose localization patterns are also indicated. Presumably, Bni1 exists in the bud cortex during the isotropic growth phase at G_2/M phase, but its concentration is too low to be detected by fluorescence microscopy. Thus, Bni1 is drawn as a dashed line around the bud cortex at the G_2/M phase. It is not clear whether Bnr1 still localizes at the bud neck after the actomyosin ring contraction and is thus indicated as a dashed line in telophase cells. (C) Septin organization in the cell cycle. Septin collar or hourglass (purple) formation involves at least three steps: septin recruitment, which requires Cdc42-GTP, its effectors Gic1 and Gic2, and possibly the polarisome; septin ring assembly, which requires Cdc42 cycling between the GDP- and GTP-bound states, its GAPs, and possibly its effector Cla4; and maturation of the septin ring into a collar, which requires Cla4 and GTP binding of the septins.

with the actin patches. Mutations in these genes cause various defects in endocytosis (135, 457). Approximately one-third of these endocytic proteins regulate actin dynamics, i.e., the assembly and the disassembly of the actin patches (135). Second, actin patches are associated with the invaginated plasma membrane (401, 475), consistent with the role of actin at the internalization step of endocytosis (299). Finally, vesicles (endosomes) labeled with the lipophilic membrane dye FM4-64 colocalize with actin patch components (236, 268).

Various cell biological approaches involving live-cell imaging of fluorophore-tagged proteins in wild-type and endocytic mutant strains have led to a working model of how the modular endocytic machinery is assembled and functions at the internalization step of clathrin- and actin-mediated endocytosis (267, 268, 398). The first module, called the coat module, consists of clathrin, Sla1 (contains four SH3 domains), Pan1 (contains two Eps15-homologous domains), End3 (contains two Eps15-homologous domains), and Sla2 (contains one epsin N-terminal homology [ENTH] domain, which may bind to phosphoinositides, and one talin-like domain) (131, 188, 384). This module may also include other ENTH domain-containing proteins such as Ent1/Ent2 and Yap1801/Yap1802 (putative clathrin adaptor proteins in yeast) (131, 384). These adaptor pro-

teins, which bind to PIP2 (phosphatidylinositol bisphosphate) via their ENTH domains (11, 250, 251), are required for the cortical association of clathrin (413). Sla1 and End3 may regulate the initiation of actin polymerization by binding to the activators of the Arp2/3 complex, such as Pan1 (560) and Las17 (413). Sla2 interacts with PIP2, clathrin, and F-actin via its N-terminal ENTH domain, the central coiled-coil region, and the C-terminal talin-like domain and thus potentially links the endocytic machinery to the actin cytoskeleton (267). Overall, the coat module likely recruits cargoes and regulates actin dynamics.

The second module, called the actin module (268), is thought to regulate coat internalization. The actin module includes actin, the capping proteins Cap1 and Cap2, which function as an obligatory heterodimer (18, 19, 281), the fimbrin Sac6 (7), the actin-binding protein Abp1 (127), and the Arp2/3 complex (617). These components target to the actin patches in an F-actin-dependent manner (268). The third module, called the WASP/Myo module, includes the yeast WASP Las17 (319, 350, 615), the type I myosins Myo3/Myo5 (139, 171, 172, 190, 303), and Bbc1/Mti1 (389, 541). This module remains immotile at the plasma membrane and is thought to regulate actin filament nucleation via the Arp2/3 complex. The last

module, called the amphiphysin module, consists of Rvs161 and Rvs167 (57, 100, 160, 334), both of which contain BAR domains that are known to tubulate membranes *in vitro* (442). The timing of the recruitment of the Rvs161-Rvs167 heterodimer (160) to the actin patches is consistent with a role in endocytic vesicle scission (382). Type I myosins and phosphoinositides are also thought to be involved in the scission process (263, 548). These four modules function in a highly coordinated manner to carry out the internalization step of endocytosis.

Cdc42 effectors and actin patches. Actin patches are concentrated at sites of polarized growth in the cell cycle (9, 457). The major question is how does Cdc42 trigger the polarization of the patches at these sites? Cdc42 is required for the docking and/or the fusion of vacuolar membranes in *S. cerevisiae* (133, 402) but does not seem to play an important role at the internalization stage of endocytosis, although this issue has not been comprehensively examined. Most of the known *cdc42*-Ts and *cdc24*-Ts mutants still form numerous actin patches at the nonpermissive temperature, but these patches are randomly distributed in the cell cortex in unbudded and budded cells (8, 9, 260), suggesting that Cdc42 is required for the polarized organization, but not the assembly, of the actin patches. However, it is still possible that Cdc42 plays a minor role in the regulation of actin patch assembly and/or dynamics in wild-type cells. This conclusion is supported by the following facts. First, permeabilized *cdc42*-Ts cells are defective in actin patch assembly *in vitro*, and this defect can be corrected by purified recombinant GTP-locked Cdc42 (320) or the recombinant PAK Ste20 (132). Second, the PAKs Ste20 and Cla4 are known to phosphorylate the type I myosins Myo3 and Myo5 (618, 619), which are thought to function downstream of Cdc42 and act together with Las17 and Vrp1 (yeast WIP, WASP interacting protein) to regulate actin patch assembly (139, 171, 303). These results led to a hierarchical pathway starting from Cdc42-GTP to PAKs, to type I myosins plus Las17 and Vrp1, and to the Arp2/3 complex, leading to actin patch assembly (302). This pathway explains why the yeast WASP Las17 does not have a Cdc42-GTP-binding motif like its mammalian counterparts. However, this PAK-mediated pathway does not play a major role in either actin patch assembly or the polarized organization of the patches, because conditional lethal alleles (Ts or ATP analog sensitive) of *CLA4* in a *ste20Δ* background do not affect the overall assembly or the polarized organization of actin patches (102, 186, 608). It is not clear whether the PAK-activated, myosin I-mediated pathway regulates the dynamics of the actin patches. In shmoos of *myo3Δ myo5Δ* cells, the life span of actin patches is increased threefold, and the number of motile patches is decreased significantly, although the velocity of the moving patches is unchanged (537). These results are consistent with the suggestion that type I myosins may be involved in the scission process of the endocytic vesicles (263).

What mediates the Cdc42-dependent polarization of the actin patches? There are two general possibilities. First, the earliest localized endocytic components, for example, those of the coat module, are good candidates for receiving the Cdc42-initiated polarization signal. Indeed, a recent study indicates that the ENTH domain of Ent1 and Ent2, redundant epsin-like proteins that are required for endocytosis and that are thought

to be components of the coat module, interacts with Cdc42 GAPs Rga1, Rga2, and Bem3 (10). These interactions are independent of the endocytic role of Ent1 and Ent2 and are believed to temporally and spatially link polarity machinery to endocytosis machinery. Ent2 also interacts with Cdc24 in a two-hybrid assay (123), although the physiological significance of this interaction is not clear. Second, a putative factor involved in actin patch clustering may be delivered via post-Golgi vesicles and actin cables to the sites of polarized growth (168). This hypothesis explains why actin patches are always clustered at the ends of actin cables (9, 274) and why a defect in secretion or actin cable assembly can cause defects in actin patch organization (168, 461). This hypothesis also implies that Cdc42 does not have to interact directly with actin patch components. Formins (Bni1 and Bnr1), which are required for the formation of actin cables (140, 458, 486, 487) and actin rings (573) (see below), are also required for the targeting of the yeast WASP Las17 to the sites of polarized growth (302). In addition, the yeast WIP Vrp1 is required for the targeting of Las17 to the actin patches (302, 537). However, it is not clear whether the formins interact directly with Vrp1 or other patch components or whether formins affect actin patch polarization indirectly through their function in actin cable formation.

Actin cables and exocytosis. Actin cables are dynamic structures that disappear within ~15 s in cells treated with the actin monomer-sequestering drug latrunculin A (LatA) (30, 274, 628). Actin cables are aligned along the cortex of the cell from the mother cell compartment to the presumptive bud site or to the daughter cell compartment. Actin cables are polarized towards sites of active cell growth, where actin patches are clustered (9, 274, 418) (Fig. 9B). For this reason, actin cables and patches are thought to mediate polarized exocytosis (9). There are two arrays of actin cables in budding yeast: one nucleated by the formin Bni1, which localizes to the bud tip, and the other nucleated by the formin Bnr1, which localizes to the bud neck (138, 140, 242, 289, 398, 399, 458, 459, 486, 487, 628, 636) (Fig. 9B). Deletion of either *BNI1* or *BNR1* is tolerated by the cell, but double deletion is lethal (138, 242, 270, 289, 587), suggesting that cells can survive with one array of actin cables directed towards either the bud tip or the bud neck. Cells carrying *bni1*-Ts *bnr1Δ* alleles lose actin cables rapidly at the nonpermissive temperature without affecting actin patch assembly, although the polarization of the patches is lost over time. As a result of the cable loss, most cells become large, round, and unbudded (140, 487). The current thought is that the formins Bni1 and Bnr1 nucleate actin filaments independently of the Arp2/3 complex. These filaments are stabilized by tropomyosins and other actin-binding proteins such as Sac6 (yeast fimbrin) (7) and ABP140 (23, 628), forming actin cables.

Exocytosis is a multistep process in which an intracellular vesicle moves to the plasma membrane, and the fusion of the vesicular membrane and plasma membrane ensues, resulting in the release of vesicular content into the extracellular space (Fig. 10). Each step of exocytosis is regulated by distinct Rab GTPases and their effector pathways (199, 419, 544). When exocytosis is instructed by a polarization signal, polarized exocytosis occurs, suggesting intimate interactions between the polarity machinery and exocytosis during polarized cell growth. The final step of exocytosis, i.e., the transport, tethering, and

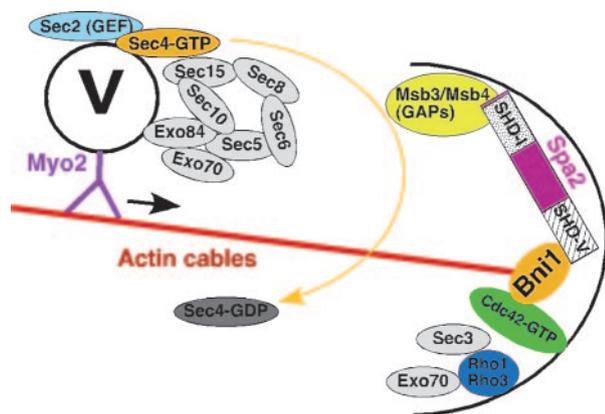


FIG. 10. Role of Cdc42 in polarization of actin cables and exocytosis. Cdc42 regulates the polarization of actin cables by directly interacting with the formin Bni1 and regulating its localization and/or activation. Cdc42 controls polarized secretion mainly via its role in actin cable organization, but it also interacts directly with the exocyst subunit Sec3, endowing the exocyst with one polarization signal. Rho1 and Rho3 also interact with Sec3 and Exo70, respectively, providing the exocyst additional polarization signals. Sec4 (brown) and its GEF, Sec2 (light blue), associate with the secretory vesicles to guarantee Sec4 in its GTP-bound form, which is required for vesicle transport from the mother cell to the bud and also for the interaction with the vesicle-associated partial exocyst through a Sec4-Sec15 interaction (all exocyst subunits are shown in light gray). Upon vesicle arrival at the bud tip, the exocyst components on the vesicle interact with Sec3 and the fraction of Exo70 already localized at the bud tip, resulting in the assembly of a complete exocyst, which is required for vesicle tethering. Thus, an exocyst is formed dynamically for each round of vesicle transport. After fulfilling its function at the plasma membrane, Sec4-GTP is hydrolyzed with the help of its GAPs Msb3 and Msb4, and Sec4-GDP returns to the cytosol. By segregating its GEF and GAPs at distinct locations, the Sec4 GTPase cycle is employed to carry out its exocytic functions efficiently. Msb3, Msb4, and Bni1 are brought to close proximity by interacting with distinct domains of Spa2. SHD, Spa2 homology domain.

fusion of the post-Golgi vesicles with the plasma membrane, should provide ample targets for such interactions. The post-Golgi vesicles are transported by the type V myosin Myo2 using actin cables as tracks (66, 460), and the transport also depends on the Rab GTPase Sec4 and its GEF, Sec2, both of which are associated with the vesicles (192, 600). Vesicle tethering is carried out by an evolutionarily conserved complex called the exocyst, which consists of eight subunits (Sec3, Sec5, Sec6, Sec8, Sec10, Sec15, Exo70, and Exo84) in *S. cerevisiae* (566). The exocyst is an effector of Sec4 (198) and is thought to form dynamically during each round of exocytosis (63). Sec4 and seven subunits of the exocyst, including Exo70, are associated with the post-Golgi vesicles, whereas Sec3 and a fraction of Exo70 are associated with the plasma membrane at sites of polarized growth (63). Upon the arrival of a vesicle at the plasma membrane, the entire exocyst is formed and tethers the vesicle to the plasma membrane for the subsequent membrane fusion event that is mediated by the SNARE proteins (63, 480). The target SNAREs for the post-Golgi fusion, including Sec9 (65, 309) and Sso1 (1, 591), localize uniformly to the plasma membrane. The vesicle SNARE Snc1 is carried on the vesicles (455) and localizes to sites of polarized growth (318). Sec1, a protein required for exocytosis (420, 508), localizes to sites of polarized growth (77) and interacts with both the exocyst and

the SNARE complexes (612). These components of the vesicle tethering and fusion machinery represent excellent targets for regulation by polarity machinery.

In the last decade, compelling evidence indicates that actin cables direct polarized secretion (66, 275, 461), whereas actin patches are involved in endocytosis (see above) (135, 398, 457). Tropomyosins are the only proteins known to decorate actin cables specifically (457). There are two isoforms of tropomyosins, the major isoform Tpm1 and the minor isoform Tpm2, both of which together define an essential role in maintaining the actin cable structures (122, 330, 331, 461). Shifting *tpm1-2 tpm2* mutant cells to the nonpermissive temperature for ~1 min results in the loss of actin cables and the depolarization of exocytosis and cell surface growth, without affecting the polarization of actin patches and endocytosis, suggesting that actin cables are responsible for exocytosis (461). In contrast, the *las17* mutant is defective in actin patch polarization and endocytosis (404, 540) but not exocytosis (275). Actin cables are thought to function as tracks along which the type V myosin Myo2 moves secretory vesicles (193, 261, 503, 505) and various organelles, including vacuoles (80–82, 224, 248), peroxisomes (227), late compartments of the Golgi apparatus (478, 479), nucleus (40, 305, 567, 630), and, perhaps, mitochondria (251, 252) from the mother cell to the daughter cell. Another type V myosin, Myo4, also uses actin cables to transport specific mRNAs from the mother cell to the daughter cells (56, 254, 530, 555). More detailed discussions of the roles of actin cables and myosin V's in exocytosis and organelle segregation can be found in several excellent reviews (66, 247, 398, 460, 504).

Cdc42 effectors and actin cables. Formins are a family of proteins that nucleate actin cables in yeast and stress fibers in mammalian cells (15, 141, 211, 223, 398, 423, 647). This family of proteins, in general, has a small GBD at the N terminus and a conserved formin homology 2 (FH2) domain at the C terminus. The FH2 domain is necessary and sufficient for the nucleation activity of the formins (458, 486), and FH2 forms a dimer, which associates with the barbed ends of actin filaments (399, 458, 626). Formins can function as a processive “leaky” cap that allows the elongation of the filament while capping the barbed end, as in the case of Bni1 in *S. cerevisiae* (647, 648). In other cases, such as the formin Cdc12 in the fission yeast *S. pombe*, it functions as a “tight” cap that blocks elongation but can be opened by binding of profilin (291). The nucleation activity of FH2 is thought to be negatively regulated by an autoinhibitory loop formed between the N-terminal GBD and the diaphanous-autoregulatory domain (DAD), which is located within the C-terminal end of the FH2 domain. Binding of an active Rho GTPase to the GBD opens up the loop, leading to the activation of formins (15, 16, 141, 211, 223, 423, 647). FH2 activity is also regulated positively by profilin, which binds to the upstream FH1 domain (291, 458, 486). In the case of Bni1, but not Bnr1, the actin-binding protein Bud6 binds to a region of Bni1 immediately downstream of the DAD and regulates FH2 activity (397, 399). Bni1 also binds to the polarity protein Spa2 (164) and to translation elongation factor 1 α (EF1 α), which binds and bundles F-actin (582). The functional significance of these interactions is not known.

The regulation of formins by Rho GTPases has been best illustrated with RhoA and the mammalian formin mDia2

(mammalian diaphanous) (16). In *S. cerevisiae*, the GBD of Bni1 has been shown to interact with Cdc42 (138) and Rho1 (289), while the GBD of Bnr1 has been shown to interact with Rho4 (242, 270). As discussed in previous sections, Cdc42 is involved mainly in the polarized organization, but not the assembly, of the actin cables and patches. Cdc42 may play a role in actin cable polarization, at least in part, by regulating Bni1 localization. The localization of Bni1 at sites of active cell growth is completely lost in a *cdc42-Ts* mutant grown at the nonpermissive temperature (425). Interestingly, multicopy *CDC42* suppresses both the lethality and the actin cable assembly defect in *rho3 rho4* mutant cells, suggesting that Cdc42 also plays a minor, but important, role in the local activation of Bni1 in the cell (116, 370). In addition to the direct interaction between Cdc42 and Bni1, Cdc42 also regulates the phosphorylation of Bni1 through its effector, the PAK Ste20 (187), although *in vitro* studies indicate that the phosphorylation of the C-terminal half of Bni1 (from FH1 to COOH) is not required for its nucleation activity (397). The functional significance of this phosphorylation is not known. It also has yet to be established whether Cdc42 affects the assembly and/or organization of the actin cables directly or indirectly via other pathways.

How does Cdc42 direct polarized exocytosis? Cdc42 is likely to contribute to this process by directing the polarized organization of the actin cables (see above) (66, 460). Accumulating evidence, however, suggests that Cdc42 may play a more direct role in exocytosis that is independent of its role in actin organization. A temperature-sensitive mutation in *CDC42*, *cdc42-6*, causes post-Golgi vesicle accumulation at 33°C, without displaying apparent defects in actin organization. Thus, Cdc42 may regulate the vesicle docking and fusion apparatus to provide maximal function at sites of polarized growth (5). In addition, Cdc42 interacts with the N-terminal domain of Sec3, a component of the exocyst, which localizes to the sites of polarized growth independently of F-actin (149), and this interaction is required for the polarized localization of Sec3 (641). Sec3 is unique among the exocyst components in that a deletion of *SEC3* does not cause cell lethality or a significant defect in secretion at 25°C (150, 613). Overexpression of Sec1 or Sec4 can suppress the lethality and secretion defect of *sec3Δ* cells at 37°C (612), suggesting that other components of the exocytic machinery, such as Sec4, Sec1, and perhaps the vesicle SNARE Snc1, may also interact with Cdc42. Msb3 and Msb4, which display GAP activity towards Sec4 (12, 13, 168), localize to sites of polarized growth, and their localization depends on Cdc42. In addition, multicopy *MSB3* and *MSB4* suppress *cdc24* and *cdc42* mutants, and Msb3 and Msb4 also interact specifically with Cdc42-GDP (51, 168, 563). These results suggest that Msb3 and Msb4 define another linkage between Cdc42 and exocytosis.

Cdc42 is likely involved in exocytosis in mammalian cells as well. Mammalian Cdc42 controls protein sorting to the basolateral plasma membrane of epithelial cells (297), and Cdc42 localizes predominantly to a brefeldin A-sensitive compartment of the Golgi apparatus (136). Mammalian Cdc42 interacts with the coatamer at the Golgi apparatus (622) and recruits the Arp2/3 complex to stimulate actin assembly, which thus promotes Golgi-derived vesicle formation (161, 547). Coatamer-bound Cdc42 also inhibits dynein recruitment to the

COPI vesicles (92). Although both yeast and mammalian Cdc42s function in exocytosis, their predominant locations of action and mechanisms of action may be different in these systems.

Septin Organization

General properties and functions of the septins. Septins are a family of GTP-binding, filament-forming proteins that are conserved from yeast to humans but are noticeably absent in plants and *Dictyostelium* (99, 144, 148, 180, 184, 205, 276, 283, 335, 336, 347, 391, 414, 485, 543, 575, 594). The number of septin genes varies among organisms, with 7 in *S. cerevisiae* and *S. pombe*, 2 in *Caenorhabditis elegans*, 5 in *Drosophila*, and 13 in mammals (594). All septins share a similar structure, including a central GTP-binding domain, a polybasic region, which binds to phosphatidylinositol polyphosphates (PIPs), and a septin-unique domain. Most septins have a coiled-coil region at their C termini and have variable-length N termini (485, 594). Septins purified from yeast, *Drosophila*, and mammals or recombinant septins form heteromeric complexes, which polymerize into filaments *in vitro* (143, 147, 157, 234, 284, 381, 519). The role of GTP binding and hydrolysis in septin functions is not well established. Some data indicate that GTP binding is essential for septin filament formation (381), while other data indicate that GTP binding and hydrolysis are not required for this process (143, 387, 519). GTP binding may regulate septin organization in *S. cerevisiae* (593), septin-septin interactions (78, 143), and septin interactions with nonseptin proteins, such as the interactions with PIPs for membrane association (78, 640).

In *S. cerevisiae*, five vegetatively expressed septins, Cdc3, Cdc10, Cdc11, Cdc12, and Shs1 (also known as Sep7), form heteromeric complexes with a stoichiometry of Cdc3:Cdc10:Cdc11:Cdc12 of 2:2:2:2 (143, 157, 392, 592, 598). The interactions among individual septins in the complexes have been determined by a variety of *in vitro* and *in vivo* approaches (592). The septin-septin interaction patterns within the complexes are remarkably conserved between *S. cerevisiae* and the fission yeast *S. pombe* (21, 594). In *S. cerevisiae*, *CDC3* and *CDC12* are essential for cell viability and are thus considered the core subunits, whereas *SHS1*, *CDC10*, and *CDC11* can be deleted under certain growth conditions. The growth defect of the deletion mutants varies from mild (in the case of the *shs1Δ* mutants) to very severe (in the case of the *cdc11Δ* mutants). *CDC11* is essential for cell viability in some strain backgrounds (592). When one of the essential septins is inactivated by a temperature-sensitive mutation, the entire septin structure is lost at the bud neck (153, 202, 279). This property makes the characterization of certain mutations in septin and in septin-associated proteins feasible.

Recent studies on live cells with the GFP-tagged septins indicate that septin collar formation involves at least three steps: septin recruitment, ring assembly, and ring maturation (Fig. 9C) (83, 253, 335). Septins are first recruited to the presumptive bud site as unorganized septin clouds or patches, which are then transformed into the first organized structure, the septin ring, within minutes (253). Indirect immunofluorescence with antibodies against native septins showed that a cortical septin ring forms in the late G₁ phase, approximately

15 min before bud emergence. Around bud emergence, the septin ring expands into a collar spanning the entire bud neck. During cytokinesis, the collar is split into two distinct rings marking the division sites at both the mother and daughter sides (153, 202, 279, 327). FRAP studies indicate that the septin structures prior to bud emergence are highly dynamic (83, 113). Upon bud emergence, the septin collar at the bud neck is stabilized. During cytokinesis, the split septin rings are dynamic again. This cell cycle-regulated septin dynamic may explain why septin-containing “neck filaments” are found only in small- to medium-budded cells but not in unbudded cells or cells undergoing cytokinesis by electron microscopy (71–73). Presumably, the labile septin structures in the latter cells are not maintained under the experimental conditions for electron microscopy but can be detected by GFP-tagged septins or indirect immunofluorescence.

Septins are best known for their role in cytokinesis. However, their requirement in this process varies tremendously from one organism to another. For example, septins play an essential role in cytokinesis in *S. cerevisiae* (215), presumably by functioning as a scaffold that tethers cytokinetic components, such as those involved in the formation of the actomyosin contractile ring and of the septum, at the bud neck (52, 325, 326, 587) and/or as a diffusion barrier to restrict the lateral movement of the cytokinetic components (112). In contrast, deletion of all four vegetatively expressed septins in the fission yeast *S. pombe* causes only a mild defect in cell separation (49, 561), perhaps by affecting the precise positioning of the septum degradation enzymes at the division site (364). Septins also play important roles in cytokinesis in other organisms such as *Drosophila* (3, 412), *C. elegans* (414), and mammals (285). In addition to cytokinesis, septins are involved in exocytosis (41, 42, 234), mitosis (542, 543, 552), integrity and motility of spermatozoa (240, 286), and apoptosis (191, 300). Recently, increased expression of septins in diverse tumor cells has been reported (205, 485, 509).

How are the septin heteromeric complexes involved in diverse cellular functions? Studies of yeast suggest that the septin collar (or hourglass) at the bud neck may serve as a scaffold (184, 335, 336) for many proteins involved in diverse cellular processes such as bud site selection (88, 491), chitin deposition (108), cytokinesis (52, 325, 326, 477, 500, 587), and the morphogenetic checkpoint (39, 338, 528). Mammalian septins are also thought to form diverse cellular scaffolds at discrete locations to perform specific functions (283, 543). For example, mammalian septin Sept2 is associated with cleavage furrow during cytokinesis (285) and with the kinetochore during mitosis (542). The Sept2/6/7 complexes regulate microtubule dynamics through an interaction of Sept2 with MAP4, a microtubule binding and bundling protein (295). The putative scaffold role of the mammalian septins in the cellular functions described above has not been extensively examined. In *S. cerevisiae*, accumulating evidence also suggests that septins form a diffusion barrier at the bud neck, which restricts the movement of membrane and membrane-associated proteins between the mother and daughter cell compartments (38, 554). These proteins include the putative factors involved in actin patch stability (38), the membrane protein Ist2, which may be involved in ion homeostasis (554), and the bud cortex-localized Lte1, the putative GEF for the small GTPase Tem1, which is essen-

tial for mitotic exit (37, 79, 439). It is worth emphasizing that the role of septins in cytokinesis is dependent on the cell cycle. Prior to the actomyosin ring contraction (from late G₁ phase to telophase), the septin collar functions as a scaffold to recruit and maintain cytokinesis components such as the type II myosin Myo1, Iqg1 (IQGAP), the *S. pombe* Cdc15 homology protein Hof1, and the formin Bnr1 at the bud neck. In telophase, the split septin rings are thought to function as diffusion barriers that sandwich the actomyosin ring and the membrane trafficking components at the bud neck (112). The septin barrier is also thought to restrict endoplasmic reticulum membrane proteins from diffusing across the bud neck (344).

Many important questions regarding septin functions remain unanswered. Although many proteins in *S. cerevisiae* are known to localize to the bud neck in a septin-dependent manner, it is not clear whether any of these proteins directly interact with the septin scaffold. In addition, the high-order structures of septins are poorly understood. Which structural organization of the septins, such as septin heteromeric complexes or the highly stable septin collar, functions as a scaffold? Several septin-associated proteins are asymmetrically localized at the mother-bud neck, while some proteins such as Bud3 and Bud4 are localized to the entire septin collar (88, 491). The proteins involved in the synthesis of bud scar chitin, such as Bni4, Chs3, and Chs4, are localized to the mother side of the bud neck (108), whereas the proteins involved in the morphogenetic checkpoint are localized to the daughter side of the bud neck (39, 338, 528). What is the structural basis for the asymmetric localization of these septin-associated proteins? Are septin filaments intrinsically polarized like actin filaments and microtubules? None of the septins possesses a transmembrane domain unlike the transmembrane domain-containing proteins in the tight junction of epithelial cells, which is a proven diffusion barrier. How do the septins then form a physical barrier at the inner surface of the plasma membrane without tightly zipping the septin structure to the plasma membrane?

Role of Cdc42 in septin organization. Because the septin collar plays important roles in numerous cellular processes as described above, it is crucial to understand how this collar structure is initially formed. The Cdc42 GTPase module appears to regulate all three stages of the formation of the septin collar (Fig. 11). Temperature-sensitive mutations in *CDC42* and *CDC24* block the recruitment of the septins to the presumptive bud site at the nonpermissive temperature, suggesting that Cdc42-GTP is required for the recruitment step (253). This role of Cdc42 is mediated at least in part by Gic1 and Gic2, a pair of Cdc42 effectors (67, 90) that interact directly with the septin Cdc12 (253). The *gic1Δ gic2Δ* mutant is temperature sensitive for growth (67, 90) and fails to recruit septins at 37°C but appears to do so normally at lower temperatures (253). It is thus likely that another pathway is involved in septin recruitment at lower temperatures (253). This parallel pathway appears to consist of the “polarisome” (521), a poorly defined network of interacting proteins, which includes the scaffold protein Spa2 (520, 521, 590), the formin Bni1 (another effector of Cdc42) (138), the actin-binding protein Bud6 (20, 399), and Pea2, a protein whose function is unknown (588). Thus, Cdc42 appears to regulate septin recruitment through

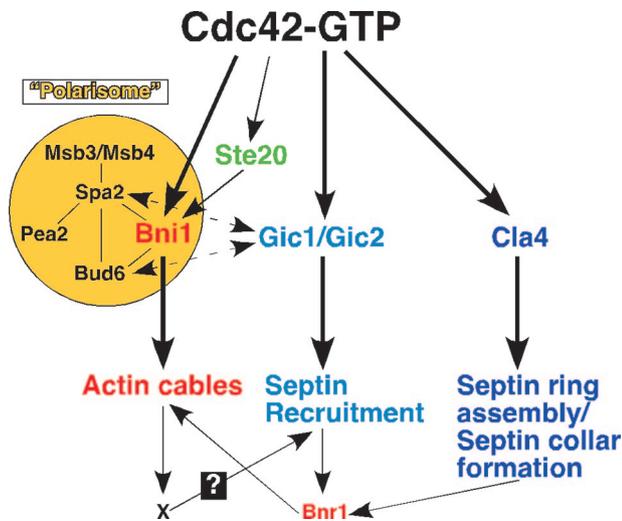


FIG. 11. Model for the role of Cdc42 in the polarized organization of the actin cytoskeleton and septins. Cdc42 controls the polarized organization of the actin cables by directly interacting and possibly activating the formin Bni1 and also by indirectly affecting the bud neck localization of the other formin Bnr1 via its role in septin recruitment and/or ring/collar assembly. Cdc42 controls polarized septin organization in late G_1 via its effectors Gic1/Gic2, Bni1, and Cla4. Ste20, another effector of Cdc42, could participate in both the actin and the septin organization by phosphorylating Bni1. Actin cables may mediate the role of Bni1 and perhaps the polarisome in septin organization by guiding the delivery of a putative factor, "X," which may be directly involved in septin recruitment as indicated by the question mark. Cdc42 effectors could also be involved in enhancing Cdc42 polarization at growth sites by interacting with each other. For simplicity, this type of potential positive feedback mechanism is not depicted here (see the text for a detailed description of the model).

the Gic proteins and the polarisome (Fig. 11). How the polarisome functions in this process is yet to be established at the molecular level.

After septin recruitment, Cdc42, its GAPs (Rga1, Rga2, and Bem3), and the PAK Cla4, an effector of Cdc42, are thought to play a role in septin ring formation (Fig. 11). Mutations affecting the cycling of Cdc42 between its GDP- and GTP-bound states confer profound defects in septin ring assembly. These mutations include *cdc42^{Q61L}* and *cdc42^{V36T,K94E}*, both of which possess decreased intrinsic GTPase activity, and *cdc42^{K186R}*, which increases the intrinsic GTPase activity (181). Deletion of all Cdc42 GAPs, *rga1Δ rga2Δ bem3Δ*, delays septin ring formation without affecting septin recruitment (83, 181, 536). The role of Cdc42 GAPs in promoting septin ring assembly may explain the surprising observation that multicopy *RGAI* and, to a lesser extent, *BEM3* and *RG2* suppress the growth and septin ring assembly defects of the *cdc12-6* mutant at the minimal restrictive temperature (30 to 32°C), in which septins are otherwise recruited to the bud tip (83, 454). It is, however, not clear why overexpression of the genes that are required for Cdc42 cycling suppresses defects in septin ring assembly. The cycling of Cdc42 may be required for efficient septin recruitment to the presumptive bud site (181). GTP hydrolysis by Cdc42 may control the quantity of localized "free septins" (released from Cdc42 effectors or other downstream pathways) that are competent for ring assembly (83, 253, 335). Alternatively, Cdc42 GAP may function as a part of the effector for

septin assembly (83, 253, 335). In addition to the *cdc42* mutants, *cla4Δ* cells fail to assemble a normal septin ring, in some cases (102, 182, 187, 265, 593, 608), suggesting that Cla4 plays a role in the initial septin ring assembly. Two other mutations in the effector domain of *CDC42*, *cdc42^{V44A}* and *cdc42^{D38E}*, also cause defects in septin ring formation (465, 467). Because these Cdc42 mutant proteins are defective in their interactions with Cdc42 effectors such as Cla4, Gic1, and Gic2 and also with Cdc42 regulators such as the GAP Bem3 (465, 467), it is difficult to attribute the septin defects associated with these *cdc42* mutants to a particular biochemical defect. Nevertheless, the studies described above indicate that Cdc42, its three GAPs, and its effector, Cla4, are involved in septin ring assembly.

Upon bud emergence, the septin ring expands into a collar structure and becomes stabilized, as indicated by FRAP (83, 113). This ring-to-collar transition in septin organization appears to be regulated by Cla4 (113, 593) and by GTP binding of the septins (593). Cla4 directly phosphorylates certain septins and efficiently suppresses the septin collar assembly defects of a *cdc10 cdc12* double mutant that is defective in GTP binding and in septin filament assembly of the septins (593). Thus, Cdc42 appears to regulate septin collar formation via Cla4. Several other kinases such as Gin4 (337) and Elm1 (60) as well as septin-interacting proteins such as Bni5 (306) localize to the bud neck in a septin-dependent manner and are also involved in septin collar formation. Cdc42 and Cla4 are required for the activation of the Gin4 kinase (568), indicating another route through which Cla4 regulates septin collar assembly.

It is worth emphasizing that the septin collar assembly is a continuous and efficient process. It is thus sometimes difficult to assign a protein function at a particular stage of septin collar assembly. However, the three-step model (Fig. 9C) helps to define the structure-function correlations for different high-order septin structures and also helps to define the functions of specific septin regulators. For example, when septins are not organized into a ring structure, such as those at the bud tip of certain *cdc42* mutants and the triple mutant lacking Cdc42 GAPs, the septins are strictly associated with elongated bud morphology (83, 182). In contrast, a highly organized septin structure, such as the septin collar in wild-type cells, is associated with normal, oval-shaped bud morphology (83, 182). Cdc42 appears to be involved in all stages of septin collar assembly. A number of other proteins described above are involved in later stages of this process. However, several questions regarding the molecular pathways involved in regulation of the high-order septin structures remain. What is the role of GTP binding and hydrolysis in septin filament formation? When are the septin filaments formed: at the recruitment stage, the ring formation stage, or the collar formation stage? What are the *in vivo* septin structures, at least at the electron microscopy level, corresponding to the three stages in septin collar assembly?

Is the role of Cdc42 in septin organization universal? Cdc42 regulates septin recruitment partly via its effectors, Gic1 and Gic2 (253), both of which contain a conserved CRIB domain, which specifically interacts with Cdc42-GTP (67, 90). Gic1 and Gic2 also interact directly with the septin Cdc12 (253). However, Gic1 and Gic2 homologs are not found anywhere else,

except in a very few yeast species belonging to *Ascomycetes* (<http://db.yeastgenome.org/homolog/images2/chr8/YHR061C.tree.gif>). Mammalian Cdc42 regulates septin organization, at least in part, via its effector, Borg3 (258). Borg3 contains a CRIB domain and interacts directly with septin complexes containing Sept6 and Sept7 (519). Cdc42 appears to inhibit the interaction between Borg3 and the septins in vitro (258, 519). Ectopic expression of Borg3 disrupts septin organization in MDCK cells (258), but the precise role of Borg3 in this process remains unknown. Interestingly, Borg3 homologs are limited to metazoans (258). Why do the Gic proteins in *S. cerevisiae* and the Borg proteins in mammals, which are capable of interacting with the highly conserved proteins Cdc42 and septins, bear strong species-specific features? The answer is likely to reflect the need to organize septins into distinct structures in different organisms, so that other organism-specific processes can be coordinated.

Model for the Roles of Cdc42 in Actin and Septin Organization

Here, we propose a model to explain how Cdc42 controls polarized organization of the actin cytoskeleton and of the septins at the beginning of the cell cycle (Fig. 11). This model synthesizes the vast and fragmented pool of information collected on individual polarity proteins into the molecular pathways that govern Cdc42-mediated cytoskeletal polarization. According to the model, Cdc42 controls the polarized organization of the actin cytoskeleton and the septins through two genetically separable, biochemically cross talking pathways: one involving the polarisome with the scaffold Spa2 and the formin Bni1 as the centerpiece and the other involving Gic1 and Gic2. The polarisome pathway mainly leads to the polarized organization of actin cables through the regulation of cellular location and perhaps the activity of Bni1. This pathway also fine-tunes septin recruitment, possibly through its role in actin organization and/or polarized exocytosis. The Gic-mediated pathway mainly regulates septin recruitment via a direct interaction with the septin complex. This pathway also affects actin cable organization by regulating the initial localization of Bnr1, which depends on the septins. In addition, the polarisome components Spa2 and Bud6 interact with the Gic proteins, allowing cross talk between these two pathways (255) (S. E. Tcheperegine and E. Bi, unpublished data) (Fig. 11).

This model is consistent with and/or can explain the following observations. (i) Polarized actin and septin organizations can occur independently (30, 153, 279, 454), but both require Cdc42 function (96, 183, 253, 454). (ii) The deletion of individual polarisome components is synthetically lethal with *gic1Δ gic2Δ* (51, 255). (iii) Mutants in the genes encoding polarisome components and the *cla4* mutant exhibit a synthetic lethal interaction (574). The deletion of any polarisome components in combination with a conditional lethal allele of *CLA4* arrests cells with septins concentrated at the bud tip under restrictive conditions (187, 265). Given that the polarisome and *Cla4* affect septin recruitment and ring/collar assembly, respectively, it is not surprising to see that the cells carrying mutations for polarisome components and *CLA4* display severe defects in septin organization. Similarly, *gic1Δ gic2Δ* is synthetically sick with *cla4Δ* (90), and the triple mutants display severe defects in

septin organization (M. Iwase and E. Bi, unpublished results). (iv) Ste20 phosphorylates Bni1 and may activate the polarisome (187). This result is also consistent with the observations that *ste20Δ* and *cla4Δ* are synthetically lethal and that the conditional inactivation of *CLA4* in the *ste20Δ* background results in severe defects in septin organization (102, 187, 265, 608). (v) Recent studies indicate that F-actin may play a fine-tuning role in septin organization (253, 265, 294). Septin recruitment and ring assembly appear to be delayed in cells treated with LatA (253). This model predicts that the simultaneous loss of F-actin and the Gic1/Gic2 effectors might completely block septin recruitment. (vi) Septins are required for the targeting of Bnr1 to the bud neck (459), suggesting that Bnr1 may function downstream of the septins. Consistent with the model, *bni1Δ* and *bnr1Δ* are synthetically lethal (587), and *bni1* mutations are synthetically lethal with the septin mutation *cdc12-5* (H. Fares and J. R. Pringle, personal communication) (459).

This model also raises several questions. Are the interactions between the Gic proteins and the polarisome (Gic-Spa2 and Gic-Bud6) essential at 37°C but dispensable at lower temperatures, given that the *gic1Δ gic2Δ* mutant is temperature sensitive for growth (67, 90)? How are these interactions regulated at the molecular level? How does F-actin affect septin recruitment? How do the septins interact with Bnr1? Do the Gic proteins affect actin organization independently of the septins and Bnr1?

The Mitotic Exit Network and Cytokinesis

The exit from mitosis, the contraction of the actomyosin ring and septum formation, and the entry into the next G₁ phase in *S. cerevisiae* are all controlled by a signaling cascade called the mitotic exit network (MEN) (Fig. 12) (for recent reviews, see references 59, 376, 531, 549, and 556). The counterpart of this network in the fission yeast *S. pombe* is called the septum initiation network (376, 531), and several key components of the MEN are also conserved in mammalian systems (34, 531, 549). The MEN starts with a small GTPase module consisting of the GTPase Tem1 (526), its putative GEF, Lte1 (524, 525), and its bipartite GAP, Bub2-Bfa1 (Fig. 12A) (173). In normal anaphase cells, Tem1 and the Bub2-Bfa1 complex localize to the spindle pole body (SPB) destined to a daughter cell (also the old SPB) (37, 156, 441), whereas Lte1 localizes to the daughter cell cortex (37, 439). This spatial segregation of the GAP and the GEF for Tem1 ensures that the MEN is not activated until the daughter-destined SPB passes through the bud neck (Fig. 12B) (37, 439). The spatially restricted activation of the MEN is further safeguarded by the protein kinase Kin4, which localizes to the SPB and the cortex of the mother cell in anaphase (104, 440). Kin4 is thought to inhibit MEN activation by counteracting the activity of the polo kinase Cdc5, which activates Tem1 by inhibiting its GAP (235). When a spindle is misaligned in the mother cell, both Kin4 and the Bub2-Bfa1 complex localize to both SPBs, inactivating Tem1 and preventing mitotic exit (104, 440). Thus, factors involved in the spatial regulation of MEN activation link spindle position to mitotic exit, which defines the spindle position checkpoint (SPOC) (54). Once Tem1 is activated, it binds to Cdc15 kinase (37, 526), which in turn activates a protein kinase complex

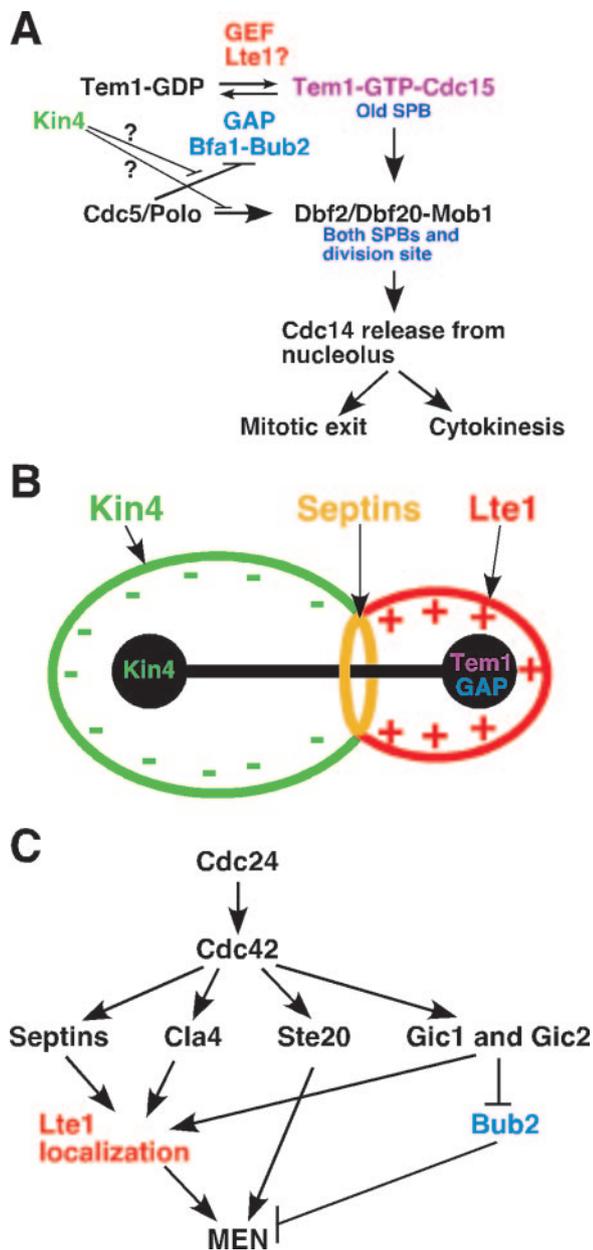


FIG. 12. Role of Cdc42 in mitotic exit. (A) The MEN in *S. cerevisiae*. The activation of Tem1 leads to the activation of a kinase cascade, resulting in the release of the phosphatase Cdc14 from the nucleolus to the nucleus, which regulates mitotic exit and cytokinesis independently. The regulators for Tem1 GTPase cycling and the localization of the key components of the MEN are indicated. (B) In normal anaphase cells, Tem1 and its GAP localize to the daughter-bound SPB (old SPB) (SPB is indicated by a solid black circle). This localization pattern ensures that Tem1 is activated only after the spindle penetrates into the bud, where Lte1 (+), the putative GEF for Tem1, is localized at the cortex. The septins and the mother cell cortex-localized inhibitor Kin4 (-) provide additional layers of regulation to further restrict the activation of Tem1 in the bud. (C) Cdc42 regulates the MEN by multiple means through its role in septin ring assembly (see the section on Cdc42 and septin organization) and its effectors Cla4, Gic1, and Gic2 to affect the localization of Lte1, through Ste20 to activate the MEN in parallel to Lte1 by an undefined mechanism, and through blocking the binding of the GAP Bub2 to Tem1 (see the text for further details).

consisting of the kinases Dbf2 and Dbf20 and their regulatory subunit, Mob1 (290, 343, 353). This cascade of kinase activation leads to the release of the protein phosphatase Cdc14 (194) from the nucleolus into the nucleus (31, 527, 597), which promotes mitotic exit by inactivating Cdc28/cyclin B (Cdk1) activity (256, 602) and also by reversing Cdk1-dependent phosphorylation (596). Cdc15 (625), Dbf2/20-Mob1 (159, 343, 633), and Cdc14 (43) also localize to the bud neck in late anaphase and play an essential role in septum formation independent of their role in mitotic exit (43, 324, 327). The lack of septum formation in MEN mutants or in mutants lacking the septum synthetic enzyme Chs2 does not affect the assembly of the actomyosin ring (50, 324, 327, 500, 587) but affects the contraction of the ring (50, 324, 500). Thus, the MEN is required for both mitotic exit and cytokinesis, and these two functions bifurcate at the level of Cdc14 (Fig. 12A). While the mechanisms underlying the role of the MEN in mitotic exit are well established, its targets in cytokinesis remain unknown.

What is the role of Cdc42 in regulating the MEN? Cdc42 affects MEN activation at multiple levels (Fig. 12C). Cdc42 and its GEF, Cdc24, are required for the asymmetric localization of Lte1 (230, 257, 517). Ras and the Cdc42 effector Cla4 act together to target Lte1 to the bud cortex (516, 632). The PAK Cla4 phosphorylates Lte1 either directly or indirectly (230, 517), thus promoting the interaction between Lte1 and Ras (516). This interaction is thought to stabilize the anchoring of Lte1 at the bud cortex. Ste20, another Cdc42 effector and a PAK, may function in parallel to Lte1 to promote mitotic exit (230), although the molecular basis for the role of Ste20 in this process remains unknown. Gic1 and Gic2 also promote MEN activation by interfering with the binding of the Bub2-Bfa1 GAP to Tem1 (231). Gic1 and Gic2 are also required for the bud cortex localization of Lte1 at 37°C (257). Septins are thought to function as a diffusion barrier at the bud neck (38, 554), which is required for restricting the localization of Lte1 to the bud cortex (79, 230, 257). Because Cdc42 is required for the initial formation of the septin ring through multiple effectors including the PAKs and the Gics (see previous sections), Cdc42 could affect Lte1 localization indirectly via septin ring assembly. Because *LTE1* is essential only at lower temperatures (524), other factors that display an Lte1-like localization and/or activity may be required for MEN activation at higher temperatures. It is not known whether Cdc42 plays any role in restricting Kin4 to the mother cell cortex. The spatial regulation of Tem1 activation represents an excellent example of a modified version of the global inhibition and local activation mechanism discussed below.

Although Cdc42 localizes to the mother-bud neck in large-budded cells (466, 651), the function of Cdc42 at the neck of large-budded cells remains unknown, as Cdc42 is not required for actin ring formation during cytokinesis (573). It has been assumed that Cdc42 may be involved in targeted membrane deposition at the bud neck to promote septum formation and/or degradation. Such a hypothesis remains to be tested. Cdc42 is indirectly involved in cytokinesis through its role in the formation of the septin ring, which is essential for the tethering of cytokinesis proteins such as the type II myosin Myo1 to the bud neck prior to late anaphase or telophase (52, 326). Septins also function as a two-ring diffusion barrier that sandwiches and restricts the lateral movement of proteins in-

volved in cytokinesis and cell separation at the bud neck during telophase (112).

Cdc42 interacts with Boi1 (47), which has been implicated in cell polarity and actin cytoskeleton reorganization. Boi1 and its functional homolog, Boi2, also interact with Bem1 and other Rho GTPases (47, 368). Boi1 and Boi2 localize to the bud neck in late anaphase (206), and their localizations depend on spindle midzone-localized Aurora kinase Ipl1 (417). Based on this and other functional analyses, Boi1 and Boi2 are proposed to function as “abscission inhibitors” in a “NoCut pathway” that delays the completion of cytokinesis in response to spindle midzone defects to prevent chromosome breakage (417). Whether this novel role of Boi1 and Boi2 is linked to their function in polarity and how exactly they inhibit cytokinesis remain to be determined.

OTHER TYPES OF CELL POLARIZATION

Cell Polarization during Mating

Haploid yeast cells are able to redirect their polarization to mate with a cell of the opposite mating type. During mating, cell polarity is determined by a pheromone gradient that is established by the position of the appropriate mating partner (Fig. 1B). Cell polarization can be induced toward an artificially produced gradient of the peptide pheromone, demonstrating that the pheromone is a sufficient chemotropic signal (510). Mating pheromones (**a** and α factors) are sensed by seven transmembrane receptors, α -factor receptor (encoded by *STE2*) of **a** cells and **a**-factor receptor (encoded by *STE3*) of α cells. Both receptors activate a G-protein heterotrimer consisting of an α subunit (Gpa1) and a $\beta\gamma$ subunit dimer (Ste4 and Ste18). Binding of a pheromone to the appropriate receptor promotes the exchange of GDP for GTP on the $G\alpha$ subunit, leading to the dissociation of the $G\beta\gamma$ heterodimer. Upon activation, $G\beta\gamma$ orchestrates several well-characterized cellular responses, including signal transduction pathways, leading to transcriptional induction, cell cycle arrest, and morphological changes, leading to the formation of a mating projection called the shmoo (Fig. 13). A major target of the $G\beta\gamma$ is a MAP kinase cascade, which includes Ste20 (a PAK-like kinase, a MAPK kinase kinase), Ste5 (a scaffold protein for the MAP kinase cascade), Ste11 (a MAPK kinase kinase), Ste7 (a MAPK kinase), Fus3 (a MAP kinase), and Ste12 (a transcription factor). Much is known about the signal transduction pathways governing these responses, and it has been extensively reviewed elsewhere (114, 545). Here, we will first discuss how cells change the direction of cell polarization from the bud site towards the mating partner and then discuss how Cdc42 is involved in MAPK signaling and polarized cell growth, leading to shmoo formation.

The Cdc42 GTPase module, which is coupled to the spatial landmark for budding by the Rsr1 GTPase module during vegetative growth, also plays a critical role in linking $G\beta\gamma$ activation to cell polarization (see below). The adaptor Far1 is necessary for linking $G\beta\gamma$ activation to the Cdc42 GEF Cdc24 (70, 408). Although the external pheromone signal normally overrides the internal signal from the bud site, the bud site is used for shmooing in the absence of a chemotropic system as in mating-specific mutants of *CDC24* or *FAR1* or in the pres-

ence of uniform pheromone concentrations. These *cdc24* (*cdc24-m1*, *cdc24-m2*, and *cdc24-m3*) and *far1* (*far1-s*) mutants still form the mating projections at wild-type efficiencies but in random directions with respect to the pheromone gradient, because these cells are defective in orienting their axes chemotropically (409, 589). When both systems that affect bud positioning and chemotropism are lacking, as in the case of the double mutant *cdc24-m1 rsr1 Δ* , cells are defective in shmoo formation. It is therefore likely that the stabilization of the growth axis during mating requires positional signaling from either the pheromone receptor or the specific bud site selection proteins in the absence of chemotropism.

During mating, cells ignore the preexisting intrinsic spatial cues and establish a new landmark in response to receptor activation. How is the spatial information of the bud site overridden in the presence of a mating partner? It is noteworthy that *Axl2* and *Bud4* are down-regulated in response to pheromones (476, 491). This decreased expression of the potential axial landmarks may allow cells to ignore the spatial cue for axial budding and polarize towards a mating partner, but it is not clear how cells also override the bipolar signal, which is apparently used for bipolar budding of *axl2* or *bud4* mutants. Thus, down-regulation of *Axl2* and *Bud4* would not be sufficient for cells to override the spatial information of the bud site. Although the underlying mechanism is not completely understood, several recent findings provide some insights into the problem. *Far1* appears to play a dual role during mating: *Far1* triggers cell cycle arrest as an inhibitor of the G_1 cyclin-Cdc28 protein kinase (443, 444) and promotes cell polarization as an adaptor protein linking $G\beta\gamma$ and Cdc24 for polarity establishment (70, 408, 589). In haploid G_1 cells, Cdc24 is in the nucleus through an association with *Far1* (411, 522, 571). In the absence of the mating pheromone, activation of the cell cycle kinase Cdc28 complexed with a G_1 cyclin (*Cln1* or *Cln2*) triggers the degradation of *Far1* at bud emergence (522), thus allowing Cdc24 to interact with *Rsr1* for polarity establishment toward the proper bud site (434, 644). It has been proposed that *Rsr1* also activates Cdc24 during budding (523). Upon pheromone treatment, the inactivation of Cdc28-*Cln* kinase leads to the stabilization of *Far1* and the relocation of the *Far1*-Cdc24 complex into the cytoplasm by the exportin *Msn5* (55). *Far1* may recruit Cdc24 to the site of activation of the heterotrimeric G protein, thus leading to the activation of Cdc24 at the site. Cdc24 exhibits a two-hybrid interaction with *Rsr1*, and the interaction is diminished by a mutation in the region that is also involved in the interaction with *Far1*, thus suggesting that *Far1* and *Rsr1* bind to the overlapping domains of Cdc24 (523). This overlapping binding site may account for the competitive binding of *Far1* and *Rsr1* to Cdc24. Thus, it has been proposed that Cdc24, when complexed with *Far1*, is unable to interact with *Rsr1*, thereby allowing the targeting of Cdc24 to the mating site instead of the bud site (197). Although this is an interesting possibility, some available data conflict with this notion, and some issues are controversial (see “Cdc24, a GEF for Cdc42” and Temporal Control of Polarity Establishment during Yeast Budding for further discussion). Cdc24 also interacts with *Bem1* (47) and activates Cdc42 at the location where pheromone receptors are clustered (29). Activated Cdc42 presumably directs polarized actin organization, exocytosis, endocytosis, and cell wall assembly as it does during

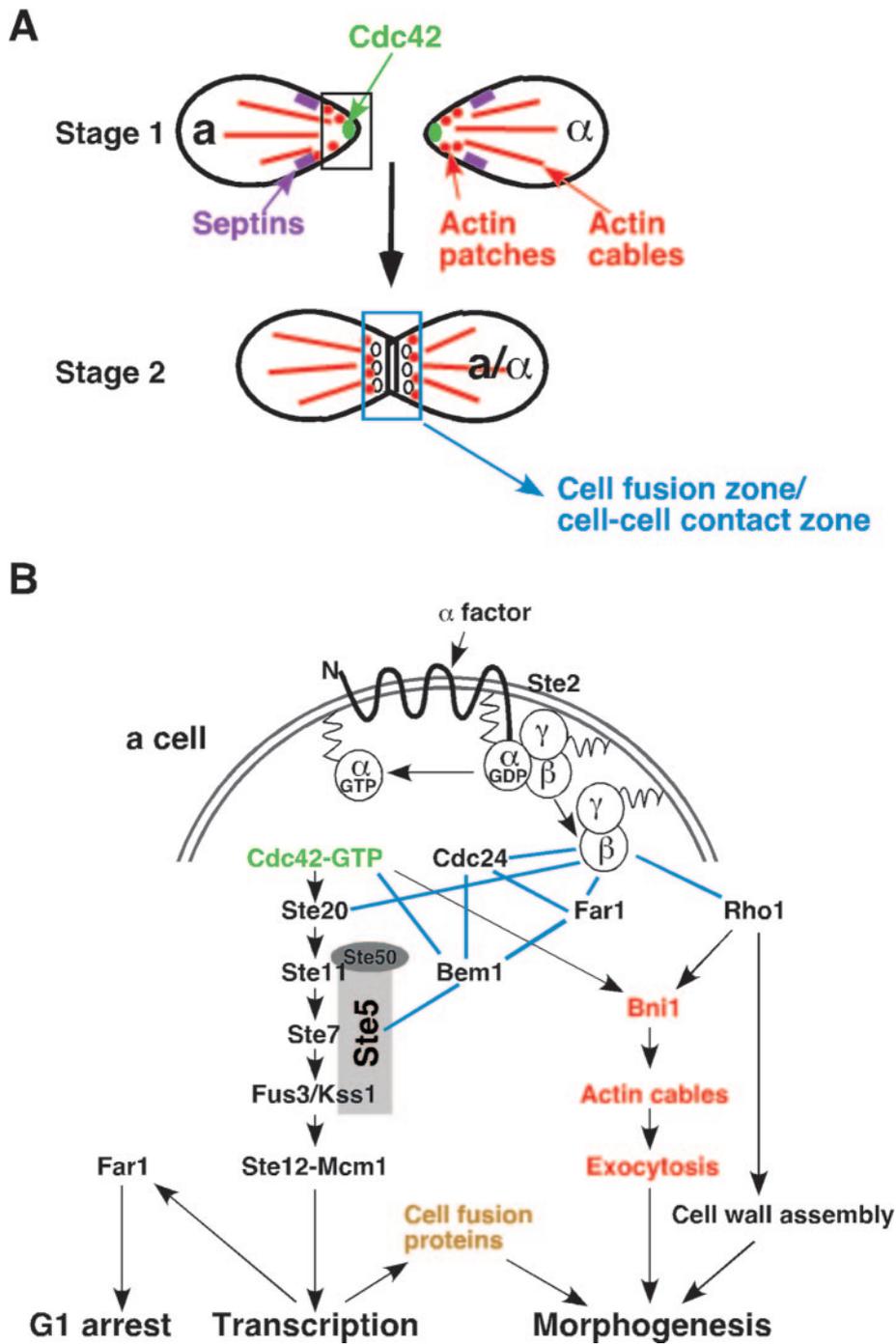


FIG. 13. Role of Cdc42 in cell polarization during mating. (A) In response to a gradient of mating pheromones secreted by cells of the opposite mating type, haploid **a** or α cells polarize to form the mating projection (stage 1). Cdc42 and other polarity proteins localize to the tip of the mating projection. Actin cables and patches are also polarized towards the tip. Septins, which may be involved in defining membrane domains, localize as patches at the base of the mating projection. The signaling events at the shmoo tip, indicated by the black box in the **a** cell at stage 1, are illustrated in panel B. During cell fusion (stage 2), the membrane protein Fus1 localizes to the cell-cell contact zone. Together with Cdc42, Bni1, and the polarisome component Spa2, Fus1 is involved in the localized delivery of secretory vesicles (black circles) to the cell-cell contact zone, leading to the eventual cell fusion. (B) Upon pheromone binding, the receptor Ste2 clusters and activates the heterotrimeric G protein, which anchors to the plasma membrane via the lipid-modified tails (indicated by the wavy line) of its α (Gpa1) and γ (Ste18) subunits. The GTP-bound α subunit dissociates from the β (Ste4)- γ complex. The $\beta\gamma$ complex then triggers the activation of the MAPK cascade, resulting in transcriptional activation and G₁ arrest. The activation of G $\beta\gamma$ also triggers polarized growth towards the pheromone gradient. The blue lines indicate protein-protein interactions (see the text for details). Rho1 has been shown to interact with the β subunit and is thus recruited to the shmoo tip. See Fig. 15 for the Rho1 pathway. For simplicity, extensive cross talks between the MAPK cascade and actin organization are not indicated here.

budding. Thus, it appears that the same Cdc42-based machinery functions in polarized morphogenesis during mating and budding in response to distinct signals, an external signal during mating and an internal signal (or cell cycle clock) during budding.

Cdc42 regulates the mating response via at least two distinct pathways: the activation of the MAPK pathway and the formation of the mating projection (Fig. 13). It is not clear whether and/or to what extent there is cross talk between these two pathways at the molecular level. Cdc42 and its GEF, Cdc24, are required for MAPK activation (532, 643) and morphogenesis, leading to the formation of the mating projection (see below). Cdc42 is likely to activate the PAK Ste20, as specific pheromone-resistant *cdc42* alleles that do not affect budding but that affect MAPK signaling and Ste20 localization were isolated (24, 400). It has also been shown that Cdc42-GTP interacts with Ste20 *in vitro* and *in vivo* (445). The polarity protein Bem1 also interacts with Ste5 (307, 345), a scaffold protein that interacts with multiple components of the MAPK pathway and endows the shared MAPK core components with signaling specificity in the mating response (64, 134, 507). This Bem1-Ste5 interaction and an interaction between Cdc42 and Bem1 are thought to enhance MAPK signal transduction (307, 345, 400). Once activated by Cdc42, Ste20 in turn phosphorylates an autoinhibitory domain at the N-terminal region of Ste11, leading to its activation (125) and consequently turning on the MAPK pathway. Ste20 interacts directly with the G-protein β -subunit Ste4, thus linking the MAPK cascade to the receptor-coupled G protein. This interaction between Ste20 and Ste4 is essential for MAPK activation (308).

In addition to shmoo formation, Cdc24 and Cdc42 are also required for the subsequent cell fusion by restricting and/or maintaining cell fusion proteins such as Fus1 to the cell-cell contact zone during mating. The cell fusion zone during mating can be viewed as two shmoo tips facing each other (stage 2) (Fig. 13A) (35, 36). Fus1, an O-glycosylated membrane protein (577), is required for the normal localization of vesicles to the cell fusion zone (166). These vesicles presumably carry enzymes for the formation and subsequent degradation of the apposing cell wall (or septum) of a prezygote, leading to cell fusion. The cytoplasmic domain of Fus1 interacts with Chs5, a late-Golgi protein required for delivering chitin synthase Chs3-containing vesicles to the plasma membrane during budding (494). The *chs5* mutants are defective in targeting Fus1 to the shmoo tip (406, 493) and fail to mate at the cell fusion step (492). This fusion defect can be partially suppressed by increasing the dosage of Fus1 in the cell (492). Fus1 also interacts with Cdc42-GTP and the formin Bni1 in two-hybrid assays (406). Mutants lacking Bni1 or other polarisome components including Spa2 and Pea2 are also defective in cell fusion (117). Spa2 is required for clustering vesicles at the cell fusion zone during mating (166). Cells lacking the tropomyosin Tpm1 are severely defective in maintaining actin cables and also fail at the cell fusion step during mating (328). Cdc42 and Bni1 are responsible for the localized formation of actin cables, which are stabilized by Tpm1. The polarized actin cables guide the Myo2-powered delivery of the Chs5-escorted, Chs3-containing vesicles to the cell fusion zone. Fus1 may be delivered to the plasma membrane via the Chs5-marked vesicles. Cdc42 then

maintains Fus1 at the plasma membrane via a direct or an indirect interaction.

Two mating-specific mutants of *CDC24*, *cdc24-m5* and *cdc24-m6*, were isolated and were found to have mutated residues in the conserved catalytic domain of Cdc24 GEF (36). Interestingly, the *cdc24-m6* mutant responds normally to pheromones and orients its growth towards a mating partner, yet it accumulates prezygotes during mating. Despite normal exocytosis, *cdc24-m6* mutant cells fail to localize proteins required for cell fusion. Thus, Cdc24 GEF activity is likely to be required for maintaining or restricting specific proteins essential for cell fusion to the cell-cell contact region. However, it is not clear why certain residues of the GEF domain are important only for mating, since Cdc24 GEF activity is also essential for budding. It remains to be seen whether these Cdc24 mutant proteins are partially defective in their GEF activity and whether higher GEF activity is required during mating in comparison to budding.

Despite the involvement of common players such as Cdc42 and its downstream targets in the polarization of the actin cytoskeleton during both budding and mating, it appears that there are some intrinsic differences between these processes. In contrast to budding in which localization of Cdc42 does not depend on actin (30), an intact actin cytoskeleton is required to maintain the polarized localization of Cdc42 and Bem1 at the shmoo tip (29). This is consistent with the finding that even after an axis of polarization is established, constant reinforcement is required to stabilize and/or maintain the polarity during mating (410). When F-actin is disrupted by LatA, Cdc42, Bem1, Spa2, and septin Cdc10 are all dispersed from shmoo tips, and pheromone receptors are no longer clustered even in the presence of their ligands (29). Receptor clustering is presumably required for the localization of many downstream components including the polarity proteins mentioned above. This result also implies that it is nearly impossible to separate the role of Cdc42 in polarized actin organization from its role in MAPK signaling. Consistent with this notion, the MAPK Fus3 phosphorylates Bni1 and is required for Bni1 localization and polarized actin organization during mating (367).

Filamentous Growth

Haploid cells penetrate agar in response to nutritional limitation. This mode of growth is termed invasive growth. Upon nitrogen starvation, diploid cells undergo a dimorphic transition known as pseudohyphal growth, which results in the formation of chains of cells with separated cytoplasm and a cell wall linkage between different cellular compartments (178, 296). Collectively, these modes of cell growth are called FG (Fig. 1C) (64), although all three terms and additional ones have been used interchangeably in many papers. The physiological function of FG is thought to enable yeast cells to forage for nutrients (178, 296). The regulatory networks involved in filamentous growth are complex and have been extensively discussed in several excellent reviews (64, 428, 483, 507). Here, we will briefly review the major molecular events involved in pseudohyphal formation.

Upon nitrogen starvation, several important cellular changes occur in a coordinated fashion: a change in the budding pattern from bipolar to unipolar (178), a G_2 delay coupled with

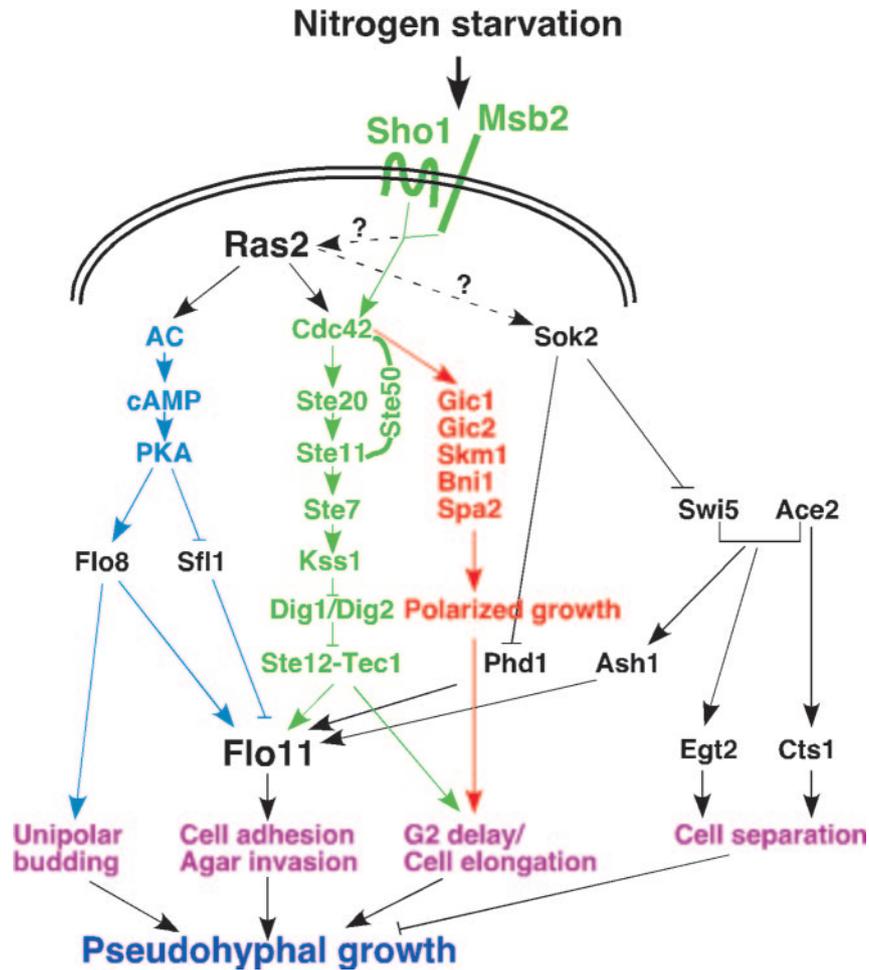


FIG. 14. Signaling pathways leading to pseudohyphal growth. Nitrogen starvation is sensed by Ras2, which controls the expression of the cell surface flocculin Flo11 via two distinct pathways, the cAMP-dependent PKA pathway (in blue) (AC, adenylyl cyclase) and the MAPK pathway (in green). PKA activates the transcriptional activator Flo8 and inactivates the transcriptional repressor Sfl1, both of which contribute to the increased expression of Flo11. Ras2 somehow activates Cdc42, which activates the MAPK cascade via its effector, Ste20. The MAP kinase Kss1 inactivates the repressors Dig1 and Dig2, thereby allowing Ste12 and Tec1 to activate the expression of Flo11 as well as of other proteins involved in cell elongation. As in budding and mating, Cdc42 regulates polarized actin organization via its effectors (in red), thereby contributing to the elongated cell morphology. In response to the nitrogen starvation signal, Sok2 negatively regulates the expression of the transcription factors Phd1 and Swi5, which activate Flo11 expression directly or via the daughter cell-specific transcription factor Ash1. The two transcription factors Swi5 and Ace2 activate the expression of the endoglucanase Egt2 and the endochitinase Cts1. Decreased expression of these key enzymes that are required for cell separation after cytokinesis causes cell attachment during filamentous growth.

cell elongation (296), the adhesion between cellular compartments as the result of the expression of new adhesive molecules on the cell surface (332, 333), and an inhibition of the expression of enzymes that degrade the cell wall and septa (428) (Fig. 14). The nutritional signal is sensed by two membrane proteins, the osmosensor Sho1 and the mucin-related glycoprotein Msb2, both of which interact with each other to regulate filamentous growth (101). In addition, Msb2 interacts with Cdc42-GTP preferentially, and this interaction is thought to trigger the activation of the FG-specific MAPK pathway (101), which consists of the same core components involved in the mating response (329). The scaffold protein Ste50 associates with Ste11, and this interaction is required for optimal invasive growth and hyperosmotic stress (high-osmolarity glycerol) signaling but has a lesser role in the pheromone response (576). Ste50 also interacts with Cdc42 via its C-terminal

“RA” (Ras association) domain (but this RA domain does not bind to Ras2) (576) and thus serves as an adaptor to tether Ste11 to the plasma membrane where Cdc42 and activated Ste20 are located, leading to the activation of Ste11 by Ste20 (576). The activation of the FG-specific MAPK pathway leads to the expression of Flo11, a glycosylphosphatidylinositol-linked cell surface protein, which mediates cell-cell adhesion (332, 333). The nutritional signal from the membrane sensors Sho1 and Msb2 is somehow sensed by Ras2 (178), which also activates the MAPK cascade (178, 396). At the same time, Ras2 activates the cyclic AMP-dependent protein kinase A (PKA), which directly regulates two functionally antagonistic transcriptional factors, Flo8 and Sfl1, to control Flo11 expression (430). Thus, Ras2 regulates Flo11 expression through both MAPK and PKA pathways. It is thought that the MAPK Fus3 is mainly

involved in mating by activating the transcriptional heterodimer Ste12-Mcm1, whereas the MAPK Kss1 is mainly involved in filamentous growth by activating the transcriptional heterodimer Ste12-Tec1 (98, 352). However, genome-wide studies indicate that the expression profiles specified by Fus3 and Kss1 overlap more than previously thought (64, 472, 639). The PKA pathway also affects the budding pattern. Both PKA and MAPK pathways may contribute to the G₂ delay, which is responsible for cell elongation (428, 483).

Epistasis analyses indicate that Cdc42 functions downstream of Ras2 and activates the MAPK cascade via Ste20 (396). Specific mutations in *CDC42* that affect filamentous growth, but not budding, were isolated. Analysis of these mutations indicate that Cdc42 effectors, including the CRIB motif-containing proteins Gic1 and Gic2 and the PAK Skm1, may be involved in filamentous growth (394). Cdc42 interacts with Sho1 and also with Msb2 to promote differential activation of the FG-specific MAPK Kss1 (101). Screens for mutations that affect pseudohyphal growth indicate that the formin Bni1, the scaffold Spa2, and the tropomyosin Tpm1 are also required for pseudohyphal growth (393). Thus, the same Cdc42-controlled polarity machinery is operating in filamentous growth as well. However, it is not clear how Ras2 activates Cdc42 and whether the role of Cdc42 in MAPK signaling can be separated from its role in polarized actin organization during filamentous growth.

OTHER SMALL GTPases AND THEIR ROLES IN POLARIZED GROWTH

Besides Cdc42, there are five other Rho proteins, Rho1 to Rho5, in *S. cerevisiae*. Among them, Rho1 has been the most extensively studied Rho protein, and its role in cell wall assembly has been discussed in detail in several excellent reviews (218, 313). Here, we will briefly discuss the functions of Rho1 in polarized cell growth. We will also discuss Rho3 and Rho4, whose functions in actin organization and exocytosis have been studied to some extent. Very little is known about Rho2 (351) and Rho5 (481), two nonessential Rho GTPases in *S. cerevisiae*. Multicopy *RHO1* or *RHO2* suppresses the temperature-sensitive growth of cells carrying a deletion of *ROM2*, which encodes a GEF for Rho1 (424) (see below). In addition, multicopy *RHO2* suppresses the growth defects of profilin (356) and *tor2* deletion strains (497), both of which are defective in actin organization (201, 498). The *tor2* deletion strain is also suppressed by multicopy *RHO1* or *ROM2* (497). Furthermore, multicopy *RHO2* plus *RHO1* suppresses the temperature-sensitive growth of a mutant allele of *BEM2* (282), which encodes a GAP for Rho1 (446, 646). Together, these genetic data suggest that Rho2 may share some functions with Rho1 and may regulate actin organization by an unknown mechanism. Rho5, which possesses an effector domain very similar to that of Cdc42/Rac1 (481), has been reported to regulate transient actin depolarization during a temperature shift to 37°C and also to down-regulate the Pkc1-dependent cell wall integrity CWI pathway (501). However, the physiological significance of these observations is not clear, as the phenotypes of *rho5* mutants appear to vary depending on the strain backgrounds (K. Singh and H.-O. Park, unpublished observations).

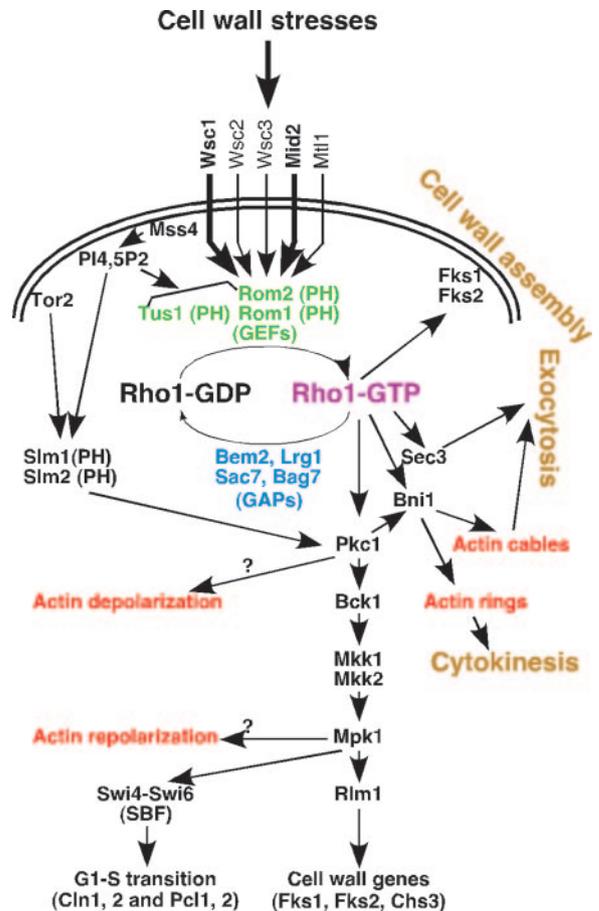


FIG. 15. Signaling pathways mediating the roles of Rho1 in cell wall assembly and actin organization. Cell wall stresses are sensed by the five type I membrane proteins Wsc1, Wsc2, Wsc3, Mid2, and Mtl1, with the major players indicated by the thick lines and boldface type. These sensors then activate the GEFs Rom1 and Rom2, which in turn activate Rho1. Activated Rho1 controls cell wall assembly in two distinct ways: as the regulatory subunit of the glucan synthases Fks1 and Fks2 and by activating the Pkc1-MAPK-mediated CWI pathway, which increases the expression of cell wall synthetic enzymes. Rho1-GTP also controls polarized exocytosis as indicated in Fig. 10. Rho1-GTP also regulates actin ring formation via Bni1 to effect cytokinesis. Mss4, the PI4P-5K, synthesizes the plasma membrane pool of PI4,5P2, which recruits the Rho1 GEFs to the plasma membrane, thus contributing to Rho1 activation. PI4,5P2 and Tor2 are involved in recruiting and/or maintaining the pair of PH domain-containing proteins, Slm1 and Slm2, at the plasma membrane. These Slm proteins are thought to function in actin organization via Pkc1 but not the MAPK pathway. In response to heat stress, Rho1-Pkc1 is thought to function in transient actin depolarization, whereas the MAPK pathway is thought to function in subsequent actin repolarization during the recovery phase (see the text for further details). SBF, Swi4/Swi6 cell cycle box binding factors.

Rho1 Regulators

Rho1 GEFs. Like Cdc42 and other small GTPases, the interconversion of Rho1 between its GDP- and GTP-bound states is regulated by at least three types of regulators: GEFs, GAPs, and GDI. There are at least three known GEFs, which include Rom1, Rom2, and Tus1 (Fig. 15). Multicopy *ROM1* or *ROM2* suppresses the cold-sensitive growth phenotype caused by a dominant negative allele of *RHO1*, *rho1*^{G22S,D125N} (424).

Like most Rho GEFs, Rom1 and Rom2 contain the DH domain, which is responsible for GEF activity for Rho GTPases, and the PH domain, which is implicated in the membrane association of the proteins and is also commonly found in Rho GEFs (424). Like Rho1 (627), Rom2 localizes to sites of polarized growth during budding and mating (355). A deletion of *ROM1* does not produce any obvious phenotype, whereas a deletion of *ROM2* causes temperature-sensitive growth at 37°C in most strain backgrounds (355, 424). The deletion of *ROM1* and *ROM2* together causes cell lethality. Like cells depleted for Rho1, cells depleted for both Rom1 and Rom2 (424) and *rom2Δ* cells grown at 37°C (355) arrest as small-budded cells. The temperature sensitivity of a *rom2Δ* strain is suppressed strongly by multicopy *RHO1* or *RHO2* and weakly by *RHO3* or *RHO4*, but not by *CDC42*, suggesting that Rom2 functions as a GEF mainly for Rho1 and Rho2. Indeed, Rom2 displays GEF activity towards Rho1 (424, 497). As described below, Rom1 and Rom2 are mainly involved in cell wall integrity by activating Rho1. In addition, multicopy *ROM2* or *RHO2* suppresses the temperature sensitivity (355) caused by the deletion of *KAR3* (380) and *CIK1* (94, 354, 427, 546), which encode a microtubule minus-end-directed kinesin and its targeting subunit, respectively. It is not clear how Rom2 and Rho2 regulate microtubule-related functions. Besides Rom1 and Rom2, Tus1 functions as a GEF for Rho1. Tus1 has a DH and a PH domain and has GEF activity towards Rho1 in vitro (496). Diploid strains homozygous for *tus1Δ* are temperature sensitive for growth at 39°C, and this temperature sensitivity is suppressed by 1 M sorbitol, by multicopy *ROM2* or *RHO2*, or by activated versions of genes encoding kinases (Pkc1, Bck1, and Mkk1) constituting the CWI pathway (496) (see below). Tus1 thus appears to regulate cell wall assembly by activating Rho1 and Rho2.

Rho1 GAPs. There are four known GAPs for Rho1: Bem2, Lrg1, Sac7, and Bag7 (Fig. 15). Bem2 was identified from a genetic screen for mutations (46) that display synthetic lethality with the deletion of *MSB1*, a common multicopy suppressor for several *cdc24-Ts* and *cdc42-Ts* mutants (45, 169). Bem2 has a Rho GAP domain, which is preceded by a PH domain, and displays GAP activity towards both Rho1 and Cdc42 in vitro (357, 446, 646). The deletion of *BEM2* causes temperature-sensitive growth at 37°C, and the mutant cells arrest mostly as large, round, unbudded cells and sometimes as large, round cells with a small bud (46, 282, 446). This phenotype resembles that caused by a loss of Cdc42 function, which is intuitively opposite to what is expected for the deletion of a Cdc42 GAP (91). The *bem2Δ* mutation is synthetically lethal with *rga1Δ*. The temperature sensitivity of *bem2Δ* cells is suppressed by increasing the dosage of the Cdc42 effector Gic1 (90) and also by simultaneously increasing the dosage of Rho1 and Rho2 (282). Like *rho1* mutants, *bem2* mutants display cell wall defects (97), and the overexpression of Bem2 down-regulates the Pkc1-Mpk1-mediated cell wall integrity pathway downstream of Rho1 (499). Together, these results suggest that Bem2 functions in both Cdc42- and Rho1-mediated processes, but the underlying mechanisms remain obscure. Lrg1, which contains a Rho GAP domain in its C-terminal region and three LIM domains in its N-terminal region, interacts with Rho1 (603) and has GAP activity towards Rho1 in vitro but not for any other Rhos and Cdc42 (151). Among other Rho GAPs tested,

Lrg1 is uniquely required for the negative regulation of Rho1-mediated glucan synthase activity (603). Lrg1 localizes to the tip of the mating projection and is required for efficient cell fusion by regulating Rho1-mediated localized glucan synthesis at the shmoo tip (151). Sac7 was originally identified from a genetic screen for mutations that suppress the temperature-sensitive growth of the actin allele *act1-4* (130). The deletion of *SAC7* causes a cold-sensitive defect in growth and actin organization (130, 497). Sac7 contains a Rho GAP domain and displays GAP activity specifically for Rho1 but not for Rho2 and Cdc42. Null mutations of *SAC7* and *ROM2* suppress each other, and the deletion of *SAC7* or overexpression of *ROM2* suppresses the temperature-sensitive mutant of *TOR2*, which is known to regulate the Rho1-Pkc1-Mpk1 cell wall integrity pathway and actin organization (497). Together, these results suggest that Sac7 is a Rho1-specific GAP. Bag7 contains a Rho GAP domain but no other obvious motifs. Bag7 interacts with Rho1, but not Rho2-Rho5 and Cdc42, and has GAP activity towards Rho1 (481, 499). A deletion of *BAG7* does not exhibit any obvious phenotype. However, the overexpression of *BAG7* suppresses the cold sensitivity of *sac7Δ* cells, suggesting that Bag7 and Sac7 may share some functions, perhaps in actin organization (497, 499). The deletion of individual Rho1 GAPs produces different growth defects, suggesting that these GAPs have differentiated roles in regulating Rho1-mediated processes. Indeed, Bem2 and Sac7 are known to regulate the Rho1-Pkc1-Mpk1 signaling pathway (362, 499), whereas Bag7 and Sac7 are thought to regulate some aspect of Rho1-mediated actin organization (499), and Lrg1 is required for Rho1-mediated regulation of glucan synthase activity (603).

Rho1 is involved in cell wall synthesis, stress responses, actin organization, and exocytosis (see below). The challenge is to figure out how the activity of Rho1 is regulated to control these distinct processes. Specifically, where and how are different Rho1 regulators such as the GEFs and GAPs localized in the cell cycle? How are the activities of the GEFs and GAPs of Rho1 regulated? Are the GEFs and GAPs regulated in responses to different environmental stimuli? Knowledge on the shared or unique role for each GEF and GAP and their regulatory mechanisms would be crucial for understanding the diverse roles of Rho1.

Rho1 Effectors and Biological Responses

Rho1 and cell wall assembly. The rigidity of the cell wall and the internal turgor pressure against the plasma membrane offer yeast cells excellent physical and chemical protection against a sudden change in osmolarity in their natural living environment (313). The cell wall is also elastic, ideal for maintaining cell shape and allowing localized surface expansion. Like the polarity proteins and the actin cytoskeleton discussed above, cell wall remodeling, including its synthesis and breakdown, must occur dynamically at sites of polarized cell growth so that plasma membrane and cell wall expansion are coordinated. The cell wall of *S. cerevisiae* consists of an electron-transparent inner layer and an electron-dense outer layer. The inner layer is composed of 1,3-β-glucan and chitin (*N*-acetylglucosamine polymers), accounting for ~50 to 60% of the wall dry weight, and this layer is mainly responsible for the mechanical strength of the wall. The outer layer is composed of heavily

glycosylated mannoproteins, accounting for 35 to 40% of the wall dry weight, and this layer mediates cell-cell interactions during mating and controls the accessibility of extracellular substances such as wall-degrading enzymes in plant tissues to the inner layer and the plasma membrane (287). The wall also consists of 1,6- β -glucan, accounting for 5 to 10% of the dry weight, whose main function is to link glycosylphosphatidylinositol-dependent cell wall proteins to β -1,3-glucan and possibly chitin to form a branched cell wall network (74, 287). The β -1,3-glucan is synthesized at the plasma membrane, and β -1,6-glucan synthesis may also occur at the plasma membrane, but it depends on the function of an intact secretory pathway (74, 287). Chitin, accounting for 1 to 2% of the wall dry weight, is synthesized at localized areas of the plasma membrane, mainly at the base of the bud from late G_1 to G_2/M phases and at the division septum in late anaphase and telophase (74). Here, we focus on how Rho1 controls β -1,3-glucan synthesis during bud growth by regulating the level and the activity of the β -1,3-glucan synthase under normal and stressed conditions.

Two related genes, *FKS1* and *FKS2*, encode the catalytic subunits of β -1,3-glucan synthase. *Fks1* is the predominantly expressed form under normal growth conditions, while *Fks2* is induced mainly under stress conditions such as high temperature, nutritional starvation, and mating pheromone treatment (313). Both *Fks1* and *Fks2* contain multiple membrane-spanning domains (119, 244, 372). The deletion of *FKS1* or *FKS2* does not cause cell lethality, but the deletion of both genes is lethal (244, 372). Rho1 regulates β -1,3-glucan synthesis via at least two distinct pathways, as a direct regulatory subunit of glucan synthase and as an upstream activator of the CWI pathway (Fig. 15) (313). These two pathways can be genetically separated from each other by specific mutations in *RHO1* (488). Temperature-sensitive mutants of *rho1* are defective in GTP-stimulated glucan synthase activity (124, 462). Both Rho1 and *Fks1* localize to sites of polarized growth (462, 627), Rho1 copurifies with *Fks1*, and GTP-bound Rho1 stimulates its enzymatic activity in vitro (124, 371, 462). Rho1-GDP and *Fks1* are transported via secretory vesicles to the sites of polarized growth (2, 374). The activation of *Fks1* at the plasma membrane is thought to be controlled by the membrane-associated GEFs Rom1 and Rom2 (2). Rho1 and *Fks1*, particularly the latter, appear to colocalize with actin patches (462, 583, 627). The actin patch-associated movement of *Fks1* may be involved in localized cell wall remodeling (583) as well as endocytosis (106). Thus, Rho1 directly regulates β -1,3-glucan synthase activity under normal growth conditions. An in vitro assay based on crude membrane preparations from *S. cerevisiae* indicates that Rho1, but not other Rhos and Cdc42, may also regulate β -1,6-glucan synthase activity (595).

Rho1 also regulates the expression of cell wall-related genes such as *FKS1*, *FKS2*, and *CHS3*, which encodes the catalytic subunit of chitin synthase III via the CWI pathway (74, 218, 313) (Fig. 15). The major components of the CWI pathway function as follows. Rho1-GTP activates its effector Pkc1 (the sole protein kinase C in *S. cerevisiae*), which activates a MAPK cascade from Bck1 (MAPK kinase kinase), Mkk1, and Mkk2 (two functionally redundant MAPK kinases) to Mpk1 (MAPK; also called Sit2). Mpk1 then activates at least two transcriptional factors, the Swi4/Swi6 cell cycle box binding factors and

Rlm1, to regulate the expression of genes required for the G_1/S transition, such as G_1 cyclins (Cln1, Cln2, Pcl1, and Pcl2), and genes required for cell wall biogenesis, such as the enzymes mentioned above. Like Rho1, Pkc1 (109), Mkk1/2, and Mpk1 (590) localize to sites of polarized growth. In addition, Pkc1 localizes to the nucleus and the mitotic spindle (109), and Mpk1 also localizes to the nucleus (590). This evidence and other evidence suggest that Pkc1 may play an Mpk1-dependent and an Mpk1-independent role in regulating microtubule-related functions (313). *PKC1* is an essential gene, and the conditional depletion of Pkc1 results in cell cycle-specific cell lysis at all growth temperatures, which is suppressed by high osmolarity, such as the presence of 1 M sorbitol (314, 432). In contrast, cells lacking each component of the MAPK cascade (or Mkk1 and Mkk2 together) exhibit a temperature-sensitive cell lysis defect, which is suppressed by osmotic stabilizing agents (304), indicating that Pkc1 has additional targets besides the MAPK pathway (313) (see below).

The CWI pathway responds to various extracellular stimuli. For example, cell wall stresses caused by calcofluor, Congo red, zymolyase, or caffeine are sensed by five known type I membrane proteins, Wsc1 (also called Hcs77 and Slg1), Wsc2, Wsc3, Mid2, and Mtl1, with the first three and last two defining two groups based mainly on sequence homology (313). Among them, Wsc1 and Mid2 play a dominant role, as the double mutant is inviable but can be rescued by high osmolarity (449, 463). The intracellular domains of Wsc1 and Mid2 interact with and activate Rom2 (449), triggering the activation of the CWI pathway. The activation of CWI is also regulated by phospholipids (218, 313). There are two essential phosphatidylinositol-4 kinases (PI4Ks) in *S. cerevisiae*, the plasma membrane-localized Stt4 and the Golgi-localized Pik1, which synthesize phosphatidylinositol-4-phosphate (PI4P) at both locations. PI4P is further converted to phosphatidylinositol-4,5-bisphosphate (PI4,5P2) by the single essential PI4P-5 kinase (PI4P-5K), Mss4. The plasma membrane pool of PI4,5P2 is required for the localization and the activation of the Rho1 GEF Rom2 via an interaction between the PIP2 and the PH domain of Rom2. This interaction is essential for the activation of the CWI pathway (25–27).

Rho1 and actin organization. Rho1 regulates actin organization via two distinct pathways: the CWI pathway and the formin Bni1 (Fig. 15). Specific *rho1*-Ts alleles display actin organization defects at the nonpermissive temperature (200, 219). Cells lacking Mpk1 also cannot polarize their actin cytoskeleton at 37°C (373). Thus, the Rho1-mediated CWI pathway is required for actin organization, at least, at 37°C. Upon a shift to 37°C, wild-type cells depolarize actin patches and cables transiently, reaching the peak at approximately 30 to 45 min after the shift, and cells repolarize their actin cytoskeleton at between 60 and 120 min (111, 322). The heat stress is thought to weaken the cell wall and thus activate the CWI pathway (269). Further analysis indicates that the CWI pathway bifurcates at Pkc1 to regulate actin depolarization and repolarization. The depolarization phase does not involve the MAPK cascade, but the repolarization phase does (107) (Fig. 15). Disruption of the actin cytoskeleton by latrunculin B also activates the CWI pathway (213). It is yet to be established how the CWI pathway regulates actin organization at the molecular level.

The CWI pathway also functions in Tor (target of rapa-

mycin)-mediated actin organization. Tor1 and Tor2, a pair of highly conserved Ser/Thr kinases, control cell growth in response to nutrients. Both Tor1 and Tor2 possess multiple domains that mediate protein-protein interactions to form Tor complexes, TORC1 and TORC2, respectively (339, 360, 623). These two complexes share essential functions in regulating translation and G₁ progression. They also perform distinct functions. TORC1 controls cell growth via a rapamycin-sensitive pathway regulating transcription, translation, and ribosome biogenesis. In contrast, TORC2 signals through a rapamycin-insensitive pathway to regulate the actin cytoskeleton (498). In *S. cerevisiae*, the CWI pathway is thought to mediate signaling from TORC2 to the actin cytoskeleton, because the overexpression of Pkc1, Bck1, Mkk1, or Mpk1 suppresses the growth and actin organization defects of the *tor2*-Ts cells (219). Because Mpk1 activation is not impaired in the *tor2*-Ts cells, it is equally possible that Tor2 and the CWI pathway signal in parallel to the actin cytoskeleton (313).

The formins Bni1 and Bnr1 are required for actin cable formation during polarized growth (140, 486) and are required for actin ring formation during cytokinesis (573, 587). Rho1 binds to Bni1 (138, 289) and is required for the activation of formin at 37°C (116). This activation requires Pkc1 but not the downstream MAPK cascade of the CWI pathway (116). Thus, it appears that Rho1-Pkc1 controls transient actin depolarization during heat stress (107) and also controls formin-mediated actin cable formation at 37°C (116). It is not clear how these two functions of Pkc1 are coordinated temporally and spatially during the temperature shift.

Phospholipids and TORC2 also act in concert to regulate actin organization. Mss4, the PI4P-5K, is required for actin organization at 37°C, suggesting that PI4,5P₂ plays an essential role in this process (111). Slm1 and Slm2, a pair of functionally redundant proteins carrying the PH domains, are targeted to the plasma membrane in a PIP₂-dependent manner. Conditional inactivation of Slm1 and Slm2 (e.g., *slm1*-Ts *slm2*Δ) results in a depolarized actin cytoskeleton, secretion, and Cdc42 and Rho1 localization (28, 142). The growth and actin organization defects of *slm1*-Ts *slm2*Δ cells are suppressed by a deletion of the Rho1 GAP Sac7 or overexpression of Pkc1 but not by its downstream components of the CWI pathway (28). In addition, both Slm1 and Slm2 physically associate with the components of the TORC2 complex and are directly phosphorylated by the Tor2 kinase (28, 142). Furthermore, temperature-sensitive mutations in TOR2 (*tor1*Δ *tor2*-Ts) are suppressed by the overexpression of *MSS4*, *ROM2*, or *PKC1* (219). Together, these data suggest that PIP₂ and TORC2 coordinately regulate the membrane association of Slm1 and Slm2, which regulates actin organization via Pkc1, but not the MAPK cascade.

Rho1 and exocytosis. As discussed above, Rho1 regulates polarized exocytosis by regulating actin cable assembly via the formin Bni1. In addition, Rho1 binds to Sec3, a component of the exocyst that is essential for the tethering of the post-Golgi vesicles to the plasma membrane. The Rho1-Sec3 interaction is thought to play a role in the spatial regulation of exocytosis (200). In addition, during heat stress, Rho1 and Pkc1, but not the MAPK components of the CWI pathway, regulate the exit of Chs3, the catalytic subunit of chitin synthase III, from the

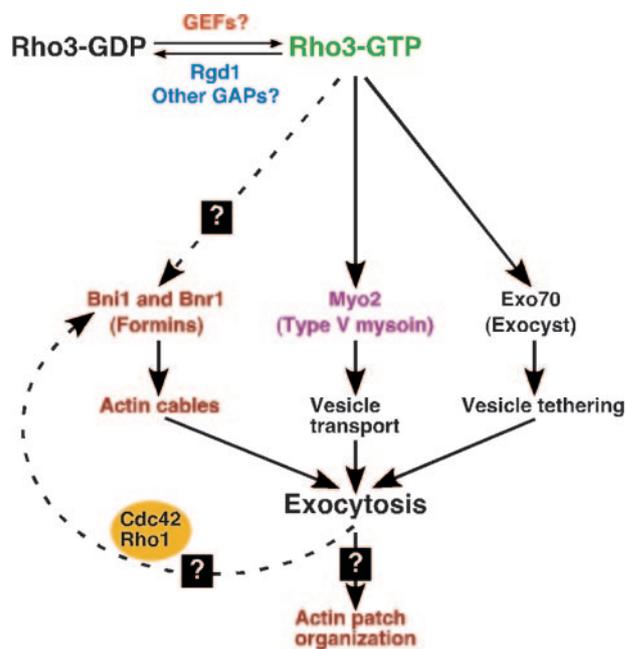


FIG. 16. Model for roles of Rho3 in exocytosis and actin organization. Activated Rho3 interacts with Myo2 and Exo70 to effect vesicle transport and vesicle tethering during exocytosis. Activated Rho3 and Rho4 (not depicted here) may activate the formins directly via their binding to the RBD of the formins (dashed line with a question mark). No physical interaction between these Rho proteins and the formins, except for the Rho4-Bnr1 interaction, has been reported. Alternatively, Rho3 and Rho4 could activate the formins indirectly and also affect actin patch polarization via their role in exocytosis (see the section on Cdc42 polarization). The specific GEFs and the GAPs, except Rgd1, of Rho3 and Rho4 are not known.

trans-Golgi network/early endosomes, which is essential for its targeting to the plasma membrane (585, 586).

Rho3, Rho4, and Polarized Cell Growth

Rho3 and Rho4 are another two small GTPases involved in polarized growth in *S. cerevisiae*. Among the nine predicted Rho GAPs (506), only Rgd1 is shown to display *in vitro* GAP activity towards Rho3 and Rho4 (115) (Fig. 16). Rgd1 contains a C-terminal Rho GAP domain and N-terminal FCH (FER/CIP4 homology) (238, 578) and DEP (disheveled, EGL-10, and pleckstrin) (359) domains, both of which are involved in membrane association. No GEFs for Rho3 and Rho4 have been identified. It is also not known whether Rdi1, the only Rho GDI in *S. cerevisiae*, acts on Rho3 and Rho4. Thus, the regulation of Rho3 and Rho4 is far from clear. The deletion of *RHO3* causes severe defects in cell growth, whereas the deletion of *RHO4* does not produce any obvious phenotype. However, the deletion of both *RHO3* and *RHO4* causes lethality at 30°C, and the overexpression of *RHO4* suppresses the growth defects of *rho3*Δ, suggesting that Rho3 and Rho4 have some overlapping functions, even though the two GTPases share only ~35% identity in their amino acid sequences (369). Depletion of Rho3 and Rho4 together results in large, round cells with a small bud and with a disorganized actin cytoskeleton (369, 370). Thus, Rho3 and Rho4 are thought to be required for the maintenance, but not the initiation, of

polarized cell growth. Because the growth defects of cells depleted for Rho3 and Rho4 are suppressed by an increased dosage of *CDC42*, *BEM1*, or *SEC4*, Rho3 and Rho4 are thought to play a role in polarized actin organization and exocytosis (241, 370).

Rho3, Rho4, and actin organization. The depletion of both Rho3 and Rho4 or specific mutations in the effector domains of Rho3 (*rho3^{Y40C}* and *rho3^{E46G}*) causes the depolarization of actin patches and the loss of actin cables (6, 370). The deletion of the Rho-binding domain (RBD) of the formin Bni1 or Bnr1 suppresses the growth and actin cable formation defects of *rho3Δ rho4Δ* cells, suggesting that Rho3 and Rho4 may activate the formins by binding to the RBDs and thus relieving a putative autoinhibitory loop formed between the RBD region at the N terminus and the DAD at the C terminus of formins (116). This idea remains to be tested. It has been shown that Rho4 interacts with Bnr1, but not with Bni1, by two-hybrid and in vitro protein-binding assays (242), but no data are available for the interaction of Rho3 with either Bni1 or Bnr1.

Rho3, Rho4, and exocytosis. Overexpression of the Rab Sec4 suppresses the growth defects of *rho3Δ rho4Δ* cells (241), suggesting that Rho3 and Rho4 are involved in exocytosis. Indeed, Rho3 interacts with both the type V myosin Myo2 and the exocyst subunit Exo70 (474). A specific mutation in the Rho3 effector domain (*rho3^{E51V}*) causes the accumulation of post-Golgi vesicles preferentially in the daughter cells at the permissive temperature and in the entire cells at the nonpermissive temperature (6). This mutant Rho3 no longer interacts with either Myo2 or Exo70. These data suggest that Rho3 plays a role in vesicle transport and tethering (6) (Fig. 16).

Polarized actin organization and exocytosis are intimately linked in *S. cerevisiae*. Depolarized actin organization such as that in *cdc24* and *cdc42* mutants results in depolarized exocytosis. Mutants defective in the late secretory pathway also exhibit a depolarized actin cytoskeleton (22, 168). Even *cdc42* and *rho3* mutants that were initially thought to display specific defects in exocytosis (5, 6) were found to have defects in actin organization (22). One intriguing possibility is that Rho3 regulates the delivery of other small GTPases that are required for Bni1 activation and/or localization via a secretory pathway and thus regulates actin organization indirectly. Although it remains to be tested, this hypothesis is consistent with the following observations. First, no direct interaction between Rho3 and Bni1 has been observed. Second, the growth defects of *rho3Δ rho4Δ* cells are suppressed by the overexpression of *Cdc42* (370), which reinforces itself at the sites of polarized growth via the actomyosin-based delivery or secretory pathway (604). Rho1 is also delivered via the secretory pathway (2, 374). Finally, *Cdc42* and Rho1 bind to Bni1, resulting in the localization and/or activation of Bni1.

There are many questions concerning the mechanisms by which Rho3 and Rho4 function. For example, where does Rho3 or Rho4 localize in the cell cycle? What are the GEFs and GAPs, in addition to Rgd1, for Rho3 and/or Rho4? What is the underlying mechanism for the cross talk between Rho3- and Rho4-mediated functions and the Rho1-Pkc1-mediated CWI pathway, as suggested by a number of genetic studies? For example, the deletion of *RGD1* is synthetically lethal with the deletion of *WSC1*, which encodes one of the membrane sensors for the CWI pathway (105). The lethality of *rgd1Δ*

wsc1Δ cells requires the presence of *RHO3* or *RHO4* (146) and is suppressed by multicopy *RHO1*, *RHO2*, *MPK1*, and *MTL1*, encoding a membrane sensor (105). As hypothesized for the coupling of the functions of Rho3 in actin organization and exocytosis described above, the genetic interactions between Rho3/Rho4 and the CWI pathway might also indicate the involvement of Rho3/Rho4 in the delivery of membrane sensors of the CWI pathway via the secretory pathway.

What Is the Role of Ras in Polarized Growth?

Ras1 and Ras2 (hereafter referred to as Ras for simplicity), two closely related Ras proteins in *S. cerevisiae* (170, 277, 562), are involved in choosing a developmental program or life style such as budding, filamentous growth, or sporulation by sensing and responding to nutrients such as carbon and nitrogen sources (502). During budding, Ras is required for cell growth and for the passage through the G₁/S transition, two intimately linked processes (311, 502, 606). Ras is regulated by the GEF Cdc25 (209, 262) and the GAPs Ira1 and Ira2 (557–559). In response to nutrients, Ras activates adenylyl cyclase to increase the cyclic AMP (cAMP) concentration, which in turn activates cAMP-dependent PKA by releasing the three catalytic subunits Tpk1 to Tpk3 from the single regulatory subunit Bcy1 (75, 569, 570), and Tpk3 then activate several transcriptional factors, thus increasing the expression of genes involved in stress responses, ribosome biogenesis, and filamentous growth (311, 502, 606). One of the major outputs of the Ras-cAMP pathway is to increase the cell size critical for passage through Start (264). During this period, the G₁ cyclin Cln3 is increased at the protein level, perhaps due to the increased rate of translation as a result of Ras-cAMP pathway activation (204). Ras triggers filamentous growth via two pathways, the Cdc42-MAPK pathway and the cAMP-PKA pathway (see above) (Fig. 14) (311, 395). The cellular cAMP concentration is down-regulated by two cAMP phosphodiesterases: the low-affinity Pde1 and the high-affinity Pde2 (311). Pde1 functions mainly during glucose readdition or intracellular acidification (346), whereas Pde2 functions mainly during filamentous growth (298, 342, 429).

The Ras-cAMP pathway and the Rho1-Pkc1 CWI pathway appear to have an antagonistic relationship, which is mediated in part by a putative transcriptional factor, Rpi1 (280, 539). There are also cross talks between the Ras-cAMP pathway and other signaling pathways involved in growth controls (311, 502, 606). In the fission yeast *S. pombe*, Ras1 regulates cell morphogenesis during vegetative growth and mating by regulating *Cdc42* and the mating MAPK pathway, respectively (431). These Ras1-mediated pathways are initiated by two distinct Ras1 GEFs (431). Similarly, Ras in *Dictyostelium* is also involved in cytoskeleton regulation and chemotaxis (323). Does Ras play a major role in polarized growth during budding in *S. cerevisiae*? A deletion of *RAS2* is synthetically lethal with a deletion of *TPM1* at 35°C, which encodes the major isoform of tropomyosin in *S. cerevisiae* (225). In addition, Ras2 and the *Cdc42* effector Cla4 function together to target Lte1 appropriately to the bud cortex to regulate the mitotic exit network (516) (see the discussion on *Cdc42* and MEN). Thus, Ras may play a more direct role in the regulation of polarized actin organization and/or growth during budding.

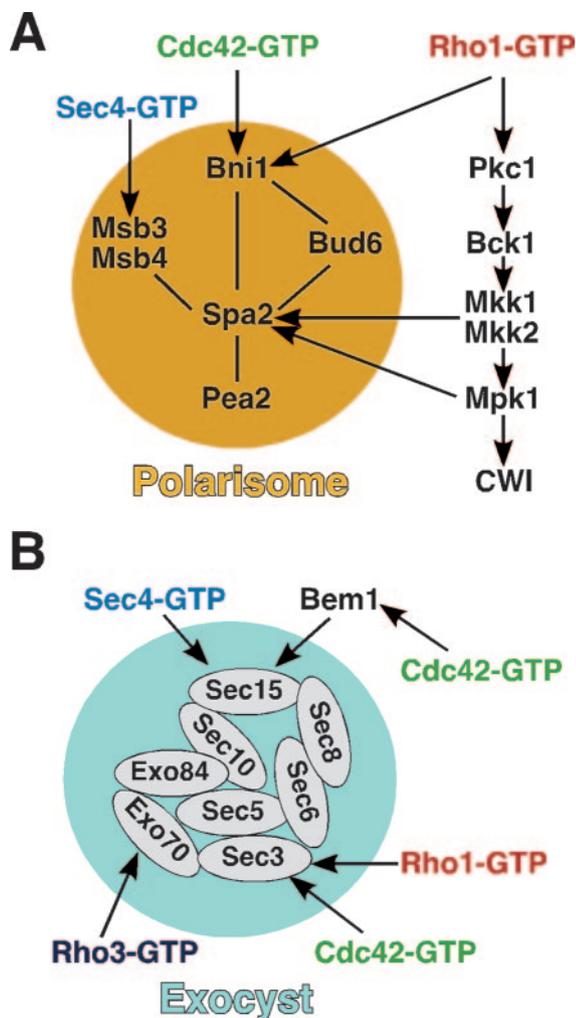


FIG. 17. Possible coordination mechanisms among small GTPases during polarized cell growth. (A) Polarisome-based signaling hub. Linkages between Cdc42 and Rho1 that are suggested by preliminary genetic and two-hybrid interactions are not depicted here. (B) Exocyst-based signaling hub (see the text for details).

COORDINATION OF Cdc42, Rho, AND Rab DURING POLARIZED GROWTH

Polarized growth occurs at discrete regions of the cell surface in *S. cerevisiae*, the presumptive bud site (late G₁), the tip of a small bud (S phase to G₂ phase), the entire bud surface (G₂/M phase to anaphase), and the bud neck of large-budded cells (late anaphase to telophase). Cdc42, different Rhos, and Rabs orchestrate the polarization of the actin cytoskeleton, cell wall assembly, and exocytosis towards these sites. Thus, the activities of these small GTPases and their regulated processes must be coordinated in time and space to generate the defined shape of the yeast cell. As discussed above, the functions of individual small GTPases in these processes are not completely understood. The coordination mechanisms among Cdc42, Rhos, and Rabs are even less clear. Nonetheless, we will attempt to summarize what is known about the possible coordination mechanisms here because of the central significance of this issue in polarized growth.

Polarisome-Based Signaling Hub

The “polarisome” consists of the scaffold protein Spa2, the formin Bni1, the actin-binding protein Bud6, Pea2, a protein of unknown function, and perhaps the Sec4 GAPs Msb3 and Msb4 (521, 563, 590) (Fig. 17A). Because the polarisome has never been consistently purified as a discrete protein complex like the Arp2/3 complex or the exocyst and because the “polarisome” simply represents a small network of physically interacting, functionally related proteins, it is sometimes difficult to determine whether a protein is a component of or just peripherally associated with the polarisome. The polarisome and the Gic1/Gic2 proteins act in parallel to regulate the processes downstream of Cdc42, such as the organization of the actin and septin cytoskeleton and exocytosis (Fig. 11 and 17A). At the bud tip, the polarisome coordinately regulates polarized growth (563). The bud tip is analogous to a focal adhesion site of an animal cell because both structures involve the polarized formation of actin cables or stress fibers, protein trafficking, which is dictated by the Rab Sec4 or Arf6, and perhaps localized activation of Cdc42, Rac, and Rho (563). The polarisome is a hub that integrates signals from Cdc42, Rho1, and the Rab Sec4 (247) (Fig. 17A). Msb3 and Msb4, a pair of dosage suppressors of *cdc24* and *cdc42* mutants (51), bind directly to the N-terminal Spa2 homology domain I (563) and also function as GAPs for Sec4 (12, 14, 168), thus linking Cdc42 to Sec4 via the polarisome. In addition, Cdc42-GTP and Rho1-GTP bind to Bni1 (138, 289), whereas these GTPases in the GDP-bound state bind to Msb3 and Msb4, thus linking Cdc42 and Rho1 together via the polarisome (563). The same Spa2 homology domain I region of Spa2 binds to Mkk1/Mkk2 and Mpk1 of the CWI pathway, thus linking Cdc42 to Rho1 again via the polarisome (521, 590).

A web of genetic interactions suggests that the function of Cdc42 and Rho1 is coordinated by several potential pathways during polarized cell growth. *ZDS1* and *ZDS2*, encoding two closely related proteins that localize to sites of polarized growth, appear to negatively regulate *CDC42* (53). A large-scale two-hybrid assay suggests that Zds1 and Zds2 interact with both Gic1 and Gic2, two closely related targets of Cdc42, and that Zds2 interacts with Rho1-GTP and Pkc1 as well (123). In addition, the overexpression of *ZDS1*, *ZDS2*, *PKC1*, or *GIC1* (but not *GIC2* or *CDC42*) suppresses the temperature-sensitive growth of *tif51A-1*, a mutant of the yeast translation initiation factor eIF5A (638). The actin organization defect of *tif51A-1* is also partially recovered by the overexpression of Pkc1 and Zds1 as well as Gic1. Taken together, these data suggest that the Pkc1-Zds-Gic interaction coordinates the function of Cdc42 and Rho1, although the underlying mechanism of this coordination is not clear. It is also unclear how eIF5A might be involved in the establishment of polarity in coordination with Pkc1 or Gic1. Given that *GIC1* encodes a putative effector of Cdc42, whereas *ZDS1* is a potential negative regulator of Cdc42, the similar phenotype of overexpression of each gene is perplexing. Interestingly, the overexpression of *PXL1*, encoding a paxillin-like protein in *S. cerevisiae*, suppresses two *cdc42*-Ts mutants but inhibits a *rho1*-Ts mutant, suggesting that Cdc42 and Rho1 may play an antagonistic role during polarized growth (169, 349). In addition, the overexpression of *ZDS1* and *MSB1*, a dosage suppressor of

cdc42-Ts mutants (45, 169), suppresses the temperature-sensitive growth of a glucan synthase mutant, *fkx2Δ fkx1*-Ts (514). Several genetic screens for genes that functionally interact with *CDC42* and *CDC24* led to the identification of *BEM4* (348), whose gene product interacts with multiple small GTPases including Rsr1, Cdc42, Rho1, Rho2, and Rho4 by two-hybrid assays (123, 348). Again, these genetic data suggest multiple cross talks between Cdc42 and Rho1, but the mechanistic details require further investigation.

Exocyst-Based Signaling Hub

The development of cell polarity is ultimately to establish distinct cellular domains or cellular asymmetry, which requires polarized secretion. The exocyst, an effector of Sec4 that consists of eight subunits (Sec3, Sec5, Sec6, Sec8, Sec10, Sec15, Exo70, and Exo84), plays an essential role in the tethering of the post-Golgi vesicles (199, 419, 544). The current idea is that Sec4, the Rab GTPase that is essential for protein trafficking from the Golgi apparatus to the plasma membrane, is required for vesicle transport and tethering and, indirectly, for the assembly of SNARE complexes leading to membrane fusion (199, 419). It has been proposed that Sec4 regulates exocytosis via two coordinated pathways (195). In one pathway, Sec4, along with its GEF, Sec2, and an incomplete version of the exocyst, is associated with the secretory vesicles and is transported towards sites of polarized growth via the actin cables and the type V myosin Myo2. Upon the arrival of the vesicles at their destination at the cell cortex, a complete exocyst complex is formed by joining the vesicle-associated and the plasma membrane-associated subunits of the exocyst (63), leading to vesicle tethering. Sec1 then mediates the binding of the exocyst to the assembled SNAREs by interacting with both of them (77, 612), leading to membrane fusion. In a parallel pathway, Sec4 interacts with another effector, Sro7 (and perhaps with its paralog, Sro77), which belongs to the lethal giant larva (*lgl*) tumor suppressor family (266, 301, 309), which in turn interacts with the target SNARE Sec9 (309) to somehow promote the function of SNARE complexes. These two pathways also talk to each other via direct interactions between Exo84 and Sro7/Sro77 (642). This hypothesis may explain complex genetic interactions observed among the components depicted in Fig. 17B.

The exocyst defines another hub that integrates the signals from many small GTPases during polarized growth (Fig. 17B). The Sec4-Sec15 interaction is thought to promote the formation and/or function of the exocyst (63, 198). Sec3 interacts with both Rho1 (200) and Cdc42 (641) in a competitive manner, and these interactions provide temporally regulated polarization signals to the exocyst. Another component of the exocyst, Exo70, interacts with Rho3 (6, 474, 482). This interaction is thought to positively regulate exocytosis, but the underlying mechanism remains unclear. In addition, Sec15 interacts with Bem1 (123, 155, 196), which interacts with Cdc42 and several other proteins involved in polarity establishment (58). The Sec15-Bem1 interaction is required for the efficient targeting of Sec15, particularly at an early stage of budding (155). Thus, the exocyst coordinates the signaling from Cdc42, Rho1, Rho3, and Sec4 during polarized growth. As in yeast, Rab and Rho family proteins have also been found to interact with the

exocyst in mammalian cells (419), suggesting that the regulation of the secretion machinery by small GTPases is evolutionarily conserved. The challenge now is to figure out how all these small GTPases regulate the exocyst at the biochemical level.

Cdc42 and Rho3 are also linked by two structurally related proteins, Boi1 and Boi2, both of which carry an SH3 domain at their N termini and a PH domain at their C termini (47, 368). It is not clear whether this Cdc42-Rho3 communication occurs through the exocyst directly, a point worthy of further investigation. Both Boi1 and Boi2 interact with the second SH3 domain of Bem1 (47), and the N-terminal region containing the first SH3 domain of Bem1 interacts with Sec15 (155), suggesting that the Boi proteins are linked to the exocyst via the Sec15-Bem1 interaction. Like other polarity proteins, Boi1 and Boi2 localize to the presumptive bud site and to the bud cortex early in the cell cycle and to the bud neck in late anaphase (206, 417). The PH domain of Boi1 is responsible for the bud cortex localization, whereas the SH3 domain is responsible for the bud neck localization (206). The deletion of either *BOI1* or *BOI2* does not cause any detectable phenotype, but the deletion of both genes causes temperature-sensitive growth, with many cells arrested as large, round mother cells with or without a bud, and the double mutant cells are prone to lysis even at the permissive temperature. Overexpression of the PH domain-containing region of Boi1 dramatically decreases cell growth, which is suppressed by the overexpression of Cdc42, and Cdc42 interacts with this region of Boi1 by a two-hybrid assay. In addition, multicopy *RHO3* effectively suppresses the temperature-sensitive growth of *boi1Δ boi2Δ* cells (47, 368). Together, these data suggest that Cdc42 and Rho3 are functionally linked via Boi1 and Boi2, although the underlying mechanism remains unknown.

UNIFYING CONCEPTS IN CELL POLARIZATIONS IN DIFFERENT ORGANISMS

So far, we have discussed the key players in polarity development in *S. cerevisiae*. Comparative studies of cell polarization in multiple organisms have indicated that the core machinery involved in the establishment and maintenance of cell polarity is conserved from yeast to humans (128, 407). Here, we outline a few key concepts for understanding cell polarization and the central roles of small GTPases in polarity development in diverse organisms.

Global and Local Cell Polarity

Global cell polarity (the mother-bud polarity) in *S. cerevisiae* is determined by intrinsic signals that direct polarized actin organization, which leads to polarized secretion for bud growth. Such polarity is maintained until late anaphase or telophase, when the growth machinery is redirected to the bud neck to promote cytokinesis and cell separation (Fig. 9) (315, 457). Local polarity (the polarity within the bud) is a result of the fine-tuning of the global polarity by several factors, such as the polarisome in response to cell cycle signals, and is responsible for the concrete bud shape (563).

In filamentous fungi, polarized growth is established during germ tube initiation from a spore and also during hyphal

branching (212, 610). Sustained polarized growth at these sites results in the elongation of the germ tube or the growing hyphae. The major elements involved in polarized growth in filamentous fungi are similar to those in *S. cerevisiae* (212, 450, 609, 610): the conserved, small GTPase-based signaling networks required for polarity establishment (212, 609); the polarized actin cytoskeleton (33, 226, 471, 579), which presumably guides secretory vesicles carrying cell wall synthetic and digestive enzymes to the growth sites; and the “Spitzenkörper” (apical body in German) (179), which may function as a vesicle supply center near the hyphal tip to control the fine shape of the tip (176, 464). The conserved polarity establishment proteins are centered on the Cdc42 GTPase module (212, 609). The polarized actin cables (sometimes called actin fibers or arrays in filamentous fungi) are thought to direct vesicle transport from the cell body to the apical region of the hypha (33) and are thus responsible for the global polarity of the hypha. As in *S. cerevisiae*, vesicles carrying cell wall synthetic and digestive enzymes are delivered to the apex of a hypha, making the hyphal tip the most active area in cell wall remodeling (529). Local cell polarity, which shapes the hyphal tip, is determined by the mode of cell wall deposition and cross-linking at the apical region, which creates a favorite site for turgor pressure to push the plasma membrane against an elastic wall at the hyphal tip. The turgor pressure may also drive the vesicles from the Spitzenkörper to the cell surface.

Despite the similarities of the major elements involved in polarity development in yeast and filamentous fungi, there are several key differences between bud and hyphal morphogenesis. First, cell cycle control of polarized growth is important in *S. cerevisiae* (315, 316) but not in filamentous fungi such as *Ashbya gossypii* (a plant pathogen whose genome is closely related to that of *S. cerevisiae*) (450, 609, 610). Second, the role of microtubules is not apparent in polarized growth in *S. cerevisiae* (9) and even in hyphal elongation in *C. albicans* (631). In contrast, microtubules and/or motors such as dynein and kinesin seem to be important in the morphogenesis of filamentous fungi (118, 243, 511–513). Microtubules modulate polarized growth in terms of the hyphal elongation rate and growth direction either by directly participating in vesicle transport (511) or by transporting some polarity factors to the hyphal tip to influence actin assembly, as was also shown for the fission yeast *Schizosaccharomyces pombe* (145, 185, 363, 366). Finally, most of the cortical markers for bud site selection in *S. cerevisiae* are either missing or poorly conserved in most filamentous fungi (212), suggesting that the mechanisms for selecting the axis of cell polarization differ significantly between *S. cerevisiae* and filamentous fungi.

Local Activation and Global Inhibition

All polarized cells, including budding yeast and human neutrophils, must develop mechanisms by which cell polarity is restricted to a single cortical site, while the rest of the cell surface is prevented from becoming additional sites of cell polarization. Although there are several possible explanations, the singularity of a polarity axis can be best explained by the hypothesis of “local activation and global inhibition,” a concept that was extensively used to explain the chemotactic behaviors of human neutrophils and the social amoeba *Dictyostelium*

(239, 379, 433). The essence of the hypothesis is that local activation at the leading edge is coupled with delivery-based positive feedback to amplify the activating signal. At the same time, a global inhibitor in the cell prevents other portions of the cell surface from becoming another leading edge. The combined actions of the localized, amplified positive signal and of the global negative signal lead to a highly polarized cell along a single anterior-posterior axis. At the molecular level, the local activators at the leading edge in both mammalian neutrophils and *Dictyostelium* are likely to be phosphoinositides such as PIP3 and small GTPases such as Cdc42 (154, 469, 620), whereas the global inhibitor is represented, at least in part, by PTEN (phosphatase and tensin homolog), a phosphatase that dephosphorylates protein and phosphoinositides including PIP3 (321, 377, 378, 624). According to the same idea, the local activators for polarized growth in *S. cerevisiae* are Cdc42 and the proteins involved in its polarization, while the global inhibitor remains to be identified. It is interesting that compartmentalized activators and inhibitors, which can be viewed as a modified version of the local activation and global inhibition model, are involved in the Cdc42-regulated mitotic exit network (Fig. 12).

Self-Organization

Self-organization has emerged as a central theme in cell biology. It can be defined simply as the capacity of a macromolecular complex or organelle to organize its own structure using a limited number of interacting components (385). Self-organized structures are in a constant state of nonequilibrium and exhibit oscillatory behaviors at the expense of energy. Thus, self-organization is intrinsically dynamic and endows biological systems with great flexibility and robustness (233, 385). Self-organization has been analyzed extensively in cytoskeleton and motor proteins. Microtubules and actin filaments, together with their motor proteins such as kinesins and myosins, can self-organize into dynamic macrostructures such as the mitotic spindle and the actomyosin contractile apparatus, which drive nuclear division, cell migration, and cytokinesis (386, 405, 489). Self-organization is also a key feature in the selection of cell division sites in *Escherichia coli* (233) and in the establishment of cell polarity in mammalian chemotactic cells (624).

The establishment and maintenance of cell polarity in *S. cerevisiae* likely involve self-organization. As discussed in the sections above, activated Cdc42 can spontaneously polarize at the cell cortex in the absence of temporal and spatial cues (84, 196, 604). The major role of Cdc42 in polarized cell growth is to direct the formation of a polarized actomyosin system, which has the features of a self-organized structure and is involved in positive feedback to reinforce Cdc42 polarization (460, 604, 605). Thus, the minimal polarity machinery in *S. cerevisiae* could include two self-organizing structures, a localized Cdc42-based signaling cap at the bud tip and a global actomyosin-based transport system that interprets and amplifies the polarization signal from the Cdc42-based cap. The validity of this hypothesis requires extensive experimental investigation and theoretical modeling.

CONCLUDING REMARKS

Substantial progress in deciphering the molecular basis of cell polarity in budding yeast has been made. Key signaling pathways that regulate polarization during the mitotic cell cycle and during mating have been identified and extensively characterized. These studies have uncovered the central roles of small GTPases in the development of cell polarity. GTPases have numerous molecular interactions with effectors and regulators and can be involved in multiple distinct signaling pathways. Some of the machinery is specific to yeast, but the general principles underlying cell polarity and the core components in the signaling pathways appear to be highly conserved throughout evolution. An understanding of the spatial and temporal control of the GTPases and their regulators in yeast is thus undoubtedly relevant to other eukaryotes. There are, however, many unanswered questions, as we tried to highlight in each section. One important, but still poorly understood, aspect is the regulation of each component, particularly regulators of GTPases such as GEFs and GAPs, involved in polarity development. Another important aspect is to understand how different GTPases are coordinated at the molecular level during polarized cell growth. The recent development of new technologies in genomics and proteomics and of fluorescence-based technology has provided a better understanding of overall as well as detailed pictures of cellular networks controlling polarity development. The major challenges are to integrate all the fragmented information into cohesive molecular pathways that can be easily understood in terms of cell physiology and function and to determine how these pathways are wired into an elegant machinery that operates with such tremendous fidelity and flexibility during the development of cell polarity.

ACKNOWLEDGMENTS

We acknowledge many contributions from the members of the Park and Bi laboratories. We thank Wei Guo for valuable discussions regarding exocytosis and E. Oakley for careful editing. We are also grateful to the anonymous reviewers for many insightful comments. We apologize for not citing all relevant work because of the broad scope and the space limitation of this review.

Our research has been supported by National Institutes of Health grants (GM56997 and GM76375) and an American Heart Association grant (0555303B) to H.-O.P. and by a National Institutes of Health grant (GM59216) and an American Cancer Society grant (RSG-02-039-05-CSM) to E.B.

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