Establishment of Cell Polarity in Yeast

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The establishment of cell polarity is a central feature of morphogenesis in many types of cells (Schnepf 1986; Horvitz and Herskowitz 1992; Rodriguez-Boulan and Nelson 1993; Shapiro 1993; Priess 1994). Polarity establishment involves selection of an axis of polarization followed by the asymmetric organization of cytoskeletal elements, membranous organelles, components of the plasma membrane, and components of the extracellular matrix or cell wall along this axis. In budding yeasts such as *Saccharomyces cerevisiae*, cell polarization is vividly manifested during the vegetative cell cycle by the appearance and selective growth of the bud, which depends on the highly polarized movement of secretory vesicles carrying new cell-surface material, and perhaps of the Golgi cisternae that generate such vesicles (Preuss et al. 1992), to the bud site and into the growing bud. This movement appears to depend primarily on the actin/myosin system (Bretscher et al. 1994; Welch et al. 1994; Govindan et al. 1995), but other cytoskeletal elements such as the cytoplasmic microtubules and the septin-containing neck filaments also polarize before bud emergence (Byers 1981; Kilmartin and Adams 1984; Ford and Pringle 1991; Kim et al. 1991; Snyder et al. 1991) and appear to play roles in modulating the pattern of cell-surface growth and/or in the segregation of organelles along the mother-bud axis (Adams 1984; Adams and Pringle 1984; Jacobs et al. 1988; Palmer et al. 1992; Li et al. 1993; Muhua et al. 1994). Thus, the central questions about the establishment of polarity in budding yeast cells concern how the axes of polarization (bud sites) are chosen, how this choice is communicated to the cytoskeletal systems, and how these processes are coordinated with other events in the cell cycle.

Yeast cells also polarize during another phase of the life cycle: During mating, a cell polarizes its cytoskeleton and cell-surface growth toward its partner of opposite mating type (Tkacz and MacKay 1979; Byers 1981; Ford and Pringle 1986; Hašek et al. 1987; Read et al. 1992; Chenevert 1994), apparently in response to the gradient of secreted mating pheromone (Jackson and Hartwell 1990; Segall 1993; Chenevert 1994; Dorer et al. 1995).

In the past few years, experimental results and ideas from our own and other laboratories have coalesced around the view that morphogenesis in the *S. cerevisiae* cell cycle is governed by a hierarchy of functions (Fig. 1) (Bender and Pringle 1989; Johnson and Pringle 1990; Chant and Herskowitz 1991; Chant and Pringle 1991, 1995; Drubin 1991; Madden et al. 1992; Chant 1994; Zahner et al. 1996). In this model, the actual morphogenetic events (only some of which are indicated) are carried out by the cytoskeletal systems working in complex ways that involve interactions both with each other and with the membranous organelles. The prerequisite polarization of the cytoskeleton is governed by signals from the "polarity-establishment functions." The paradigmatic proteins in this class (Cdc24p and Cdc42p) were defined by conditional-lethal mutations that result in an inability to polarize the cytoskeletal systems or cell-surface growth, and hence in a failure to bud. Although the polarity-establishment proteins can apparently operate at any point on the cell surface, the cell normally selects bud sites in one or the other of two nonrandom patterns (Winge 1935; Freifelder 1960; Streiblová 1970; Hicks et al. 1977; Sloat et al. 1981). In the axial budding pattern (as typically displayed by **a** or α cells, such as normal haploids), mother and daughter cells both produce new buds near the preceding division site. In the bipolar budding pattern (as typically displayed by **a**/α cells, such as normal diploids), the daughter cell usually buds at the pole distal to the division site, and the mother cell can bud at either pole. These budding patterns are controlled by the bud-site-selection functions, which are not essential for polarity establishment per se. The "general site-selection functions" are necessary for either the axial or the bipolar budding pattern. In contrast, the "axial-specific functions" and "bipolar-specific functions" are necessary only for the generation of one or the other cell-type-specific pattern.

In this paper, we review the key evidence that has led to the model of Figure 1, focusing on the logic of viewing the morphogenetic system as a hierarchy, the nature of the known components at different levels in the hierarchy, and the communication between one level and the next. We also provide some new data bearing on these issues. Finally, we discuss briefly how polarity establishment is coordinated with other events in the cell cycle and how the hierarchy controlling

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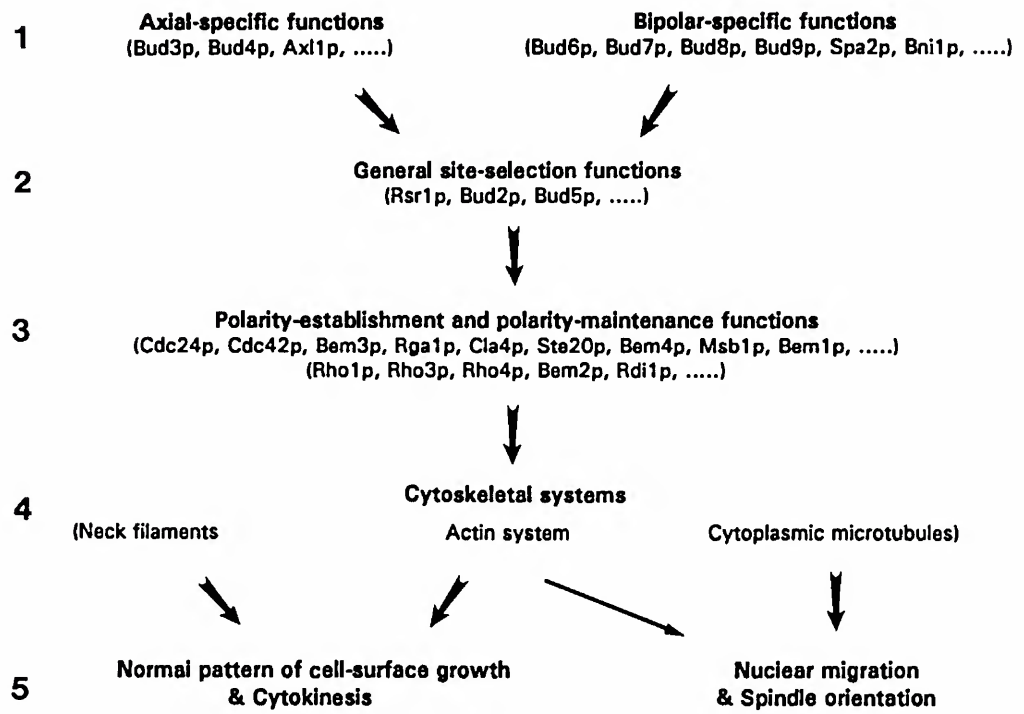

Figure 1. Morphogenetic hierarchy in the *S. cerevisiae* cell cycle. According to this model, cortical positional signals specific to the axial or bipolar budding patterns (level 1) localize the action of the general site-selection functions (level 2), which in turn localize the action of the polarity-establishment functions (level 3), which in turn direct the polarization of the cytoskeletal elements (level 4), which carry out the actual morphogenetic events (level 5). The pattern of cell-surface growth is determined primarily by the actin system but is influenced by the neck filaments, which are also essential for cytokinesis. Nuclear migration and spindle orientation are determined primarily by the cytoplasmic microtubules, but are also influenced by the actin system. See text for references and additional details.

polarization during budding relates to that controlling polarization during mating.

MATERIALS AND METHODS

Microbiological methods. Standard techniques were used for the growth and genetic manipulation of yeast strains (Guthrie and Fink 1991).

Antibody preparation. To prepare Cdc24p-specific antibodies, a glutathione *S*-transferase (GST)-fusion protein containing amino acids 472–854 of Cdc24p was produced in *Escherichia coli* using the vector pGEX-3X (Pharmacia) and was used to immunize rabbits according to standard procedures. Cdc24p-specific antibodies were obtained by two steps of affinity purification on nitrocellulose blots (Pringle et al. 1989). The first step used a fusion of amino acids 472–854 of Cdc24p to MalE (prepared in *E. coli* using vector pMAL-c2 [New England BioLabs]), and the second step used the GST-fusion protein. The purified antibodies recognized essentially only one band (or closely spaced pair of bands) in immunoblots of proteins from yeast expressing *CDC24* under control of the *GAL1* promoter, and the intensity of this band varied in the expected way with the carbon source (data not shown). Antibodies specific for Bem1p were prepared similarly, using fusions of approximately the carboxy-terminal two thirds of Bem1p to β-galactosidase (using vector pUR289 [Rüther and Müller-Hill 1983)] and to anthranilate synthase (using vector pATH3 [Koerner et al. 1991]). Immunoblots using the affinity-purified antibodies on extracts of wild-type cells, cells deleted for *BEM1*, and cells carrying a high-copy-number *BEM1* plasmid confirmed the specificity of the antibodies for Bem1p (data not shown). Antibodies to GST were purchased from Molecular Probes and affinity-purified on nitrocellulose blots of the GST-Cdc24p fusion protein.

Cytological techniques. Staining of bud scars with Calcofluor and of actin with Rhodamine-phalloidin, immunofluorescence, and fluorescence microscopy were performed essentially as described previously (Pringle et al. 1989).

Two-hybrid analyses. Two-hybrid analyses (Fields and Sternglanz 1994) were performed using the system described by Gyuris et al. (1993). DNA fragments to be fused to the LexA DNA-binding domain in plasmid pEG202 or to the activation domain in plasmid pJG4-5 were generated by polymerase chain reaction using the cloned genes as templates. For quantitative assessment of interactions, strain EGY48 containing the *lexAop-*

lacZ reporter plasmid pSH18-34 was co-transformed with a pEG202 derivative and a pJG4-5 derivative. β-Galactosidase activities were measured in three to six different isolates of each strain after growth for 16 hours at 30°C in minimal medium containing 2% galactose and 1% raffinose.

RESULTS AND DISCUSSION

Axial- and Bipolar-specific Positional Signals in the Cell Cortex

The general features of the axial and bipolar budding patterns have been known for many years (see above). However, until recently, we did not have a sufficiently precise description of either pattern to allow us to think critically about possible mechanisms. In particular, we could not discriminate among three possible models for the axial pattern, namely (1) each new bud forms near the proximal (or birth-scar) pole, (2) each new bud forms adjacent to some previous division site, and (3) each new bud forms adjacent to the immediately preceding division site. The details of the bipolar pattern were even more obscure. For example, we did not know whether a daughter cell can ever produce its first bud at the proximal pole, what rules govern the choice of poles by mother cells, or whether the pattern of successive bud sites around the proximal pole is the same as seen in axially budding cells. (If it were, one could imagine that the axial pattern is produced simply by an additional restriction—elimination of the distal pole as a possible budding site—on an underlying bipolar pattern.)

To address these issues, we characterized both budding patterns in detail (Chant and Pringle 1995). The results showed that in exponentially growing, axially budding cells, each new bud site is adjacent to the immediately preceding division site. This suggested that in each cell cycle, the division site on both mother and daughter cells is marked by a transient positional signal that can direct bud-site selection in the next cell cycle but not in later cell cycles. The hypothesis of a transient positional signal was supported by the observations that temporary arrest of the cell cycle causes cells to form their next buds in nonaxial positions (usually at the distal pole) (Madden and Snyder 1992; Chant and Pringle 1995), after which axial (adjacent) budding then resumes in the new location.

The *BUD3* and *BUD4* gene products appear to be involved specifically in determining the axial budding pattern: *bud3* and *bud4* mutations, including deletions, cause **a** or α cells to bud bipolarly but have no detectable effect on cell growth or on the bipolar budding of **a**/α cells (Chant and Herskowitz 1991; Chant et al. 1995; S. Sanders and I. Herskowitz, pers. comm.). Immunolocalization of Bud3p (Chant et al. 1995) revealed that it behaves as predicted for the transient positional signal marking the division sites for axial budding. No Bud3p signal was detected in cells with small buds, but cells with large buds display an apparent double ring of Bud3p at the mother-bud neck (Fig. 2A). Upon cell division, a single ring of Bud3p is left on both mother and daughter cells (Fig. 2A); these rings remain until approximately the time at which the new bud sites assemble. Several lines of evidence suggest that Bud3p assembles onto the preexisting neck-filament structures: The localization of Bud3p in large-budded and unbudded cells is indistinguishable from that of the neck-filament-associated septin proteins (Haarer and Pringle 1987; Ford and Pringle 1991; Kim et al. 1991; Chant et al. 1995); Bud3p localization is lost rapidly when a temperature-sensitive septin mutant is

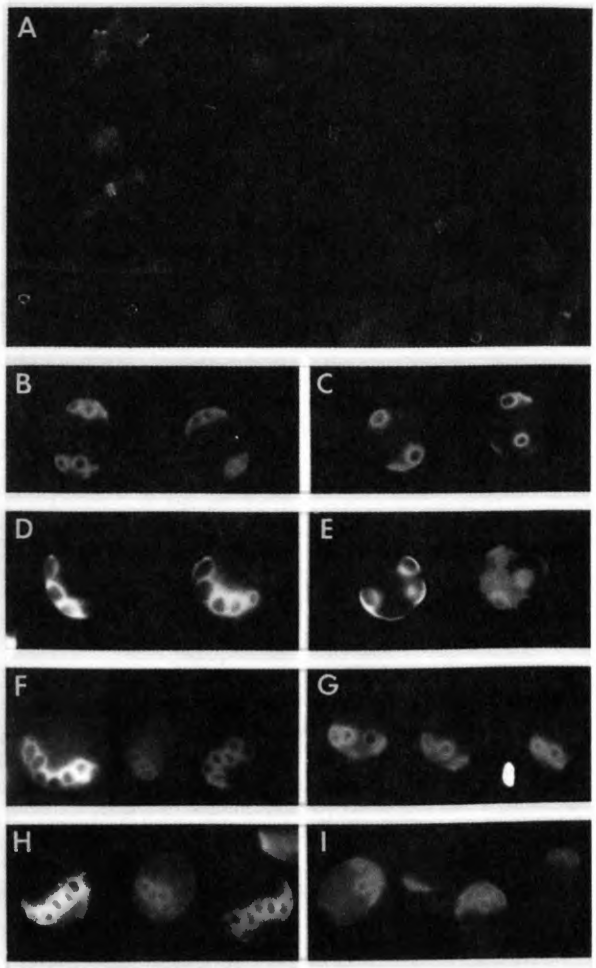

Figure 2. (*A*) Localization of Bud3p to rings at the mother-bud necks of large-budded cells and to the preceding division sites of recently divided unbudded cells. (Reprinted, with permission, from Chant et al. 1995 [copyright Rockefeller University Press].) (*B,C*) Bipolar budding of a normal **a**/α diploid (*B*) and of an α *bud3*Δ haploid (*C*). (*D–I*) Mutants with specific defects in bipolar budding. (*D,F,H*) Normal axial budding of α *BUD3* strains carrying a *bud6* (*D*), *bud8* (*F*), or *bud9* (*H*) mutation. (*E,G,I*) Aberrant budding patterns of α *bud3*Δ or **a**/α *BUD3/BUD3* strains carrying a *bud6* (*E*, random budding), *bud8* (*G*, unipolar budding, proximal pole; birth scars are obscured by the bud scars), or *bud9* (*I*, unipolar budding, distal pole; note birth scars at the proximal poles) mutation.

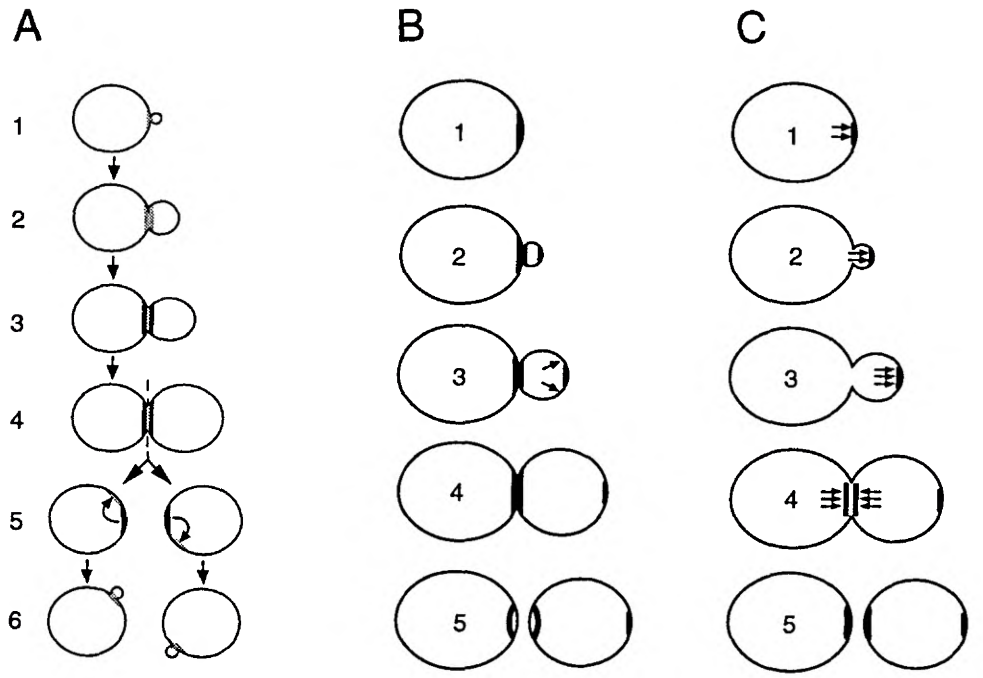

Figure 3. (*A*) Generation of the axial budding pattern by a cycle involving the neck-filament-associated proteins (*light shading*) and the Bud3p-containing axial positional signal (*dark shading*). The neck-filament-associated proteins are assembled early in the cell cycle as a ring around the mother-bud neck (1, 2). Later, the axial signal molecules assemble onto this preexisting structure (3). At division, the structures at the neck split (4), leaving superimposed rings of axial signal molecules and neck-filament-associated proteins at the division site on each progeny cell (5). The axial signal molecules then determine (by means of the general site-selection and polarity-establishment functions) the position of a new polarity axis (including a new ring of neck-filament-associated proteins) adjacent to the previous division site (5). As the new buds form, the old rings of axial signal molecules and neck-filament-associated proteins disassemble (6). (*B,C*) Two possible models to explain the apparent localization of the positional signals used in bipolar budding. Shading indicates the postulated positional signal(s); arrows indicate the sites of active cell-surface growth, which in the model of panel *B* is supposed to exclude the patch at the very tip of the bud that is occupied by the bipolar signal molecules. See text and Chant and Pringle (1995) for details. (Reprinted, with permission, from Chant et al. [1995] and Chant and Pringle [1995] copyright Rockefeller University Press.)

shifted to restrictive temperature (Chant et al. 1995); and septin mutations can cause a loss of axial budding (Flescher et al. 1993; Chant et al. 1995). Thus, Bud3p and the neck-filament-associated proteins appear to be linked in a cycle in which each determines the localization of the other (Fig. 3A). Bud4p appears to behave similarly (S. Sanders and I. Herskowitz, pers. comm.) and thus is probably also a component of the axial-specific cortical signal.

Surprisingly, Bud3p and Bud4p are expressed and localized in **a**/α cells just as they are in **a** or α cells (Chant et al. 1995; S. Sanders and I. Herskowitz, pers. comm.). Similarly, several lines of evidence (including the bipolar budding of *bud3* and *bud4* mutants and the apparent use of bipolar sites by **a** or α cells following cell-cycle arrest) suggest that the bipolar positional signals (as discussed in more detail below) are expressed in **a** and α cells as well as in **a**/α cells. Thus, it is not clear how cell type determines the budding pattern. An important clue to this conundrum has recently been provided by the identification of a third axial-specific gene, *AXL1*, which is expressed in **a** and α cells but not in **a**/α cells (Fujita et al. 1994), presumably reflecting control by the **a**1/α2 repressor (Herskowitz et al. 1992).

The detailed analysis of bipolar-budding cells showed that both daughter cells and mother cells can bud at either pole during any cell cycle, although there are interesting (and unexplained) biases affecting the choice of poles. Moreover, the patterns of bud sites at both the proximal and distal poles are nonaxial (i.e., a new bud site at a given pole is not always adjacent to the immediately preceding bud site at that pole). In contrast to the disruption of axial budding by temporary arrest of the cell cycle (see above), such arrest has little effect on bipolar budding. However, a few cells form buds in nonpolar positions during the recovery from starvation, and these atypically positioned bud sites then appear to represent new poles, in the sense that future bud sites can also be selected in their vicinity. Taken together, these observations suggest that bipolar budding depends on persistent or permanent positional signals that are distinct from the transient signal used for axial budding. These signals appear to be present in newborn daughter cells both in a zone corresponding to their birth scars at their proximal poles and at the tips of their distal poles; in mother cells, signal also appears to be present at each previous bud site. Thus, in any cell cycle, a bipolar-

budding cell can bud at the tip of its distal pole (if this site has not already been used), in a zone around its proximal pole, or adjacent to any previous bud site. The observations can be accomodated by either of two simple models (Chant and Pringle 1995). In one model (Fig. 3B), the postulated bipolar signal molecules are localized to the presumptive bud site, partitioned between the tip and the base of the bud at bud emergence, and further partitioned between the mother and daughter cells at cytokinesis. In the other model (Fig. 3C), the signal molecules are proposed to be left as remnants of the cell-surface-growth machinery, which is concentrated early at the bud tip and later at the division site.

This analysis of the bipolar budding pattern suggested that it should be possible to isolate mutants that are specifically defective in bipolar budding; such mutants might be defective either in the postulated bipolar positional signal(s) or in factors necessary for the localization or recognition of such signals. To allow detection of recessive mutations, we used a haploid *bud3*Δ strain, which buds bipolarly like a normal **a**/α strain (see above; Fig. 2B,C). To detect the widest range of possible phenotypes (including potentially unexpected ones), and to do so in a genetic background that was relatively simple, we developed streamlined methods for screening large numbers of individual mutagenized clones for their patterns of bud scars by Calcofluor staining and fluorescence microscopy.

In a screen of 20,500 mutagenized clones, we recovered 30 that had clear alterations in budding pattern due to single-gene mutations (Zahner et al. 1996). Upon transformation with a *BUD3*-containing plasmid, 19 of these mutants continued to display aberrant budding patterns, indicating that they are defective in general site-selection functions (see Fig. 1 and further discussion below). However, the remaining 11 mutants displayed normal axial budding in the presence of *BUD3* (Fig. 2D,F,H), indicating that they indeed have defects in bipolar-specific functions. Of these mutants, 6 bud randomly (Fig. 2E) or in a complex, heterogeneous pattern, whereas 2 mutants bud almost exclusively from the proximal pole (Fig. 2G) and 3 bud almost exclusively from the distal pole (Fig. 2I). Genetic analysis revealed that the 6 random or heterogeneous mutants harbor defects in six different genes, four of which (*BUD2*, *BUD5*, *SPA2*, and *BNI1*) were known previously, and two of which (named *BUD6* and *BUD7*) are novel. *BUD2* and *BUD5* are discussed further in the following two sections. Although *SPA2* has been studied intensively by M. Snyder and coworkers (Snyder 1989; Snyder et al. 1991; Costigan et al. 1992; Flescher et al. 1993), the precise role of Spa2p remains unclear. However, it is intriguing that this protein localizes to presumptive bud sites, bud tips, and the mother-bud neck at various times in the cell cycle (Snyder 1989; Snyder et al. 1991), very much as postulated for the bipolar-specific positional signal(s) (Fig. 3B,C). *BNI1* was first identified (H.F. Fares and J.R. Pringle, in prep.) on the basis of a genetic interaction with *CDC12*, which encodes one of the neck-filament-associated septin proteins; thus, the apparent role of Bni1p in bipolar budding suggests (not surprisingly, in view of the models of Fig. 3B,C) that proteins associated with the neck filaments are involved in positioning the bipolar signal(s) as well as in positioning the Bud3p-containing axial signal (see above and Fig. 3A). Analyses of the Bud6p and Bud7p sequences and of the localization of these proteins (in progress) should provide clues to the nature of their roles in bipolar budding.

The 2 proximal-pole mutants define one novel gene (*BUD8*), and the 3 distal-pole mutants define one novel gene (*BUD9*). *BUD8* and *BUD9* might encode proteins that are specific components of the distal-pole signal and the proximal-pole signal, respectively; however, the *bud8 bud9* double mutant does not bud randomly but instead predominantly from the proximal pole, implying that the *bud9* mutation does not simply eliminate an essential component of the proximal-pole signal. Thus, we think it is more likely that *BUD8* and *BUD9* encode proteins that are essential for the normal positioning (or partitioning) of the bipolar positional signal (see Fig. 3B,C). Analyses of protein sequences and localization are in progress.

Other evidence suggests that the actin cytoskeleton may also be involved in positioning the signals used for bipolar budding. Several mutations affecting actin structure cause a randomization of budding pattern in **a**/α cells (Drubin et al. 1993) while having little or no effect on the axial budding of **a** or α cells (D. Drubin, pers. comm.). In addition, mutations in *RVS161*, *RVS167*, and several interacting genes appear both to affect the organization of the actin cytoskeleton and to disrupt the bipolar but not the axial budding pattern (Bauer et al. 1993; Sivadon et al. 1995), although the complex, pleiotropic phenotypes of these mutants (Bauer et al. 1993; Desfarges et al. 1993) complicate the interpretation of these observations.

General Site-selection Functions: A GTPase Signaling Module

The general site-selection proteins encoded by *RSR1* (or *BUD1*), *BUD2*, and *BUD5* were defined by mutations (including deletions of the genes) that randomize the budding patterns of **a**, α, and **a**/α cells but have no obvious effect on growth rate or other aspects of cell morphology (Bender and Pringle 1989; Chant and Herskowitz 1991; Chant et al. 1991; Park et al. 1993). These phenotypes suggest that the gene products are dedicated to bud-site selection and play no role in polarity establishment per se; this distinction is a critical part of the logical basis for viewing the morphogenetic functions as a hierarchy (Fig. 1). (It is conceivable that the general bud-site-selection proteins play another role that is not revealed by the deletion mutations because of functional redundancy. However,

this seems unlikely because a dominant-negative allele of *RSR1* also randomizes bud-site selection without affecting growth rate [Ruggieri et al. 1992].) Rsr1p is a Ras-related GTPase (Bender and Pringle 1989), and strong evidence from sequence similarities (Chant et al. 1991; Park et al. 1993), in vitro biochemistry (Park et al. 1993; Zheng et al. 1995), and in vivo genetic analysis (Powers et al. 1991; Bender 1993) indicates that Bud5p and Bud2p are the guanine-nucleotide-exchange factor (GEF) and GTPase-activating factor (GAP), respectively, that regulate Rsr1p.

Immunofluorescence observations suggest that Rsr1p is patchily distributed over the entire cell surface (J. Chant et al., unpubl.). This suggests that the role of Rsr1p in communicating spatial information to the polarity-establishment functions (see further discussion below) is mediated by localized regulation of its GTPase cycle rather than by localization of the protein itself. Because loss of Rsr1p-GEF activity (Chant et al. 1991), loss of Rsr1p-GAP activity (Chant and Herskowitz 1991; Bender 1993; Park et al. 1993), and loss of intrinsic Rsr1p GTPase activity (by mutation of glycine 12 to valine; Ruggieri et al. 1992) all produce the same phenotype as loss of Rsr1p itself, Rsr1p function appears to require cycling between the GTP- and GDP-bound forms, rather than simply generating an adequate amount of activated (i.e., GTP-bound) Rsr1p. This view of Rsr1p function is supported by the observations that overproduction of Rsr1p does not noticeably affect the budding pattern (Ruggieri et al. 1992), whereas overproduction of Bud5p partially randomizes budding (Chant et al. 1991), and that Bud5p and Bud2p appear to be involved in communication with the axial-specific and bipolar-specific positional signals (as discussed further below). It is not yet clear whether the localized regulation of the Rsr1p GTPase cycle is achieved by localization of the Bud5p and Bud2p proteins themselves or by localized regulation of their activities.

Although Rsr1p, Bud5p, and Bud2p appear to form a tidy functional module, it is almost certain that the original screens that identified these genes were not saturated (Chant and Herskowitz 1991; Chant et al. 1991). Indeed, several of the general site-selection mutants identified in the screen for bipolar-specific mutants (see above) appear to have mutations in novel genes (V. Snell and J.R. Pringle, unpubl.). How the functions of these additional genes relate to the Rsr1p GTPase cycle is not yet known.

Communication between the Cortical Positional Signals and the General Site-selection Functions

The arguments above suggest that the axial and bipolar budding patterns are generated by communication between the corresponding positional signals in the cell cortex and the Rsr1p regulatory factors. Additional strong support for this hypothesis came from the identification of special alleles of *BUD5* and *BUD2* among the mutations having specific effects on the bipolar budding pattern (see above). Presumably, these alleles encode products that have lost the ability to interact with the bipolar positional signal(s) but retain the ability to interact with the Bud3p- and Bud4p-containing axial positional signal. Identification and analysis of additional bipolar-specific *BUD5* and *BUD2* alleles and of the (expected) corresponding axial-specific alleles should allow the Bud5p and Bud2p domains involved in these interactions to be mapped, as well as facilitate the identification of specific components of the cortical signals with which the interactions occur.

Polarity-establishment and Polarity-maintenance Functions: Additional GTPase Signaling Modules

In sharp contrast to the bud-site-selection proteins, the paradigmatic polarity-establishment proteins Cdc24p and Cdc42p are essential for viability. At restrictive temperature, temperature-sensitive *cdc24* and *cdc42* mutants accumulate uniformly as large, round, unbudded cells in which the deposition of cell-surface components is nearly or entirely isotropic (Hartwell et al. 1974; Sloat and Pringle 1978; Field and Schekman 1980; Sloat et al. 1981; Adams et al. 1990); the cells continue to grow and eventually burst. Moreover, neither the neck-filament-associated septin proteins nor proteins of the actin cytoskeleton undergo polarized assembly in these mutants (Adams and Pringle 1984; Adams et al. 1990; Amatruda and Cooper 1992; Li et al. 1995; H. Kim et al., unpubl.). Normally, both sets of proteins assemble at the presumptive bud site about 15 minutes before bud emergence (Ford and Pringle 1991; Kim et al. 1991; Snyder et al. 1991; Amatruda and Cooper 1992). The two sets of proteins appear to assemble independently of each other: The relative timing of assembly is variable in individual wild-type cells, and each set of proteins can assemble in mutants unable to assemble the other (Adams and Pringle 1984; H. Kim et al., unpubl.). Thus, the observation that both sets of proteins require the functions of Cdc24p and Cdc42p for their assembly constitutes a formal justification for considering the polarity-establishment functions to comprise a level in the morphogenetic hierarchy distinct from that of the cytoskeletal elements per se.

Molecular justification for this view comes from the evidence that Cdc24p and Cdc42p have signal-transduction rather than structural roles; in particular, Cdc42p is a Rac/Rho-type GTPase (Johnson and Pringle 1990), whereas Cdc24p appears to be a GEF that operates on Cdc42p (Zheng et al. 1994b). Thus, the control of cytoskeletal polarization by Cdc24p is presumably mediated at least in part by its activation of Cdc42p (Fig. 4). However, it remains unclear whether Cdc24p has targets other than Cdc42p. For example, Cdc24p might also have GEF activity toward one or more of the other Rho proteins (Fig. 1; and see

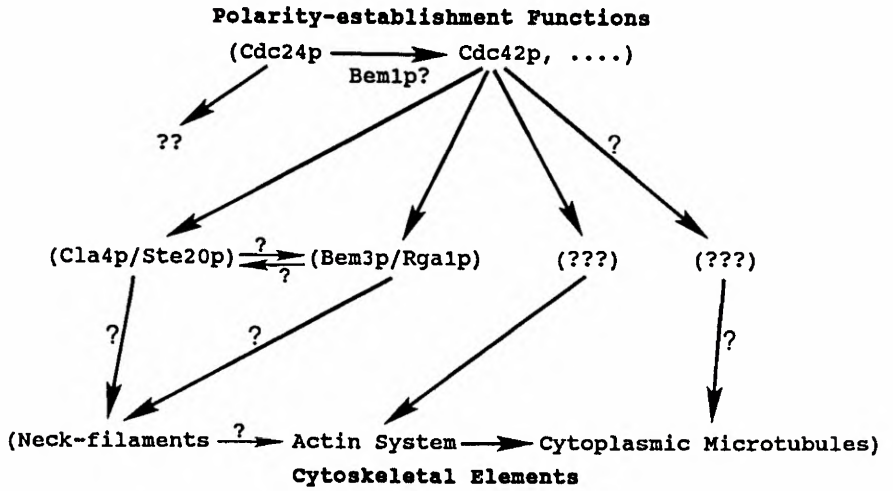

Figure 4. Diagram highlighting the few clues and the many uncertainties as to how the polarity-establishment proteins promote the polarized assembly of the cytoskeleton. Cdc24p activates Cdc42p but may also have additional targets. Bem1p probably facilitates the Cdc24p-Cdc42p interaction as well as interactions involving other bud-site-selection and polarity-establishment proteins. Cdc42p interacts both with the Cla4p/Ste20p protein kinases and with the Bem3p/Rga1p GAPs, but whether these interactions represent two separate effector pathways or different aspects of a single pathway is unclear. Loss of Cla4p/Ste20p function affects localization of the neck-filament-associated septin proteins, but whether this represents a direct effect on septin assembly or an indirect effect reflecting a subtle influence on the targeting of cell-surface growth is unclear. Cdc42p function is necessary for polarized assembly of the actin cytoskeleton, but effectors in this pathway have not been identified. The neck filaments are not necessary for polarized actin assembly but may influence the degree of actin polarization (and hence the pattern of cell-surface growth). The orientation of the cytoplasmic microtubules is influenced by the actin system, but it is not clear if the polarity-establishment proteins also control microtubule polarization more directly. The roles of other putative polarity-establishment proteins (not shown) are even more obscure at present, and no attempt has been made to incorporate the probable interactions between polarity-establishment and polarity-maintenance functions. See text for references and more detailed discussion.

below), a possibility that is lent some credibility by the lack of reports to date of other Rho-GEFs in yeast (see below). Although no GEF activity of Cdc24p upon Rho1p was detected in vitro (Zheng et al. 1994b), it is possible that such activity occurs in vivo or that one of the other Rho proteins is a Cdc24p target. In addition, the domains of Cdc24p implicated in its GEF activity (amino acids 281–518 [Hart et al. 1991; Miyamoto et al. 1991; Zheng et al. 1994b]) and in its interactions with Bem1p (amino acids 780–854 [Peterson et al. 1994]) and Rsr1p (amino acids 473–854 [H.-O. Park and I. Herskowitz, pers. comm.]) appear unlikely to account for all of its 854 amino acids. In particular, the roles of the amino-terminal 280 amino acids, of the pleckstrin-homology (PH) domain (Peterson et al. 1994), and of the putative Ca^{++}-binding site (Ohya et al. 1986; Miyamoto et al. 1991) remain obscure, although the recent observation that Ca^{++} interferes with the Cdc24p-Bem1p interaction (Zheng et al. 1995; and see below) may be an important clue to the last of these questions. One attempt to screen genetically for Cdc24p targets other than Cdc42p has to date been unsuccessful (E. Bi and J. Pringle, unpubl.): In a search for genes whose overexpression would improve the previously observed (Bender and Pringle 1989) multicopy suppression of *cdc24*^{ts} by *CDC42*, we recovered only *MSB1* and *CLA4*, both of whose products appear to interact with Cdc42p (Bender and Pringle 1989; Cvrčková et al. 1995; A. Bender and Y. Matsui, pers. comm.; and see below) and therefore are unlikely to define a separate effector pathway.

Control of the Cdc42p GTPase cycle presumably also involves other regulatory factors. The identification of the putative Cdc42p-GAPs Bem3p and Rga1p (Fig. 1), and their possible roles (along with the Cdc42p-associated protein kinases Cla4p and Ste20p) as Cdc42p effectors, are discussed below. It is not yet clear whether the Rho GDP-dissociation inhibitor (GDI) Rdi1p operates on Cdc42p (Masuda et al. 1994), although it does interact strongly with prenylatable Cdc42p in two-hybrid tests (Table 1).

Other proteins have been provisionally assigned to the polarity-establishment class (Fig. 1) based on mutant phenotypes, genetic interactions, or both; however, it should be noted that none of these other proteins produces such a clear-cut loss-of-polarity phenotype when defective as do Cdc24p and Cdc42p. For example, *BEM4* displays a variety of genetic interactions with *CDC24* and *CDC42*, and deletion of *BEM4* causes (at least in some genetic backgrounds) temperature-sensitive growth with the accumulation of large, unbudded cells whose actin cytoskeletons appear depolarized (Y. Matsui and A. Bender, pers. comm.). However, the sequence of Bem4p has so far been uninformative, and its precise role remains obscure. Similarly, *MSB1* displays numerous genetic interac-

Table 1. Two-hybrid Evidence for Interaction of Cdc42p with Protein Kinases and GAPs

(a) *Specificity of interactions for Cdc42p vs. other Rho proteins*

	Activation-domain fusions						
DNA-binding-domain fusions	Rdi1p-A	Ste20p-A	Cla4p	Bem3p	Rga1p	Msb2p	none
Cdc42p	2564	232	1	3	16	4	2
Cdc42p^{C188S}	129	3506	248	12	191	41	40
Cdc42p^{Q61L,C188S}	150	4826	1663	1798	3956	205	311
Rho1p^{C206S}	2002	22	25	17	14	33	30
Rho3p^{C228S}	3	4	1	18	1	14	10

(b) *Specificity of interaction for activated Cdc42p*

	Activation-domain fusions				
DNA-binding-domain fusions	Cdc42p	Cdc42p^{C188S}	Cdc42p^{G12V,C188S}	Cdc42p^{Q61L,C188S}	none
Ste20p-B	781	2490	4895	5196	38
Cla4p-A	57	1777	4550	4777	5
Bem3p	3	1	4	268	5
Rga1p	2	26	561	2016	5
Msb2p	2	7	3	3	2
None	20	18	26	18	17

Two-hybrid assays were conducted as described in Materials and Methods. Numbers are the average β-galactosidase activities (in Miller units) from 3–6 different isolates. Except as noted, all constructs encode fusions of the DNA-binding domain or activation domain to the full-length proteins. Rdi1p-A contains amino acids 3–202 of Rdi1p; Ste20p-A and Ste20p-B contain amino acids 319–646 and 319–496, respectively, of Ste20p; and Cla4p-A contains amino acids 174–294 of Cla4p. The Cdc42p^{C188S}, Rho1p^{C206S}, and Rho3p^{C228S} proteins lack the prenylation sites and thus should be freer to engage in two-hybrid interactions because of a lack of membrane association; the Cdc42p^{G12V} and Cdc42p^{Q61L} proteins should be defective in GTPase activity and thus locked in the GTP-bound form (Ziman et al. 1991). Constructs containing Msb2p (Bender and Pringle 1992) were included as negative controls, as were the vectors without inserts (labeled "none"). Full details of the constructions and additional related data can be found in Simon et al. (1995), Cvrčková et al. (1995), and Stevenson et al. (1995).

tions with *CDC24*, *CDC42*, *BEM4*, and *BEM1* (Bender and Pringle 1989, 1991; A. Bender and Y. Matsui, pers. comm.). However, deletion of *MSB1* produces no obvious phenotype, and the sequence of Msb1p has so far been uninformative (Bender and Pringle 1991).

Somewhat more information is available about *BEM1*. This gene was identified independently on the bases of (1) temperature-sensitive-lethal loss-of-polarity mutants that proved to be *bem1 bud5* double mutants (Chant et al. 1991), (2) mutants synthetically lethal with a deletion of *MSB1* (Bender and Pringle 1991), (3) a mutant suppressed by multicopy *CDC42* (A. Bender and Y. Matsui, pers. comm.), and (4) mutants showing an inability to polarize growth in response to α factor (Chenevert et al. 1992). Deletion of *BEM1* in an otherwise wild-type background results in temperature-sensitive lethality; the arrested population contains many large, unbudded cells but is more heterogeneous morphologically than is the *bem1 bud5* double mutant (Chant et al. 1991; Chenevert et al. 1992). It has also been shown by two-hybrid analysis and by in vitro analysis using bacterially expressed proteins that Bem1p associates directly both with Cdc24p and with Rsr1p (Peterson et al. 1994; H.-O. Park and I. Herskowitz, pers. comm.). Bem1p contains two SH3 domains (Chenevert et al. 1992), but these are not involved in the interaction with Cdc24p (Peterson et al. 1994) and thus presumably interact with some other component(s) such as the Boi1p and Boi2p proteins recently identified by two-hybrid analysis (A. Bender, pers. comm.). The multiplicity of its interactions suggests that Bem1p might serve a scaffolding role in organizing multiple components at the site of polarization (see also below). This hypothesis is supported by observations in *Schizosaccharomyces pombe*, where it has been observed that overexpression of the Bem1p homolog Ral3p/Scd2p can facilitate two-hybrid interactions both between Ral1p/Scd1p (a Cdc24p homolog) and Ras1p (an Rsr1p homolog) and between Ral1p/Scd1p and *S. pombe* Cdc42p (Chang et al. 1994).

In contrast to the paradigmatic polarity-establishment mutants, mutants defective in *RHO1* or in *RHO3* and *RHO4* (whose functions seem to be at least partially redundant) accumulate small-budded cells (Matsui and Toh-e 1992; Yamochi et al. 1994). This suggests that the Rho-type GTPases encoded by these genes are involved in the maintenance of cytoskeletal polarity or of polarized cell-surface growth after bud emergence, rather than in the initial establishment of polarity. Thus, these Rho proteins are provisionally assigned to a separate class of "polarity-maintenance functions" (Fig. 1). However, it should be noted that the distinction between polarity-establishment functions and polarity-maintenance functions may not be sharp; in particular, Cdc24p and Cdc42p may function in both processes. This was suggested for Cdc24p by the apparent loss of polarized bud growth when budded cells were shifted to restrictive temperature and by the abnormal-shaped buds produced during growth at semi-permissive temperatures (Sloat et al. 1981). Similar observations have been made on *cdc42*

mutants, and several lines of genetic evidence also suggest that Cdc42p has a function in polarity maintenance that overlaps with the function of Rho3p and is distinct from its function in polarity establishment (Y. Matsui, pers. comm.).

It remains unclear how the GTPase cycles of Rho1p, Rho3p, and Rho4p are regulated. Although no GEFs for these proteins have been reported, sequence homology, in vitro assays, and in vivo genetic interactions all suggest that the *BEM2* (or *IPL2*) gene product is a GAP for Rho1p (and perhaps the other Rho proteins) (Kim et al. 1994; Peterson et al. 1994; Zheng et al. 1994b). In addition, the *RDI1* gene product functions as a Rho1p-GDI in vitro (Masuda et al. 1994) and interacts strongly even with nonprenylatable Rho1p in two-hybrid tests (Table 1). It is also unclear how the regulation of the Rho proteins is controlled by (or coordinated with) the function of Cdc24p and Cdc42p, although the synthetic lethality of *bem2* mutations with both *msb1* mutations and *bem1* mutations (Bender and Pringle 1991; Peterson et al. 1994), as well as general arguments about the structure of the morphogenetic hierarchy (see above), suggest strongly that such control (or coordination) must occur. The immediate effectors of the Rho proteins also await identification. One clue to either the regulation or function of the Rho proteins comes from the genetic interactions between *BEM2* and genes controlling protein phosphatase function (Healy et al. 1991; Kim et al. 1994), which suggest (not too surprisingly) that protein phosphorylation/dephosphorylation plays a role.

Communication between the General Site-selection and Polarity-establishment Functions

Multiple lines of evidence now suggest that the communication of positional information from the general site-selection proteins to the polarity-establishment proteins, and thus polarity establishment at appropriate locations, occurs by means of an interaction between GTP-bound Rsr1p and Cdc24p. *RSR1* was originally identified as a multicopy suppressor of a $cdc24^{ts}$ mutation (Bender and Pringle 1989). Suppression is not observed with a *cdc24* null mutation, suggesting that it may involve stabilization of the thermolabile Cdc24p by interaction with Rsr1p. $rsr1^{G12V}$ (whose product should be locked in the GTP-bound form) retains suppression ability despite its incompetence for normal bud-site selection, whereas $rsr1^{K16N}$ (whose product should be unable to bind GTP) does not (Ruggieri et al. 1992); moreover, suppression by *RSR1*, but not by $rsr1^{G12V}$, requires the Rsr1p-GEF Bud5p (Ruggieri et al. 1992; Bender 1993). In addition, certain $cdc24^{ts}$ mutants display random budding at permissive temperatures (where bud formation and overall cell morphology appear essentially normal; Sloat et al. 1981); strikingly, among five independent $cdc24^{ts}$ alleles tested, only the two with this random-budding phenotype were suppressed at restrictive temperature by overexpression of *RSR1* (E. Bi and J.R. Pringle, unpubl.). Recently, these genetic inferences have been supported by biochemical data showing a specific interaction between Rsr1p-GTP and Cdc24p (Zheng et al. 1995; H.-O. Park and I. Herskowitz, pers. comm.).

Like Rsr1p (see above), Cdc24p appears to have a circumcellular distribution (Fig. 5A,B). Thus, one possible model is that in wild-type cells, localized *activation* of Rsr1p leads to localized *activation* of Cdc24p, which leads to localized activation of Cdc42p. The latter step may be coupled to localization of Cdc42p itself, as it, in contrast to Rsr1p and Cdc24p, is clearly concentrated at presumptive bud sites and the tips of small buds (Ziman et al. 1993). However, this model faces several difficulties. First, Zheng et al. (1995) did not detect any effect of Rsr1p-GTP upon the Cdc42p-GEF activity of Cdc24p as measured in vitro. This difficulty might be avoided if an activation that occurs in vivo were dependent on components not present in these in vitro assays. A perhaps deeper problem is that the model does not readily explain how Cdc24p would become activated at a single (random) site, and at the proper time in the cell cycle, in a mutant lacking Rsr1p. Thus, a more attractive model may be that Cdc24p is activated (directly or via an effect on some associated protein) by some form of the cell-cycle-controlling Cdc28p-cyclin protein kinase, but that this activation (or subsequent productive interaction with Cdc42p) is normally restricted spatially to the site at which com-

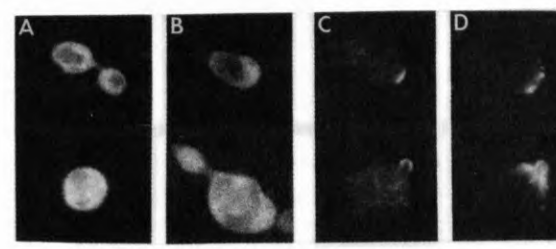

Figure 5. (*A*,*B*) Localization of Cdc24p. Strain NV10-EB (*MAT***a** *cdc24::LEU2 ade2 his3 leu2 lys2 trp1 ura3* [pEGKT-CDC24]) contains a high-copy-number plasmid derived from pEG(KT) (Mitchell et al. 1993) that expresses a fusion of GST to full-length Cdc24p under control of the *GAL1/GAL10* upstream activator sequence. NV10-EB cells were grown on medium containing 2% glucose + 2% galactose. Under these conditions, the fusion protein is produced at levels comparable to that of Cdc24p in a wild-type strain (as judged by immunoblotting) and fully complements the *cdc24::LEU2* disruption for growth at 30°C. Immunofluorescence was performed using Cdc24p-specific antibodies (*A*) or GST-specific antibodies (*B*) (see Materials and Methods). Cdc24p appears to have a circumcellular distribution with a concentration near or at the cell surface. Similar results were obtained when the Cdc24p-specific antibodies were used to stain wild-type cells. The GST-specific antibodies showed essentially no staining of control cells. (*C*,*D*) Cells of wild-type strain C276 were double-labeled using Bem1p-specific antibodies (*C*; see Materials and Methods) and Rhodamine-phalloidin to visualize actin (*D*).

plex formation with Rsr1p-GTP occurs. Formation of this complex might involve Bem1p (see above), a hypothesis supported by the observation that Bem1p also is localized to presumptive bud sites and the tips of small buds (Fig. 5C,D) (K. Corrado and J. Pringle, unpubl.). In the absence of Rsr1p, Bem1p might be able to support the formation of complexes involving activated Cdc24p and Cdc42p at random sites. An attractive feature of this model is that it would explain the synthetic lethality observed (Chant et al. 1991) between *bud5* mutations (which would reduce the level of Rsr1p-GTP) and *bem1* mutations.

Other proteins may also be involved in the Rsr1p-Cdc24p interaction. In a G1-cyclin-deficient background, *bud2* mutations are lethal and cause arrest like that seen with *cdc24* or *cdc42* mutants (Benton et al. 1993; Cvrčková and Nasmyth 1993). The lethality is relieved by deletion of *RSR1*, and an $rsr1^{G12V}$ mutation produces a similar effect in a G1-cyclin-deficient *BUD2* strain. One model to explain these results is that when Rsr1p is stuck in the GTP-bound form, some component that is necessary for Cdc24p function and limiting in the G1-cyclin-deficient background becomes sequestered into unproductive complexes. The putative limiting component does not appear to be Cdc24p (B. Benton and F. Cross, pers. comm.) and might be Bem1p or some novel protein.

Communication between the Polarity-establishment Functions and the Cytoskeleton

It has long been clear that the activities of Cdc24p and Cdc42p are necessary for the establishment of cytoskeletal and growth polarity (see above). However, until very recently, there have been few clues as to how these proteins actually communicate positional information to the cytoskeleton. Although this remains far from clear, recent evidence suggests that the communication involves both a family of protein kinases that interact with, and are activated by, Cdc42p-GTP and a set of proteins with Cdc42p-GAP activity.

The protein kinase connection was revealed serendipitously during a genetic screen for mutations lethal in a G1-cyclin-deficient background (Cvrčková et al. 1995). One of the genes identified, *CLA4*, encodes a protein kinase similar in structure to Ste20p, which was itself known from its role at the head of the "MAP kinase cascade" that transmits the signal during response to mating pheromone (Sprague and Thorner 1992; Herskowitz 1995; Levin and Errede 1995). Deletion of *CLA4* in a wild-type background produces morphological abnormalities but is nonlethal. However, such deletion is lethal in a *ste20*Δ background, implying that in addition to its role in pheromone signaling, Ste20p plays (or at least can play) a role in vegetative growth that is redundant with that of Cla4p. A $cla4^{ts}$ *ste20*Δ strain has a remarkable phenotype at restrictive temperature (Cvrčková et al. 1995). It appears to polarize its actin cytoskeleton normally and displays polarized cell-surface growth. Moreover, the neck-filament-associated septin proteins appear to initiate normal polarized assembly, forming a ring at the presumptive bud site as in wild-type cells. However, as the bud begins to grow, the septins fail to remain in a normal ring at the mother-bud neck, and instead become dispersed over the surface of the growing bud. Associated with this delocalization of the septins is the production of abnormally wide mother-bud necks and a failure of cytokinesis. It is unclear whether the primary defect in the mutant involves a late stage in septin assembly or a subtle effect on the organization of the actin cytoskeleton and hence on the precise localization of cell-surface growth relative to the septin ring.

Cla4p and Ste20p are similar in structure to a human protein kinase, $p65^{PAK}$, that binds in vitro to human Cdc42Hs-GTP and is activated by this interaction (Manser et al. 1994). Consistent with these observations, a combination of gel-overlay binding assays (Cvrčková et al. 1995), two-hybrid data (Table 1) (Cvrčková et al. 1995; Simon et al. 1995), and in vitro protein kinase assays (Simon et al. 1995) suggests that both Cla4p and Ste20p interact specifically with Cdc42p-GTP in yeast and are activated by that interaction. Several observations suggest that these interactions are physiologically significant. First, *cla4* and *cdc42* mutations are synthetically lethal (Cvrčková et al. 1995). Second, simultaneous overexpression of *CLA4* and *CDC42* gives much stronger suppression of a $cdc24^{ts}$ mutation than does overexpression of *CDC42* alone (E. Bi and J. Pringle, unpubl.). Third, data suggest that Cdc24p and Cdc42p are involved in transmitting the mating pheromone signal from the βγ component of the heterotrimeric G protein to the MAP kinase cascade (Simon et al. 1995; Stevenson et al. 1995).

In the pathway for polarity establishment during budding, from the available evidence it appears that the Cdc42p-Cla4p/Ste20p link is *not* involved either in the initial polarized organization of the septins or in the polarization of the actin cytoskeleton (Fig. 4). However, it should be noted that a third yeast member of the Cla4p/Ste20p family, more similar in structure to Cla4p than to Ste20p, has recently been identified by the genome-sequencing project. In two-hybrid assays, this protein behaves very much like Cla4p (cf. Table 1) (C. De Virgilio and J. Pringle, unpubl.). It seems likely that deleting this gene (perhaps in combination with deletions of *CLA4* and/or *STE20*) will produce an earlier disruption of septin organization, an effect on actin polarization, or both. Another important goal clearly is to identify the substrates of this family of protein kinases. In this regard, it should be noted that the immediate target of Ste20p in the pheromone-response pathway appears to be the MAP kinase kinase kinase Ste11p (Sprague and Thorner 1992; Herskowitz 1995; Levin and Errede 1995), and that the effects of *cla4* mutations can be suppressed by high osmolarity if the elements of a second MAP kinase pathway (the so-called HOG pathway, involved in resistance to osmotic

stress) are intact (Cvrcková et al. 1995). Thus, although other routes to activation of the HOG pathway have been identified (Maeda et al. 1995), it seems possible that one immediate target of Cla4p/Ste20p during budding is the pair of HOG pathway MAP kinase kinase kinases Ssk2p and Ssk22p (Levin and Errede 1995; Maeda et al. 1995).

Meanwhile, new evidence suggests that Bem3p and Rga1p, a pair of putative Cdc42p-GAPs, may also be effectors of septin assembly. *BEM3* was identified as a multicopy suppressor of a mutation in *BEM2* (Bender and Pringle 1991), which itself appears to be a GAP for other Rho proteins, but not for Cdc42p (see above). In contrast, several lines of evidence (reviewed below) suggest that Bem3p is a GAP for Cdc42p. *RGA1* was identified (Stevenson et al. 1992, 1995) by a mutation that allowed a modest level of expression of the pheromone-signaling pathway even in the absence of Gβγ, normally an essential positive transducer of the pheromone signal (Sprague and Thorner 1992). As several other lines of evidence (reviewed below) suggest that Rga1p is a GAP for Cdc42p, this phenotype presumably reflects the role of Cdc42p in transmission of the pheromone signal (see above): The loss of a Cdc42p-GAP presumably results in a higher basal level of activated (GTP-bound) Cdc42p, which allows some activity of the signaling pathway even in the absence of the normal stimulus.

Bem3p and Rga1p both have sequence homology with other known Rho-GAPs, and Bem3p has been shown to have Cdc42p-GAP activity in vitro (Zheng et al. 1994b; Stevenson et al. 1995). In addition, both Bem3p and Rga1p interact specifically with Cdc42p, and not with other Rho proteins, in two-hybrid assays (Table 1) (Stevenson et al. 1995); as expected for a GAP, the interaction is much stronger (indeed, for Bem3p only detectable) when Cdc42p is locked in the GTP-bound form by a G12V or Q61L mutation. In addition to the effect of *rga1* mutation on the pheromone-signaling pathway (see above), several other in vivo tests are also consistent with the hypothesis that Bem3p and Rga1p can reduce the levels of activated Cdc42p. First, overexpression of either *BEM3* or *RGA1* eliminates the suppression otherwise seen (Bender and Pringle 1989) of *cdc24^{ts}* mutations by multicopy *CDC42* (Fig. 6A); presumably, the suppression depends on having an adequate amount of Cdc42p in the GTP-bound form, a condition that is prevented by overexpression of a GAP. Second, overexpression of either *BEM3* or *RGA1* also exacerbates the phenotype of *cdc24^{ts}* or *cdc42^{ts}* mutations (i.e., reduces the maximum temperature at which the strains can survive) (Fig. 6B, sectors 3 and 4) (Stevenson et al. 1995), whereas deletion of *BEM3* has a weak effect in the opposite direction (E. Bi and J. Pringle, unpubl.).

Given this background and the known role of Cdc42p in promoting polarized assembly of the septins, we were surprised to find that overexpression of either *BEM3* or *RGA1* could *suppress* temperature-sensitive

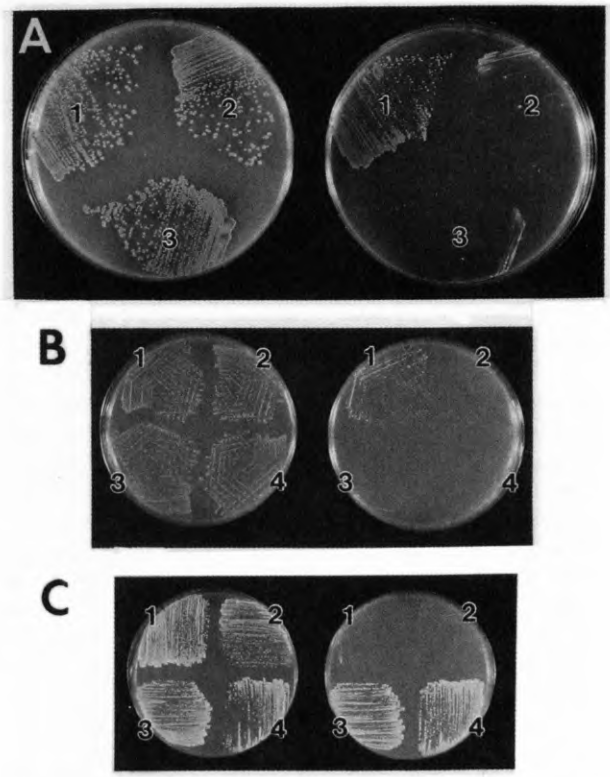

Figure 6. Effects of overexpression of putative Cdc42p-GAPs. (*A*) Strain YEF323 (*MATα ade2 his4 leu2 trp1 ura3 cdc24-H^{ts}*) harboring a *CDC42*-containing plasmid (YEp352-CDC42) was transformed with the high-copy-number plasmids YEp13 (vector control; sectors 1), YEp13-RGA1 (sectors 2), or YEp13-BEM3 (sectors 3) and incubated at 23°C (left) or 37°C (right) on YEPD medium containing 1 M sorbitol (cf. Bender and Pringle 1989). (*B*) Strain YEF316 (*MAT***a** *ade2 his3 leu2 lys3 trp1 ura3 cdc24-B^{ts}*) was transformed with high-copy-number plasmids YEp13 (sectors 1), YEp13-ZDS1 (sectors 2), YEp13-BEM3 (sectors 3), or YEp13-RGA1 (sectors 4) and incubated at 23°C (*left*) or 34°C (*right*) on YEPD medium. (*C*) Strain YML17 (*MAT***a** *leu2 ura3 cdc12-6^{ts}*) was transformed with high-copy-number plasmids YEp13 (sectors 1), YEp13-ZDS1 (sectors 2), YEp13-BEM3 (sectors 3), or YEp13-RGA1 (sectors 4) and incubated at 23°C (*left*) or 30° (*right*) C on YEPD medium.

mutations in *CDC12*, one of the septin-encoding genes (Fig. 6C, sectors 3 and 4) (E. Bi and J. Pringle, unpubl.). Such suppression of *cdc12^{ts}* is not displayed by at least one other apparent negative regulator of Cdc42p (Fig. 6C, sector 2), whose genetic interactions with *CDC24* and *CDC42* are similar to those described above for *BEM3* and *RGA1* (Fig. 6B, sector 2) (E. Bi and J. Pringle, unpubl.). In addition, deletion of *BEM3*, although producing little or no phenotypic effect on its own (Zheng et al. 1994b; Stevenson et al. 1995), is synthetically lethal with the *cdc12^{ts}* mutation (E. Bi and J. Pringle, unpubl.), and deletion of both *BEM3* and *RGA1*, although not lethal, produces morphological abnormalities consistent with the hypothesis that septin assembly has been compromised (Stevenson et

al. 1995). The simplest interpretation of the results is that the Cdc42p-GAP activity of Bem3p and Rga1p is a concomitant of a role as positive effectors of septin assembly at the presumptive bud site (Fig. 4). It is not yet clear whether Bem3p and Rga1p function as components of the Cla4p/Ste20p effector pathway or as an independent effector pathway (Fig. 4), and the viability of the *bem3 rga1* double mutant suggests that Bem3p and Rga1p may not be the only Cdc42p-GAPs.

To date, although the possibility that each system is more than twofold redundant (see above) is an important caveat, neither the Cla4p-family protein kinases nor the Cdc42p-GAPs are implicated in the polarization of the actin cytoskeleton (Fig. 4). Indeed, there are still few clues as to how this centrally important process occurs. It is possible that Cdc42p operates through one or more of the other Rho GTPases in a "GTPase cascade" to achieve actin polarization (Chant and Stowers 1995); this would parallel a model developed for animal cells that proposes sequential action of Cdc42, Rac, and Rho in organizing the actin cytoskeleton (Nobes and Hall 1995). However, there is little evidence for the existence of such a pathway in yeast; indeed, as noted above, the available evidence instead suggests that Rho1p, Rho3p, and Rho4p are required not for initial polarization but for subsequent maintenance of polarity during early bud growth (Matsui and Toh-e 1992; Yamochi et al. 1994; K. Nishimura et al., in prep.). Another possibility is that Cdc42p functions by regulating phosphoinositide 3-kinase, as has been suggested for mammalian cells (Zheng et al. 1994a). However, it is not clear that this mechanism could produce the tight spatial localization of actin cytoskeletal assembly that is actually observed. Thus, it seems more likely that the communication between Cdc42p and the actin cytoskeleton will involve novel elements; these elements are likely to be discovered either by additional genetic studies or by use of the permeabilized-cell system developed by Li et al. (1995), which exhibits Cdc42p-dependent actin assembly.

Coordination with the Cell Cycle and Relationship to Polarity Establishment during Mating

Successful cellular reproduction clearly requires that the morphogenetic events of the cell cycle be coordinated temporally both with each other and with other events such as the duplication and division of the nucleus. As the unbudded cells produced by division at first grow isotropically (Pringle and Hartwell 1981), it has long been presumed that the onset of cell polarization must be triggered by the cell-cycle-commitment event known as Start. Indeed, there is now good evidence that the activation of the Cdc28p protein kinase by G1 cyclins (the molecular equivalent of Start) is responsible for the initiation of polarization (Lew and Reed 1993). (However, it should also be noted that the orientation of this polarization appears to respond to positional signals that were placed in the cell cortex during previous cell cycles, as described above.) Moreover, it appears that subsequent transitions in the organization of the cytoskeleton are also triggered by changes in the activities of the Cdc28p kinase as it associates with other cyclins during the cell cycle (Lew and Reed 1993), and that coordination of the budding pathway with the nuclear cycle is mediated by a checkpoint that delays nuclear division (by means of inhibitory phosphorylation of Cdc28p) if bud emergence or growth has been delayed (Lew and Reed 1995).

The substrates upon which Cdc28p operates to exert these controls have not been identified. It would not be surprising to find that they include the Rsr1p regulatory factors Bud5p and/or Bud2p, and, indeed, the lethality of *bud2* mutations in a G1-cyclin-deficient background (Benton et al. 1993; Cvrčková and Nasmyth 1993) strongly suggests that the Cdc28p-G1-cyclin complexes directly or indirectly affect the Rsr1p-Cdc24p interaction (see also above). However, this cannot be the whole story, as cells lacking *RSR1* (Bender and Pringle 1989), *BUD5* (Chant et al. 1991), or *BUD2* (Park et al. 1993) appear to have normal coordination of budding with the cell cycle. One possible model is that control by the Cdc28p-G1-cyclin protein kinase is exerted *both* on the Rsr1p regulatory factors and on a polarity-establishment protein such as Cdc24p or Bem1p (or both).

When an **a** cell encounters an α cell, the endogenous program for establishing polarity adjacent to the previous bud site (axial budding) is overridden by the pheromone signals, and the cells polarize growth toward their mating partners (see the introduction). It is of interest to ask what part of the hierarchy controlling polarization during budding (Fig. 1) is also involved in the control of polarization during mating. Mutations in the bud-site-selection genes do not appear to affect the ability to polarize toward a mating partner (Chenevert 1994), implying that the products of these genes are involved only in site selection during budding. In contrast, mutations in *CDC24* prevent mating (Reid and Hartwell 1976) and the polarization of growth and secretion in response to mating pheromone (Field and Schekman 1980). Interpretation of these observations has recently been complicated by the evidence that Cdc24p and Cdc42p are apparently involved in transmission of the pheromone signal from the G protein βγ subunits (Sprague and Thorner 1992) to Ste20p at the head of the MAP-kinase cascade (Simon et al. 1995; Stevenson et al. 1995; M. Peter and I. Herskowitz, pers. comm.); that is, the failure to polarize in response to pheromone could reflect a general failure to respond to pheromone rather than a specific involvement of the proteins in polarization under these conditions. However, the isolation of special *cdc24* and *bem1* mutants that fail to polarize in response to pheromone but are otherwise normal in pheromone response (Chenevert et al. 1992, 1994) suggests that Cdc24p and Bem1p (and probably Cdc42p)

are directly involved in polarization in response to pheromone in addition to their apparent role in pheromone signal transmission. It is interesting that Ste20p also appears to function in polarity establishment during budding, at least under conditions in which its homolog Cla4p is deficient (Cvrčková et al. 1995; and see above). It seems likely that these apparent complications (from the point of view of the investigator) are actually a simplification (from the point of view of the cell) in facilitating the coordination of the several different pathways in which these proteins participate.

CONCLUDING REMARKS

Although many mysteries remain, the morphogenetic hierarchy depicted in Figure 1 seems likely to provide a reliable guide for further thinking and experimentation on the generation of cell polarity in yeast. Although other methodologies have played a role, the progress in this area has been due largely to the power of genetic methods to uncover novel and often unanticipated functions, even when the proteins providing these functions are nonabundant in the cell, and to elucidate the functional relationships between gene products. It remains to be seen to what extent the general organization of functions and specific mechanisms elucidated in yeast cells will apply also to other kinds of cells. However, given such hints as the role of cortical markers in orienting division planes in *Caenorhabditis elegans* embryos (Hyman 1989; Goldstein 1995), the similar organization of GTPase modules and GTPase cascades (Chant and Stowers 1995) and the remarkable similarity of Cdc42 proteins in different organisms (Johnson and Pringle 1995), and the apparently widespread communication of Cdc42 proteins with Ste20p-like protein kinases (Manser et al. 1994; Simon et al. 1995; Cvrčková et al. 1995), it seems likely that studies in yeast will be of considerable assistance to attempts to elucidate the corresponding mechanisms in other types of cells.

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REFERENCES

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

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

Adams, A.E.M., D.I. Johnson, R.M. Longnecker, B.F. Sloat, and J.R. Pringle. 1990. *CDC42* and *CDC43*, two additional genes involved in budding and the establishment of cell polarity in the yeast *Saccharomyces cerevisiae*. *J. Cell Biol.* **111:** 131.

Amatruda, J.F. and J.A. Cooper. 1992. Purification, characterization, and immunofluorescence localization of *Saccharomyces cerevisiae* capping protein. *J. Cell Biol.* **117:** 1067.

Bauer, F., M. Urdaci, M. Aigle, and M. Crouzet. 1993. Alteration of a yeast SH3 protein leads to conditional viability with defects in cytoskeletal and budding patterns. *Mol. Cell. Biol.* **13:** 5070.

Bender, A. 1993. Genetic evidence for the roles of the bud-site-selection genes *BUD5* and *BUD2* in control of the Rsr1p (Bud1p) GTPase in yeast. *Proc. Natl. Acad. Sci.* **90:** 9926.

Bender, A. and J.R. Pringle. 1989. Multicopy suppression of the *cdc24* budding defect in yeast by *CDC42* and three newly identified genes including the *ras*-related gene *RSR1*. *Proc. Natl. Acad. Sci.* **86:** 9976.

———. 1991. Use of a screen for synthetic-lethal and multicopy-suppressee mutants to identify two new genes involved in morphogenesis in *S. cerevisiae*. *Mol. Cell. Biol.* **11:** 1295.

———. 1992. A Ser/Thr-rich multicopy suppressor of a *cdc24* bud emergence defect. *Yeast* **8:** 315.

Benton, B.K., A.H. Tinkelenberg, D. Jean, S.D. Plump, and F.R. Cross. 1993. Genetic analysis of Cln/Cdc28 regulation of cell morphogenesis in budding yeast. *EMBO J.* **12:** 5267.

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

Byers, B. 1981. Cytology of the yeast life cycle. In *The molecular biology of the yeast* Saccharomyces: *Life cycle and inheritance* (ed. J.N. Strathern et al.), p. 59. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Chang, E.C., M. Barr, Y. Wang, V. Jung, H.-P. Xu, and M.H. Wigler. 1994. Cooperative interaction of *S. pombe* proteins required for mating and morphogenesis. *Cell* **79:** 131.

Chant, J. 1994. Cell polarity in yeast. *Trends Genet.* **10:** 328.

Chant, J. and I. Herskowitz. 1991. Genetic control of bud site selection in yeast by a set of gene products that constitute a morphogenetic pathway. *Cell* **65:** 1203.

Chant, J. and J.R. Pringle. 1991. Budding and cell polarity in *Saccharomyces cerevisiae*. *Curr. Opin. Genet. Dev.* **1:** 342.

———. 1995. Patterns of bud-site selection in the yeast *Saccharomyces cerevisiae*. *J. Cell Biol.* **129:** 751.

Chant, J. and L. Stowers. 1995. GTPase cascades choreographing cellular behavior: Movement, morphogenesis, and more. *Cell* **81:** 1.

Chant, J., K. Corrado, J.R. Pringle, and I. Herskowitz. 1991. Yeast *BUD5*, encoding a putative GDP-GTP exchange factor, is necessary for bud site selection and interacts with bud formation gene *BEM1*. *Cell* **65:** 1213.

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

Chenevert, J. 1994. Cell polarization directed by extracellular cues in yeast. *Mol. Biol. Cell* **5:** 1169.

Chenevert, J., N. Valtz, and I. Herskowitz. 1994. Identification of genes required for normal pheromone-induced cell polarization in *Saccharomyces cerevisiae*. *Genetics* **136:** 1287.

Chenevert, J., K. Corrado, A. Bender, J.R. Pringle, and I. Herskowitz. 1992. A yeast gene (*BEM1*) necessary for cell polarization whose product contains two SH3 domains. *Nature* **356:** 77.

Costigan, C., S. Gehrung, and M. Snyder. 1992. A synthetic lethal screen identifies SLK1, a novel protein kinase homolog implicated in yeast cell morphogenesis and cell growth. *Mol. Cell. Biol.* **12:** 1162.

Cvrčková, F. and K. Nasmyth. 1993. Yeast G1 cyclins *CLN1* and *CLN2* and a GAP-like protein have a role in bud formation. *EMBO J.* **12:** 5277.

Cvrčková, F., C. De Virgilio, E. Manser, J.R. Pringle, and K. Nasmyth. 1995. Ste20-like protein kinases are required for normal localization of cell growth and for cytokinesis in budding yeast. *Genes Dev.* **9:** 1817.

Desfarges, L., P. Durrens, H. Juguelin, C. Cassagne, M. Bonneau, and M. Aigle. 1993. Yeast mutants affected in viability upon starvation have a modified phospholipid composition. *Yeast* **9:** 267.

Dorer, R., P.M. Pryciak, and L.H. Hartwell. 1995. *Saccharomyces cerevisiae* cells execute a default pathway to select a mate in the absence of pheromone gradients. *J. Cell Biol.* **131:** 845.

Drubin, D.G. 1991. Development of cell polarity in budding yeast. *Cell* **65:** 1093.

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

Field, C. and R. Schekman. 1980. Localized secretion of acid phosphatase reflects the pattern of cell surface growth in *Saccharomyces cerevisiae*. *J. Cell Biol.* **86:** 123.

Fields, S. and R. Sternglanz. 1994. The two-hybrid system: an assay for protein-protein interaction. *Trends Genet.* **10:** 286.

Flescher, E.G., K. Madden, and M. Snyder. 1993. Components required for cytokinesis are important for bud site selection in yeast. *J. Cell Biol.* **122:** 373.

Ford, S.K. and J.R. Pringle. 1986. Development of spatial organization during the formation of zygotes and shmoos in *Saccharomyces cerevisiae*. *Yeast* **2:** S114.

———. 1991. Cellular morphogenesis in the *S. cerevisiae* cell cycle: Localization of the *CDC11* gene product and the timing of events at the budding site. *Dev. Genet.* **12:** 281.

Freifelder, D. 1960. Bud position in *Saccharomyces cerevisiae*. *J. Bacteriol.* **80:** 567.

Fujita, A., C. Oka, Y. Arikawa, T. Katagai, A. Tonouchi, S. Kuhara, and Y. Misumi. 1994. A yeast gene necessary for bud-site selection encodes a protein similar to insulin-degrading enzymes. *Nature* **372:** 567.

Goldstein, B. 1995. Cell contacts orient some cell division axes in the *Caenorhabditis elegans* embryo. *J. Cell Biol.* **129:** 1071.

Govindan, B., R. Bowser, and P. Novick. 1995. The role of Myo2, a yeast class V myosin, in vesicular transport. *J. Cell Biol.* **128:** 1055.

Guthrie, C. and G.R. Fink, eds. 1991. *Methods Enzymol.*, vol. 194.

Gyuris, J., E. Golemis, H. Chertkov, and R. Brent. 1993. Cdi1, a human G1 and S phase protein phosphatase that associates with Cdk2. *Cell* **75:** 791.

Haarer, B.K. and J.R. Pringle. 1987. Immunofluorescence localization of the *Saccharomyces cerevisiae CDC12* gene product to the vicinity of the 10-nm filaments in the mother-bud neck. *Mol. Cell. Biol.* **7:** 3678.

Hart, M.J., A. Eva, T. Evans, S.A. Aaronson, and R.A. Cerione. 1991. Catalysis of guanine nucleotide exchange on the CDC42Hs protein by the *dbl* oncogene product. *Nature* **354:** 311.

Hartwell, L.H., J. Culotti, J.R. Pringle, and B.J. Reid. 1974. Genetic control of the cell division cycle in yeast. *Science* **183:** 46.

Hašek, J., I. Rupeš, J. Svobodová, and E. Streiblová. 1987. Tubulin and actin topology during zygote formation of *Saccharomyces cerevisiae*. *J. Gen. Microbiol.* **133:** 3355.

Healy, A.M., S. Zolnierowicz, A.E. Stapleton, M. Goebl, A.A. DePaoli-Roach, and J.R. Pringle. 1991. *CDC55*, a *S. cerevisiae* gene involved in cellular morphogenesis: Identification, characterization, and homology to the B subunit of mammalian type 2A protein phosphatase. *Mol. Cell. Biol.* **11:** 5767.

Herskowitz, I. 1995. MAP kinase pathways in yeast: For mating and more. *Cell* **80:** 187.

Herskowitz, I., J. Rine, and J. Strathern. 1992. Mating-type determination and mating-type interconversion in *Saccharomyces cerevisiae*. In *The molecular and cellular biology of the yeast* Saccharomyces: *Gene expression* (ed. E.W. Jones et al.), p. 583. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Hicks, J.B., J.N. Strathern, and I. Herskowitz. 1977. Interconversion of yeast mating types. III. Action of the homothallism (*HO*) gene in cells homozygous for the mating type locus. *Genetics* **85:** 395.

Horvitz, H.R. and I. Herskowitz. 1992. Mechanisms of asymmetric cell division: Two Bs or not two Bs, that is the question. *Cell* **68:** 237.

Hyman, A.A. 1989. Centrosome movement in the early divisions of *Caenorhabditis elegans:* A cortical site determining centrosome position. *J. Cell Biol.* **109:** 1185.

Jackson, C.L. and L.H. Hartwell. 1990. Courtship in *S. cerevisiae:* Both cell types choose mating partners by responding to the strongest pheromone signal. *Cell* **63:** 1039.

Jacobs, C.W., A.E.M. Adams, P.J. Szaniszlo, and J.R. Pringle. 1988. Functions of microtubules in the *Saccharomyces cerevisiae* cell cycle. *J. Cell Biol.* **107:** 1409.

Johnson, D.I. and J.R. Pringle. 1990. Molecular characterization of *CDC42*, a *Saccharomyces cerevisiae* gene involved in the development of cell polarity. *J. Cell Biol.* **111:** 143.

———. 1995. Cdc42p. In *Guidebook to the small GTPases* (ed. M. Zerial and L. Huber), p. 283. Oxford University Press, Oxford.

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

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

Kim, Y.-J., L. Francisco, G. Chen, E. Marcotte, and C.S.M. Chan. 1994. Control of cellular morphogenesis by the Ipl2/Bem2 GTPase-activating protein: Possible role of protein phosphorylation. *J. Cell Biol.* **127:** 1381.

Koerner, T.J., J.E. Hill, A.M. Myers, and A. Tzagoloff. 1991. High-expression vectors with multiple cloning sites for construction of *trpE* fusion genes: pATH vectors. *Methods Enzymol.* **194:** 477.

Levin, D.E. and B. Errede. 1995. The proliferation of MAP kinase signaling pathways in yeast. *Curr. Opin. Cell Biol.* **7:** 197.

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

———. 1995. A cell cycle checkpoint monitors cell morphogenesis in budding yeast. *J. Cell Biol.* **129:** 739.

Li, R., Y. Zheng, and D.G. Drubin. 1995. Regulation of cortical actin cytoskeleton assembly during polarized cell growth in budding yeast. *J. Cell Biol.* **128:** 599.

Li, Y.-Y., E. Yeh, T. Hays, and K. Bloom. 1993. Disruption of mitotic spindle orientation in a yeast dynein mutant. *Proc. Natl. Acad. Sci.* **90:** 10096.

Madden, K. and M. Snyder. 1992. Specification of sites for polarized growth in *Saccharomyces cerevisiae* and the influence of external factors on site selection. *Mol. Biol. Cell* **3:** 1025.

Madden, K., C. Costigan, and M. Snyder. 1992. Cell polarity and morphogenesis in *Saccharomyces cerevisiae*. *Trends Cell Biol.* **2:** 22.

Maeda, T., M. Takekawa, and H. Saito. 1995. Activation of yeast PBS2 MAPKK by MAPKKKs or by binding of an SH3-containing osmosensor. *Science* **269:** 554.

Manser, E., T. Leung, H. Salihuddin, Z. Zhao, and L. Lim. 1994. A brain serine/threonine protein kinase activated by Cdc42 and Rac1. *Nature* **367:** 40.

Masuda, T., K. Tanaka, H. Nonaka, W. Yamochi, A. Maeda, and Y. Takai. 1994. Molecular cloning and characterization of yeast *rho* GDP dissociation inhibitor. *J. Biol. Chem.* **269:** 19713.

Matsui, Y. and A. Toh-e. 1992. Yeast *RHO3* and *RHO4 ras* superfamily genes are necessary for bud growth, and their defect is suppressed by a high dose of bud formation genes *CDC42* and *BEM1*. *Mol. Cell. Biol.* **12:** 5690.

Mitchell, D.A., T.K. Marshall, and R.J. Deschenes. 1993. Vectors for the inducible overexpression of glutathione S-transferase fusion proteins in yeast. *Yeast* **9:** 715.

Miyamoto, S., Y. Ohya, Y. Sano, S. Sakaguchi, H. Iida, and Y. Anraku. 1991. A *DBL*-homologous region of the yeast *CLS4/CDC24* gene product is important for Ca^{2+}-modulated bud-site assembly. *Biochem. Biophys. Res. Commun.* **181:** 604.

Muhua, L., T.S. Karpova, and J.A. Cooper. 1994. A yeast actin-related protein homologous to that in vertebrate dynactin complex is important for spindle orientation and nuclear migration. *Cell* **78:** 669.

Nobes, C.D. and A. Hall. 1995. Rho, Rac, and Cdc42 GTPases regulate the assembly of multimolecular focal complexes associated with actin stress fibers, lamellipodia, and filopodia. *Cell* **81:** 53.

Ohya, Y., S. Miyamoto, Y. Ohsumi, and Y. Anraku. 1986. Calcium-sensitive *cls4* mutant of *Saccharomyces cerevisiae* with a defect in bud formation. *J. Bacteriol.* **165:** 28.

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

Park, H.-O., J. Chant, and I. Herskowitz. 1993. *BUD2* encodes a GTPase-activating protein for Bud1/Rsr1 necessary for proper bud-site selection in yeast. *Nature* **365:** 269.

Peterson, J., Y. Zheng, L. Bender, A. Myers, R. Cerione, and A. Bender. 1994. Interactions between the bud emergence proteins Bem1p and Bem2p and Rho-type GTPases in yeast. *J. Cell Biol.* **127:** 1395.

Powers, S., E. Gonzales, T. Christensen, J. Cubert, and D. Broek. 1991. Functional cloning of *BUD5*, a *CDC25*-related gene from *S. cerevisiae* that can suppress a dominant-negative *RAS2* mutant. *Cell* **65:** 1225.

Preuss, D., J. Mulholland, A. Franzusoff, N. Segev, and D. Botstein. 1992. Characterization of the *Saccharomyces* Golgi complex through the cell cycle by immunoelectron microscopy. *Mol. Biol. Cell* **3:** 789.

Priess, J.R. 1994. Establishment of initial asymmetry in early *Caenorhabditis elegans* embryos. *Curr. Opin. Genet. Dev.* **4:** 563.

Pringle, J.R. and L.H. Hartwell. 1981. The *Saccharomyces cerevisiae* cell cycle. In *The molecular biology of the yeast* Saccharomyces: *Life cycle and inheritance* (ed. J.N. Strathern et al.), p. 97. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Pringle, J.R., R.A. Preston, A.E.M. Adams, T. Stearns, D.G. Drubin, B.K. Haarer, and E.W. Jones. 1989. Fluorescence microscopy methods for yeast. *Methods Cell Biol.* **31:** 357.

Read, E.B., H.H. Okamura, and D.G. Drubin. 1992. Actin- and tubulin-dependent functions during *Saccharomyces cerevisiae* mating projection formation. *Mol. Biol. Cell* **3:** 429.

Reid, B.J. and L.H. Hartwell. 1976. Regulation of mating in the cell cycle of *Saccharomyces cerevisiae*. *J. Cell Biol.* **75:** 355.

Rodriguez-Boulan, E. and W.J. Nelson, eds. 1993. Epithelial and neuronal cell polarity and differentiation. *J. Cell Sci. Suppl.* **17.**

Ruggieri, R., A. Bender, Y. Matsui, S. Powers, Y. Takai, J.R. Pringle, and K. Matsumoto. 1992. *RSR1*, a *ras*-like gene homologous to Krev-1(*smg21A/rap1A*): Role in the development of cell polarity and interactions with the Ras pathway in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **12:** 758.

Rüther, U. and B. Müller-Hill. 1983. Easy identification of cDNA clones. *EMBO J.* **2:** 1791.

Schnepf, E. 1986. Cellular polarity. *Annu. Rev. Plant Physiol.* **37:** 23.

Segall, J.E. 1993. Polarization of yeast cells in spatial gradients of α mating factor. *Proc. Natl. Acad. Sci.* **90:** 8332.

Shapiro, L. 1993. Protein localization and asymmetry in the bacterial cell. *Cell* **73:** 841.

Simon, M.-N., C. De Virgilio, B. Souza, J.R. Pringle, A. Abo, and S.I. Reed. 1995. Role for the Rho-family GTPase

Cdc42 in yeast mating pheromone signal pathway. *Nature* **376:** 702.

Sivadon, P., F. Bauer, M. Aigle, and M. Crouzet. 1995. Actin cytoskeleton and budding pattern are altered in the yeast *rvs161* mutant: The Rvs161 protein shares common domains with the brain protein amphiphysin. *Mol. Gen. Genet.* **246:** 485.

Sloat, B. and J.R. Pringle. 1978. A mutant of yeast defective in cellular morphogenesis. *Science* **200:** 1171.

Sloat, B.F., A. Adams, and J.R. Pringle. 1981. Roles of the *CDC24* gene product in cellular morphogenesis during the *Saccharomyces cerevisiae* cell cycle. *J. Cell Biol.* **89:** 395.

Snyder, M. 1989. The *SPA2* protein of yeast localizes to sites of cell growth. *J. Cell Biol.* **108:** 1419.

Snyder, M., S. Gehrung, and B.D. Page. 1991. Studies concerning the temporal and genetic control of cell polarity in *Saccharomyces cerevisiae*. *J. Cell Biol.* **114:** 515.

Sprague, G.F., Jr. and J.W. Thorner. 1992. Pheromone response and signal transduction during the mating process of *Saccharomyces cerevisiae*. In *The molecular and cellular biology of the yeast* Saccharomyces: *Gene expression* (ed. E.W. Jones et al.), p. 657. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Stevenson, B.J., N. Rhodes, B. Errede, and G.F. Sprague, Jr. 1992. Constitutive mutants of the protein kinase STE11 activate the yeast pheromone response pathway in the absence of the G protein. *Genes Dev.* **6:** 1293.

Stevenson, B.J., B. Ferguson, C. De Virgilio, E. Bi, J.R. Pringle, G. Ammerer, and G.F. Sprague, Jr. 1995. Mutation of *RGA1*, which encodes a putative GAP for the polarity-establishment protein Cdc42p, activates the pheromone reponse pathway in the yeast *Saccharomyces cerevisiae*. *Genes Dev.* **9:** 2949.

Streiblová, E. 1970. Study of scar formation in the life cycle of heterothallic *Saccharomyces cerevisiae*. *Can. J. Microbiol.* **16:** 827.

Tkacz, J.S. and V.L. MacKay. 1979. Sexual conjugation in yeast. Cell surface changes in response to the action of mating hormones. *J. Cell Biol.* **80:** 326.

Welch, M.D., D.A. Holtzman, and D.G. Drubin. 1994. The yeast actin cytoskeleton. *Curr. Opin. Cell Biol.* **6:** 110.

Winge, Ø. 1935. On haplophase and diplophase in some Saccharomycetes. *C.R. Lab. Carlsberg Ser. Physiol.* **21:** 77.

Yamochi, W., K. Tanaka, H. Nonaka, A. Maeda, T. Musha, and Y. Takai. 1994. Growth site localization of Rho1 small GTP-binding protein and its involvement in bud formation in *S. cerevisiae*. *J. Cell Biol.* **125:** 1077.

Zahner, J.E., H.A. Harkins, and J.R. Pringle. 1996. Genetic analysis of the bipolar pattern of bud-site selection in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* (in press).

Zheng, Y., S. Bagrodia, and R.A. Cerione. 1994a. Activation of phosphoinositide 3-kinase activity by Cdc42Hs binding to p85. *J. Biol. Chem.* **269:** 18727.

Zheng, Y., A. Bender, and R.A. Cerione. 1995. Interactions among proteins involved in bud-site selection and bud-site assembly in *Saccharomyces cerevisiae*. *J. Biol. Chem.* **270:** 626.

Zheng, Y., R. Cerione, and A. Bender. 1994b. Control of the yeast bud-site assembly GTPase Cdc42. Catalysis of guanine nucleotide exchange by Cdc24 and stimulation of GTPase activity by Bem3. *J. Biol. Chem.* **269:** 2369.

Ziman, M., J.M. O'Brien, L.A. Ouellette, W.R. Church, and D.I. Johnson. 1991. Mutational analysis of *CDC42Sc*, a *Saccharomyces cerevisiae* gene that encodes a putative GTP-binding protein involved in the control of cell polarity. *Mol. Cell. Biol.* **11:** 3537.

Ziman, M., D. Preuss, J. Mulholland, J.M. O'Brien, D. Botstein, and D.I. Johnson. 1993. Subcellular localization of Cdc42p, a *Saccharomyces cerevisiae* GTP-binding protein involved in the control of cell polarity. *Mol. Biol. Cell* **4:** 1307.