The Glc7p-Interacting Protein Bud14p Attenuates Polarized Growth, Pheromone Response, and Filamentous Growth in *Saccharomyces cerevisiae*

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A genetic selection in *Saccharomyces cerevisiae* for mutants that stimulate the mating pathway uncovered a mutant that had a hyperactive pheromone response pathway and also had hyperpolarized growth. Cloning and segregation analysis demonstrated that *BUD14* was the affected gene. Disruption of *BUD14* in wild-type cells caused mild stimulation of pheromone response pathway reporters, an increase in sensitivity to mating factor, and a hyperelongated shmoo morphology. The *bud4* mutant also had hyperfilamentous growth. Consistent with a role in the control of cell polarity, a Bud14p-green fluorescent protein fusion was localized to sites of polarized growth in the cell. Bud14p shared morphogenetic functions with the Ste20p and Bni1p proteins as well as with the type 1 phosphatase Glc7p. The genetic interactions between *BUD14* and *GLC7* suggested a role for Glc7p in filamentous growth, and Glc7p was found to have a positive function in filamentous growth in yeast.

One output of eukaryotic signal transduction pathways is a redirection of polarized growth and an attendant change in cell morphology. The yeast *Saccharomyces cerevisiae* provides several examples of this phenomenon, for example, during mating and in the transition to filamentous growth. Coordination between signal transduction and oriented growth is therefore essential for the appropriate execution of a morphological response. However, the mechanisms that underlie this coordination are incompletely understood.

Polarized or apical growth in yeast is initiated at specific sites that are marked by bud-site-selection proteins localized to the cell surface (12, 63, 64). Recognition of these cues by a core GTPase module is an important step in the establishment of polarized growth at specific cellular locations (33, 52, 60). Once a site has been chosen, recruitment of the polarity establishment protein Cdc42p and associated proteins initiates polarized growth by directing actin polymerization, polarized secretion, and growth toward the established site (32, 54, 65). In part, these events are accomplished by interaction of Cdc42p with the Gic1p/Gic2p proteins (8, 31) and with the polarisome (21, 70). Polarisome components include Bni1p and Bud6p (2, 21, 70, 78, 95) and Pca2p and Spa2p (13, 23, 77). Polymerization of the actin cytoskeleton by the polarisome is an important regulatory step in directed growth (22, 71, 89) and is dynamic. For example, the Hsl1p and Hsl7p proteins attenuate polarized growth by interaction with the Svw1p protein kinase and the septin ring, at which site septin assembly is monitored (14, 45, 47, 80).

At least two signaling pathways in yeast are capable of re-directing polarized growth: the mating or pheromone response pathway and the filamentous growth pathway. Indeed, these pathways share common components, although the morphological output of the two pathways is quite distinct (42, 67). Activation of the mating pathway by binding of pheromone to its cognate receptor leads to reorientation of cell polarity toward the perceived mate and the formation of a shmoo (29, 40, 48, 75). Several proteins have been characterized that facilitate communication between the polarized growth machinery and the mating pathway. In particular, Far1p links the pheromone receptor and heterotrimeric G-protein to Cdc24p, the guanine nucleotide exchange factor for Cdc42p (9, 55, 82, 96). The Cdc24p-Far1p complex is exported from the nucleus to the site of incipient shmoo formation upon pheromone treatment, where the complex promotes polarization of the actin cytoskeleton (55–57, 79, 88). The Bem1p and (possibly) Mgd1p proteins stabilize Cdc24p at sites of polarized growth (10, 39), and interactions between Bem1p and mating signaling proteins have been described (41, 46). The Akr1p septin-interacting protein also promotes both pheromone-dependent signaling and shmoo formation (19, 27, 35, 36), as does the Akr1p protein (28, 66). Not surprisingly, polarisome components are also required for shmoo formation (13, 21).

Filamentous growth is a response to nutrient limitation characterized by the formation of elongated cells that remain connected in branched chains (15, 26, 67; for reviews, see references 37, 50, and 51). The cues that direct cell polarity during filamentous growth are the same as for vegetative growth (17, 87). Cell elongation during filamentous growth is driven, in part, by the extension of the G2 phase of the cell cycle (1, 38). How the filamentous growth pathway causes the G2 extension is not clear, but it may be mediated by Ste12p-dependent expression of the Cln1p cyclin (43, 49). Multiple signaling pathways are required for filamentous growth, a fact that underscores the complexity of this morphogenetic response (19).

To identify new components that interface between the pheromone response and/or filamentous growth pathway and the cell polarity machinery, we performed a genetic selection for mutants that exhibit enhanced pheromone response path-
way activity and screened among these mutants for those with altered morphology. We isolated one mutant, defective in the **BUD14** gene, that displayed a hypersensitive response to pheromone, enhanced filamentous growth, and hyperpolarized growth. Genetic analysis suggests that Bud14p attenuates polarized growth by a mechanism independent of the Hsl1p and Hsp70 proteins but dependent upon the polarisome complex. Genetic analysis also supports a connection between Bud14p and the phosphatase Glc7p in polarized growth in yeast.

**MATERIALS AND METHODS**

**Strains, media, and microbiological techniques.** The yeast strains used in this study are listed in Table 1. One set of strains is isogenic with SY2002, a derivative of Sc252 (provided by J. Hopper [85]). Another set was derived from HYL33 and HYL334 of the filamentous S.2978b background (provided by G. Fink [15]). Disruption of the **BUD14** gene was performed using pSL2000. Disruption of **BNI1** was performed using plasmid pSL321, which was provided by C. Boone (21). Disruption of the PE42 and SPA2 genes was performed using plasmids pNV44 and p210, which were provided by I. Herskowitz (91). Deletion of **STE4** was performed using plasmids pSL14, ste11::URA3, ste11::URA3, ste12::URA3, ste25::URA3, ste5::URA3, ste20::URA3, and ste4::LEU2 constructs. HSL1 and HSL7 were disrupted using plasmids pslb1::URA3 and psl7::URA3, which were provided by M. Grunstein (47). Disruption of **PSB2** was performed using pSL2002 (85). SY2428 was made. **ADE1** by isolation of the **ADE1** gene from plasmid pSL2001, followed by transformation and selection for **ADE**+ colonies. Strains containing alleles of **GLC7** were provided by M. J. Stark (3) and K. Tatchell (5, 7). A subset of gene disruptions was performed by PCR-based methods that removed the entire open reading frame and replaced it with auxotrophic markers from **Candida glabrata** (for **TRP1**, **LEU2**, and **HIS3**) or **Kluyveromyces lactis** (for **URA3**). Plasmids containing these markers were provided by I. Herskowitz. Other gene disruptions, integrated green fluorescent protein (GFP) fusions, and G418 promoter fusions were made by PCR-based methods using plasmids provided by J. Pringle (44). Gene disruptions and integrated promoter and protein fusions were confirmed by PCR Southern analysis and by phenotype. Yeast and bacterial strains were propagated using standard methods (76). YPD and SD media have been described elsewhere (83).

Disruption of the **BUD14** gene was cloned by transformation of **YEPD** to SCD-His, and **SAD1** and **HSL1** background (provided by G. Fink [15]). Disruption of the **BUD14** gene was performed using a one- or two-step gene replacement integration using the plasmid pRS313, -314, -315, and -316 have been described previously (67), except that equal concentrations of cells were spotted onto YPD solid agar medium for 2 days at 30°C.

Disruption of the plasmid demonstrated that **BUD14** is the complementing gene. Linkage analysis to the fact that **BUD14** is adjacent to **ADE1**. A wild-type strain **ADE1 BUD14** [SY37/2] was mated to the bud14-1 ade1 mutant (SY37/1), and the resulting diploid was subjected to segregation analysis. In 20 tetrads, the bud14-1 phenotypes (His+ and elongated morphology) cosegregated with the Ade phenotype. Tests for mating-specific functions were performed as described previously (83).

**Protein localization.** Indirect immunolocalization of the Cdc3p protein was performed using polyclonal anti-Cdc3p antibodies (provided by J. Pringle [34]) that were purified as described elsewhere (34). Cells were grown to mid-log phase, fixed, permeabilized, and probed using anti-Cdc3p primary and Alexa (A594)-conjugated goat anti-rabbit secondary antibodies (Molecular Probes, Eugene, Ore. [62]). The localization of GFP-Bud14p was determined by using a fluorescein isothiocyanate (FITC) filter.

**Microscopy.** Standard light differential interference contrast (DIC) and fluorescence microscopy using rhodamine and FITC filter sets was performed using an Axiosplan 2 microscope (Zeiss, Jena, Germany), a black and white Orca II digital camera (Hamamatsu, Japan), and Openlab software program (Improvis, Coventry, United Kingdom). Only brightness and contrast digital adjustments were performed on photographs.

**RESULTS**

Identification of **BUD14** as an attenuator of the mating pathway. We sought to identify factors that impinge upon both the pheromone response pathway and morphogenesis. As a primary selection, mutants were isolated that restored signaling of mating pathway-dependent reporters (**FUS1-lacZ** and **FUS1-HIS3**) in a strain lacking a basal signal (**ste4**). A number of dominant and recessive mutations have been isolated and characterized by using this screen (16, 64, 85). As a secondary screen, His+ colonies were examined by microscopy for those exhibiting interesting morphological phenotypes. One such mutant was identified (referred to as bud14-1). It was **His**+, had higher **FUS1-lacZ** expression than wild type (Table 2), and also had an elongated cell morphology, a nonaxial budding pattern, and conferred agar invasion to the Sc252 strain background. Each of these phenotypes was recessive. Complementation and segregation analysis confirmed that the mutant was distinct from other mutations isolated in the selection (e.g., **rga1**) and that both the morphological and **FUS1** reporter activation phenotypes cosegregated.

To identify the genetic gene, the mutant was transformed with a CEN-based genomic library (68, 69), and the resulting colonies were screened for complementation of the His+ phenotype. A single DNA fragment was isolated that complemented both the His+ and cell morphological phenotypes. Deletion analysis of the plasmid demonstrated that **BUD14** is the complementing gene, and linkage analysis confirmed that a defect in **BUD14** was responsible for the phenotypes observed in the original mutant.

Enhanced mating pathway activity and hyperpolarized shmoos morphology in the **bud14** mutant. The **BUD14** gene was disrupted to examine the null phenotype and investigate the genetic requirements for the mutant phenotype. The **bud14** mutant was slightly more sensitive to pheromone than wild type, as determined by halo assay (Fig. 1A). Quantitation of the rate of shmo formation in saturating pheromone confirmed bud14's enhanced pheromone sensitivity (Fig. 1B). Microscopic examination of cells exposed to mating pheromone showed that the bud14 mutant had elongated shmoos with
narrow necks (Fig. 1C), and in some cases the shmoo tips were irregular. Cells containing numerous vacuoles were also observed (Fig. 1C): 20% of bud14 cells had multiple vacuoles after a 3-h exposure to pheromone, compared to ~1% for wild type. Staining using a vacuole-specific dye, FM4-64, confirmed that the vesicles observed in the bud14 mutant were vacuoles (unpublished results).

Overexpression of BUD14 also affected shmoo morphology. In this case, the cells failed to form defined projections (Fig. 1C). Overexpression of BUD14 did not affect FUS1 expression.
or sensitivity to pheromone (unpublished results) but caused slow growth and morphological phenotypes (see below).

The genetic requirements for FUS1-reporter activation in the bud14 mutant were examined. Disruption of STE20 mostly blocked FUS1 expression in the bud14 mutant, and disruption of STE50, STE11, and STE12 completely blocked it (Table 2). In contrast, disruption of STE4 or STE5 did not completely prevent FUS1 expression, similar to the results obtained with the original bud14-1 isolate (Table 2). The bud14 mutant phenotypes are reminiscent of those of the rga1 mutant (82a, 85). Therefore, we examined the phenotype of an rga1 bud14 double mutant. This mutant exhibits higher FUS1 expression than observed in either single mutant (Table 2), and it also had new morphological defects, including cells with wide bud necks (Table 2). Loss of Pbs2p, the mitogen-activated protein kinase kinase for the HOG pathway (61), is known to cause enhanced signaling in the rga1 mutant and other mutants that stimulate FUS1 expression (15, 59, 85). Disruption of PBS2 in the bud14 mutant caused significantly higher FUS1 expression than that observed in either single mutant, as well as morphological defects (Table 2).

Morphological consequences of deletion or overexpression of BUD14 in ste mutants. The effect of ste mutations on the morphology of the bud14 mutant was examined, based on the supposition that the elongated morphology of bud14 was due to activation of the pheromone response pathway. However, disruption of STE genes failed to suppress the elongated morphology of the bud14 mutant (Table 2). In fact, disruption of STE20 exacerbated the elongated morphology (Fig. 2A) and caused other morphological abnormalities that were not observed in other bud14 ste double mutants (Table 2). The consequence of overexpression of BUD14 in ste mutants was also examined. In ste50, ste20, ste11, and ste12 mutants, but

| Strain   | Genotype         | FUS1 expression | AT (mM) | Cell morphology |
|----------|------------------|-----------------|---------|-----------------|
| SY2431   | ste4Δ            | 0.1             | His⁻    | WT              |
| SY3871   | ste4Δ bud14-1    | 0.5             | His⁺    | Elongated       |
| SY3874   | ste4Δ bud14Δ     | 0.45            | His⁺    | Elongated       |
| SY3875   | ste5Δ bud14Δ     | 0.5             | His⁺    | Elongated       |
| SY3876   | ste20Δ bud14Δ    | 0.25            | His⁻    | Irregular       |
| SY3877   | ste50Δ bud14Δ    | 0.1             | His⁺    | Elongated       |
| SY3878   | ste11Δ bud14Δ    | 0.1             | His⁻    | Elongated       |
| SY3879   | ste12Δ bud14Δ    | 0.1             | His⁻    | Elongated       |
| SY2002   | Wild type        | 19              | His⁺    | WT              |
| SY3873   | bud14Δ           | 33              | 1       | Elongated       |
| SY3912   | rga1Δ            | 48              | 1       | Elongated       |
| SY3880   | bud14Δ rga1Δ     | 193             | 15      | Irregular       |
| SY2002   | Wild type c      | 18              | His⁺    | WT              |
| SY3913   | pbs2Δ            | 50              | 1       | WT              |
| SY3913   | bud14Δc          | 38              | 1       | Elongated       |
| SY3880   | bud14Δ pbs2Δc    | 444             | 15      | Irregular       |

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a β-Galactosidase activity was measured as described in Materials and Methods. The reported values are the average of three independent determinations, and standard deviation was less than 20%.

b The highest concentration of AT (aminotriazole) tested that allowed growth on synthetic medium lacking histidine. His⁺ refers to growth on SD-His medium but not on SD-His plus 1 mM AT.

c Cells were grown in synthetic medium, which showed the maximal induction of the bud14 pbs2 double mutant.

d WT, wild-type morphology.
not in ste4 or ste5 mutants, overexpression of BUD14 caused the formation of a long thin bud. This phenotype was particularly striking for the ste20 mutant (Fig. 2A). Buds were longer and thinner than for the other ste mutants, and a higher percentage of the cells exhibited the phenotype (>90% for ste20 compared to 40% for ste12). Overexpression of BUD14 in the ste20 mutant also caused mislocalization of the septin ring in 90% of the cells (Fig. 2B), a phenotype reminiscent of the terminal phenotype of a ste20 cla4 double mutant (18). Septin ring mislocalization was also observed in wild-type cells overproducing Bud14p, but at a lower frequency (10%; see below). Deletion or overexpression of BUD14 in the ste20 mutant also caused slow growth phenotypes not observed in other ste mutants (Fig. 2C).

**Hyperfilamentous growth in the bud14 mutant.** We speculated that Bud14p might be involved in filamentous growth because of the elongated morphology of the bud14 mutant. Moreover, the original bud14-1 isolate exhibited nonaxial budding and agar invasion in the Sc252Ja background, whereas the wild-type strain did not. Disruption of BUD14 in the filamentous (Sc1278b) background caused hyperinvasive growth as assessed by the plate-washing assay (Fig. 3A). The bud14 mutant colonies were more ruffled than the wild type, another characteristic of filamentous growth (Fig. 3A) (17). The single cell invasive growth assay (17) showed that the bud14 mutant had distal-pole budding and had elongated cells under glucose-rich conditions, in contrast to axial budding and spherical cells observed for the wild type (Fig. 3B). Under glucose-limiting conditions, bud14 cells were longer than the wild type (Fig. 3B). Prolonged agar invasion, which accentuates the elongated morphology of filamentous cells (17), confirmed that bud14 cells were longer and thinner than the wild type (Fig. 3C). Since the bud14 mutant exhibits hyperfilamentous growth, we infer that Bud14p acts in a manner antagonistic to filamentous growth.

**Hyperpolarized growth and distal-pole budding in the bud14 mutant.** The genetic requirements underlying the morphology of the bud14 mutant were investigated. The bud14 mutant had an elongated morphology during vegetative growth (Fig. 1C, 2A, and 4A). In an exponential culture, 60% of the bud14 cells were elongated compared to 2% for wild type, and 4% of cells were at least twice as long as wild type. The possibility that Bud14p functions with Hsl proteins in attenuation factors of polarized growth, was investigated. Disruption of BUD14 in hsl1p and hsl7p, two known attenuators of polarized growth, was investigated. Disruption of BUD14 in hsl1 and hsl7 mutants exacerbated their elongated cell morphology (Fig. 4A). The more pronounced phenotypes of the bud14 hsl1 and bud14 hsl7 double mutants imply that Bud14p and Hsl proteins influence polarized growth by different mechanisms.

To determine whether the polarisome was required for the elongated morphology of the bud14 mutant, PEA2 and BNI1 were disrupted. Loss of either PEA2 or BNI1 suppressed the hyperpolarized growth of the bud14 mutant (Fig. 4B). In fact, suppression was reciprocal. The bni1 single mutant exhibited abnormal cell morphology in 30% of cells (Fig. 4B), and this phenotype was suppressed in the bni1 bud14 double mutant. Bni1p and Bud14p exhibit another genetic interaction: the slow growth phenotype observed in strains overexpressing BUD14 was exacerbated by bni1 (Fig. 4C). These phenotypes suggest that a morphogenetic function is shared between
Bud14p and the polarisome and define a requirement for the polarisome in promoting polarized growth in the bud14 mutant.

**BUD14** was originally identified in a genetic screen for mutants that in diploid cells exhibit bipolar bud-site-selection defects (58). We also observed a budding defect in haploid cells lacking BUD14. Bud scar staining of the bud14 mutant confirmed an increase in distal-pole budding in haploid cells (Table 3). The distal-pole budding pattern may be a consequence of the hyperpolarized growth of the bud14 mutant, which is known to promote distal-pole budding (17, 78).

**Localization of Bud14p to the mother-bud neck and its role in cytokinesis.** The localization of Bud14p was investigated to gain insight into its biological function. A galactose-inducible N-terminal GFP-BUD14 fusion was integrated at the BUD14 locus in an otherwise wild-type strain. GFP-Bud14p was observed at the distal tips of cells, at the mother-bud neck, and in the cytoplasm (Fig. 5A). During the course of our analysis, independent confirmation of the localization of Bud14p to these sites was reported using a functional GFP-Bud14p fusion under the control of the BUD14 promoter (58). The localization of Bud14p to the mother-bud neck suggested a role in cytokinesis; thus examination of the septin ring in cells lacking or overproducing Bud14p was performed. In cells overexpressing BUD14, large septin rings were observed in 30% of cells (Fig. 5B), and 10% of the cells had mislocalization of the septin ring (Fig. 5B). No septin defects were observed in the bud14 mutant, but bud14 combined with other mutations including rga1, pbs2 (unpublished results), and partial loss-of-function alleles of glc7 (see below) caused wide bud neck phenotypes.

**A shared function for Bud14p and Glc7p in polarized growth.** Bud14p interacts with the type 1 protein phosphatase Glc7p by two-hybrid analysis (90) and by direct physical interaction (F. Dubouloz and C. De Virgilio, personal communication; M. J. Stark, personal communication). Glc7p is an essential phosphatase required for diverse cellular processes, including glucose control (73, 74), glycogen accumulation (94), cell cycle progression (6), pachytene exit (4), chromosome segregation (30), and ion homeostasis (93). In addition, alleles of GLC7 have been isolated that affect morphogenesis (3).

To determine if Bud14p and Glc7p share a function in polarized growth, double mutant analysis was performed using partial loss-of-function alleles of the essential GLC7 gene (provided by K. Tatchell). The enhanced polarized growth conferred by a bud14 mutation was dramatically exacerbated when combined with the partial loss-of-function allele glc7-132 (Fig. 6A). Cells with wide mother-bud necks were observed in the bud14 glc7-132 double mutant, presumably indicative of cytokinesis defects (Fig. 6A). Moreover, the bud14 glc7-132 double mutant had a conditional growth defect not observed in either single mutant (Fig. 6B). Similar, although less striking, additive effects in the bud14 mutant were observed with partial loss-of-function alleles glc7-133 and glc7-127; no additive effects were observed with a hyperactive allele of GLC7, glc7-109 (data not shown). The glc7-132 allele also partially suppressed the morphological (Fig. 6A) and slow growth (Fig. 6B) defects associated with overexpression of BUD14. Thus, Glc7p and Bud14p share a function related to cell polarity and cytokinesis.

**A requirement for Glc7p in filamentous growth in yeast.** Given that Bud14p has a role in filamentous growth and given the genetic and physical interactions between Bud14p and Glc7p, we suspected that Glc7p might have a role in filamentous growth. Partial loss-of-function alleles of GLC7 (provided by M. J. Stark) were tested and were found to confer a defect in agar invasion (Fig. 7A) and in unipolar budding and cell elongation (Fig. 7B). Other partial loss-of-function alleles,
glc7-127 and glc7-132, also had a filamentous growth defect (data not shown). Deletion of GAC1, whose product interacts with Glc7p and is required for glycogen accumulation (94), did not affect filamentous growth, suggesting that the glycogen storage defects of glc7 mutants are not responsible for the filamentous growth defect.

### TABLE 3. Bud-site-selection defect in the haploid bud14 mutant

| Strain               | Axial (%) | Random (%) | Bipolar (%) |
|----------------------|-----------|------------|-------------|
| Wild type            | 87        | 4          | 9           |
| bud14                 | 27        | 13         | 60          |

a Wild-type (SY2002) and bud14 (SY3873) cells were grown to mid-log phase in YPD at 30°C and stained with Calcofluor as described in Materials and Methods. Cells containing multiple bud scars were classified as axial (three or more scars at one pole), random (at least one scar in the middle third of the cell), or bipolar (three or more scars at both poles); at least 200 cells were counted for each strain.
DISCUSSION

Bud14p has a function antagonistic to mating and filamentous growth. A genetic selection designed to isolate components that impinge upon both the pheromone response pathway and morphogenesis uncovered a mutant defective in Bud14p function. The bud14 mutant showed enhanced basal signaling of the pheromone response pathway and enhanced filamentous growth, suggesting an antagonistic role for Bud14p in both pathways. The two pathways share components, and possibly Bud14p impinges upon a factor common to both pathways. One candidate is the p21-activated kinase Ste20p (Fig. 8), which is the most upstream component required to mediate Bud14p-dependent expression of FUS1. Moreover, BUD14 and STE20 shared genetic interactions not observed with other STE genes. Ste20p also has a role in polarized growth distinct from other Ste proteins (17, 42, 78) and may regulate polarisome function (A. Goehring, unpublished data) (Fig. 8).

Bud14p attenuates polarized growth. We have shown that Bud14p is required for normal cell morphology and attenuates polarized growth. Bud14p’s inhibition of polarized growth is distinct from that of Hsl1p and Hsl7p, whose mutant phenotypes on the surface resemble those of the bud14 mutant. This distinction is based on the pronounced phenotype of hsl bud14 double mutants compared to either single mutant and on the observation that the enhanced polarized growth of hsl mutants is suppressed under glucose-rich conditions (17), whereas the bud14’s hyperpolarized growth is not. Moreover, Hsl1p and Hsl7p do not influence FUS1 activity (unpublished observations).

We speculated that bud14’s hyperpolarized growth is a consequence of stimulation of the pheromone response pathway. This possibility was excluded based on the observation that ste mutants (which abolish mating pathway signaling) failed to suppress the elongated morphology of the bud14 mutant. Genetic evidence did, however, suggest that the polarisome was required for the hyperpolarized growth observed in the bud14 mutant (Fig. 8). Thus, Bud14p may be an attenuator of polarized growth distinct from those previously characterized.

Bud14p localizes to sites in the cell where polarized growth occurs (this report and reference 58). For example, Bud14p is
localized to the distal pole of the cell, which would position it appropriately to impede polarized growth. The localization of Bud14p to the mother-bud neck may be an alternative way that Bud14p impinges on polarized growth, in that Bud14p may promote cytokinesis. Overproduction of Bud14p caused a defect in cytokinesis, and cytokinesis defects were observed when the bud14 mutation was combined with other mutations. Since overproduction of Bud14p did not affect the morphology of the septin ring itself, Bud14p may have a regulatory function in cytokinesis, as opposed to a role in septin ring biogenesis or stability. It is noteworthy that Bud14p has a putative SH3 domain. Such domains are known to mediate the assembly of large multiprotein complexes, including cell polarity complexes (53).

Glc7p and Bud14p share a function in polarized growth. The type 1 protein phosphatase Glc7p has previously been implicated in polarization of the actin cytoskeleton and morphogenesis (3), and evidence herein suggests that Glc7p may influence polarized growth by a mechanism involving Bud14p (Fig. 8). Disruption of BUD14 in strains containing partial loss-of-function alleles of glc7 caused a morphological defect more severe than either single mutation, and the morphological abnormalities caused by overexpression of BUD14 were partly suppressed by glc7 alleles. Glc7p interacts with Bud14p, and it is plausible that Glc7p dephosphorylates Bud14p to modulate its function. Whether Bud14p is a target of Glc7p or is otherwise involved in Glc7p function remains to be determined, but Bud14p does share other functions with Glc7p, including glycogen accumulation (unpublished results) and growth under vegetative conditions. Indeed, Bud14p and Glc7p both localize to the mother-bud neck (this report and references 7 and 58), suggesting that Glc7p may play a regulatory role in cytokinesis during vegetative growth. Glc7p is known to be required for septin organization during meiosis (86).

Finally, we showed that Glc7p has a role in filamentous growth. Glc7p appears to have a positive role in filamentous growth, whereas Bud14p has a negative role (Fig. 8). Although these proteins seem to have functionally antagonistic roles in filamentous growth, Bud14p and Glc7p both attenuate polarized growth (Fig. 8). That Bud14p and Glc7p apparently function antagonistically in some settings and in concert in other settings is an intriguing puzzle. Resolution of this puzzle will require identification of interacting proteins and an examination of their functions.

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REFERENCES

1. Ahn, S. H., A. Acuño, and S. J. Kron. 1999. Regulation of G2/M progression by the STE11 mitogen-activated protein kinase pathway in budding yeast filamentous growth. Mol. Biol. Cell 10:3301–3316.

2. Amberg, D. C., J. E. Zahnber, J. W. Mulhallon, J. R. Pringle, and D. Botstein. 1997. Aip3p/Bud6p, a yeast actin-interacting protein that is involved in morphogenesis and the selection of bipolar budding sites. Mol. Biol. Cell 8:279–273.

3. Andrews, P. D., and M. J. Stark. 1995. A role for the Swe1 checkpoint kinase in the cell cycle of budding yeast Saccharomyces cerevisiae. J. Cell Sci. 118:507–520.

4. Baker, S. H., D. L. Frederick, A. Bloecher, and K. Tatchell. 1999. Aly1p, a gene that regulates fungal development and virulence. FEMS Microbiol. Rev. 23:621–626.

5. Bloecher, A., and K. Tatchell. 1999. Defects in Saccharomyces cerevisiae phenotype type I activate the spindle/kinetochore checkpoint. Genes Dev. 13:517–522.

6. Butty, A. C., and K. Tatchell. 2000. Dynamic localization of protein phosphatase type I in the mitotic cell cycle of Saccharomyces cerevisiae. J. Cell Biol. 149:125–140.

7. Butty, A. C., P. M. Pryciak, L. S. Huang, I. Herskowitz, and M. Peter. 1998. The role of Arp1p in linking the heterotrimeric G protein to polarity establishment proteins during yeast mating. Science 282:1511–1515.

8. Butty, A. C., N. Perrinjaquet, A. Petit, M. Jaquenoud, J. E. Segall, K. Hofmann, C. Zawahlen, and M. Peter. 2002. A positive feedback loop stabilizes the guanine-nucleotide exchange factor Cdc42 at sites of polarization. EMBO J. 21:1565–1576.

9. Chant, J., and J. R. Pringle. 1995. Patterns of bud-site selection in the yeast Saccharomyces cerevisiae. J. Cell Biol. 129:791–756.

10. Chant, J. 1999. Cell polarity in yeast. Annu. Rev. Cell Dev. Biol. 15:365–391.

11. Chenevert, J., N. Valtz, and I. Herskowitz. 1999. Studies of the SH3-domain protein Bem1p with proteins of the MAP kinase cascade and actin. Science 287:1204.

12. Cid, V. J., M. Jaquenoud, M. P. Gulli, J. Chant, and M. Peter. 1997. Novel Cdc42-binding proteins Cdc16 and Gic2 control cell polarity in yeast. Genes Dev. 11:2972–2982.

13. Cullen, J. L., M. A. McEachern, and J. Thorner. 1995. Afr1 promotes polarized morphogenesis in Saccharomyces cerevisiae. Genetics 145:845–862.

14. Cullen, J. L., A. M. Preto-Evangelista, M., D. Pruyne, D. C. Amberg, C. Boone, and A. Bretscher. 2002. Formins directly interact with Aip3p/Bud6p to assemble polarized actin cell growth in yeast. Nat. Cell Biol. 4:32–41.

15. Fujitani, T., K. Kominami, S. Nishida, Y. Takai, and Y. Tanaka. 1998. Phosphorylation of Bni1p by Cdc42 and Spac2p: implication in localization of Bni1p at the bud site and regulation of the actin cytoskeleton in Saccharomyces cerevisiae. Mol. Biol. Cell 9:1221–1233.

16. Gagnon, P., L. V. Loeb, J. D. Kerentseva, T. Pan, M. Sepulveda-Becerra, and H. Liu. 1999. Saccharomyces cerevisiae G1 cyclins are differentially involved in invasive and pseudohyphal growth independent of the filamentation mitogen-activated protein kinase pathway. Genetics 153:1535–1546.

17. Giot, L., and J. B. Konopka. 1997. Functional analysis of the interaction between Arf1p and the Cdc12p septin, two proteins involved in morphogenesis and cell cycle progression. Genetics 145:845–862.

18. Givan, S. A., and G. F. Sprague, Jr. 1997. The ankyrin repeat-containing protein Ack1p is required for the endocytosis of yeast pheromone receptors. Mol. Biol. Cell 8:1317–1327.

19. Herskowitz, I. 1997. Building organs and organisms: elements of morphogenesis exhibited by budding yeast. Cold Spring Harbor Symp. Quant. Biol. 62:57–63.

20. Herskowitz, I. 1997. Building organs and organisms: elements of morphogenesis exhibited by budding yeast. Cold Spring Harbor Symp. Quant. Biol. 62:57–63.

21. Loeb, J. D., T. A. Kerentseva, T. Pan, M. Sepulveda-Becerra, and H. Liu. 1999. Saccharomyces cerevisiae G1 cyclins are differentially involved in invasive and pseudohyphal growth independent of the filamentation mitogen-activated protein kinase pathway. Genetics 153:1535–1546.

22. Lu, M. A. McEachern, and J. Thorner. 1999. Studies of the SH3-domain protein Bem1p with proteins of the MAP kinase cascade and actin. Science 287:1204.

23. Liu, H., C. A. Styles, and G. R. Fink. 1993. Elements of the yeast pheromone response pathway required for filamentous growth of diploids. Science 262:1744–1744.

24. Loeb, J. D., T. A. Kerentseva, T. Pan, M. Sepulveda-Becerra, and H. Liu. 1999. Saccharomyces cerevisiae G1 cyclins are differentially involved in invasive and pseudohyphal growth independent of the filamentation mitogen-activated protein kinase pathway. Genetics 153:1535–1546.

25. Giot, L., and J. B. Konopka. 1997. Functional analysis of the interaction between Arf1p and the Cdc12p septin, two proteins involved in morphogenesis and cell cycle progression. Genetics 145:845–862.

26. Givan, S. A., and G. F. Sprague, Jr. 1997. The ankyrin repeat-containing protein Ack1p is required for the endocytosis of yeast pheromone receptors. Mol. Biol. Cell 8:1317–1327.

27. Herskowitz, I. 1997. Building organs and organisms: elements of morphogenesis exhibited by budding yeast. Cold Spring Harbor Symp. Quant. Biol. 62:57–63.

28. Herskowitz, I. 1997. Building organs and organisms: elements of morphogenesis exhibited by budding yeast. Cold Spring Harbor Symp. Quant. Biol. 62:57–63.

29. Herskowitz, I. 1997. Building organs and organisms: elements of morphogenesis exhibited by budding yeast. Cold Spring Harbor Symp. Quant. Biol. 62:57–63.

30. Herskowitz, I. 1997. Building organs and organisms: elements of morphogenesis exhibited by budding yeast. Cold Spring Harbor Symp. Quant. Biol. 62:57–63.

31. Loeb, J. D., T. A. Kerentseva, T. Pan, M. Sepulveda-Becerra, and H. Liu. 1999. Saccharomyces cerevisiae G1 cyclins are differentially involved in invasive and pseudohyphal growth independent of the filamentation mitogen-activated protein kinase pathway. Genetics 153:1535–1546.

32. Giot, L., and J. B. Konopka. 1997. Functional analysis of the interaction between Arf1p and the Cdc12p septin, two proteins involved in morphogenesis and cell cycle progression. Genetics 145:845–862.

33. Givan, S. A., and G. F. Sprague, Jr. 1997. The ankyrin repeat-containing protein Ack1p is required for the endocytosis of yeast pheromone receptors. Mol. Biol. Cell 8:1317–1327.

34. Herskowitz, I. 1997. Building organs and organisms: elements of morphogenesis exhibited by budding yeast. Cold Spring Harbor Symp. Quant. Biol. 62:57–63.

35. Givan, S. A., and G. F. Sprague, Jr. 1997. The ankyrin repeat-containing protein Ack1p is required for the endocytosis of yeast pheromone receptors. Mol. Biol. Cell 8:1317–1327.
