A Genomic Study of the Bipolar Bud Site Selection Pattern in *Saccharomyces cerevisiae*

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A genome-wide screen of 4168 homozygous diploid yeast deletion strains has been performed to identify nonessential genes that participate in the bipolar budding pattern. By examining bud scar patterns representing the sites of previous cell divisions, 127 mutants representing three different phenotypes were found: unipolar, axial-like, and random. From this screen, 11 functional classes of known genes were identified, including those involved in actin-cytoskeleton organization, general bud site selection, cell polarity, vesicular transport, cell wall synthesis, protein modification, transcription, nuclear function, translation, and other functions. Four characterized genes that were not known previously to participate in bud site selection were also found to be important for the haploid axial budding pattern. In addition to known genes, we found 22 novel genes (20 are designated *BUD13-BUD32*) important for bud site selection. Deletion of one resulted in unipolar budding exclusively from the proximal pole, suggesting that this gene plays an important role in diploid distal budding. Mutations in 20 other novel *BUD* genes produced a random budding phenotype and one produced an axial-like budding defect. Several of the novel Bud proteins were fused to green fluorescence protein; two proteins were found to localize to sites of polarized cell growth (i.e., the bud tip in small budded cells and the neck in cells undergoing cytokinesis), similar to that postulated for the bipolar signals and proteins that target cell division site tags to their proper location in the cell. Four others localized to the nucleus, suggesting that they play a role in gene expression. The bipolar distal marker Bud8 was localized in a number of mutants; many showed an altered Bud8-green fluorescence protein localization pattern. Through the genome-wide identification and analysis of different mutants involved in bipolar bud site selection, an integrated pathway for this process is presented in which proximal and distal bud site selection tags are synthesized and localized at their appropriate poles, thereby directing growth at those sites. Genome-wide screens of defined collections of mutants hold significant promise for dissecting many biological processes in yeast.

INTRODUCTION

Polarized cell division is a fundamental process in which cells divide along specific cleavage planes; this process is essential for the development of both eukaryotes and prokaryotes. Polarized cell divisions can mediate appropriate cell-cell contacts and partition cytoplasmic components asymmetrically between daughter cells. Polarized cell divisions occur during the life cycle of many organisms, including early embryogenesis in *Caenorhabditis elegans* (Hyman and White, 1987), neurogenesis in *Drosophila* and mammals (Kraut *et al.*, 1996), spore development in *Bacillus subtilus* (Shapiro, 1993), and development of the snail body plan (Freeman and Lundelius, 1982). The mechanisms for selecting sites for polarized growth and division as well as for directing growth toward these sites are only beginning to be understood.

The budding yeast *Saccharomyces cerevisiae* is an excellent model for studying polarized cell division. *S. cerevisiae* exists in different forms, each with a specific cell morphology and division pattern (Roemer *et al.*, 1996b; Costigan and Snyder, 1998; Madden and Snyder, 1998). During growth in rich media, both haploid and diploid yeast cells are ellipsoid and select bud sites according to their mating locus (*MAT*) and pedigree (Freifelder, 1960; Snyder, 1989; Chant and Pringle, 1995). Haploid *MATa* and *MATα* cells use an axial budding pattern in which cells bud adjacent to the preceding site of cytokinesis (i.e., the proximal poles). Diploid *MATa/MATα* cells exhibit a more complex bipolar budding pattern: daughter cells bud at distal poles (180° from the birth site), whereas mother cells bud either distal or proximal to the birth site, with new mothers exhibiting a bias for budding at

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the distal site (i.e., near the previous site of cytokinesis). The different budding patterns are thought to fulfill different purposes. The diploid bipolar pattern is thought to maximize exposure of a growing microcolony to nutrients (Gimeno and Fink, 1992; Gimeno et al., 1992; Madden et al., 1992); in contrast, the haploid pattern is thought to position cells in a microcolony derived from a homothallic cell that switches its mating type close to one another to facilitate mating and diploid formation (Nasmyth, 1982). It is thought that cortical tags mark future bud sites and serve as spatial cues for the initiation of new buds (Chant et al., 1991; Snyder et al., 1991; Flescher et al., 1993; Chant and Pringle, 1995).

Three distinct classes of proteins important for bud site selection have been identified (Snyder, 1989; Chant and Herskowitz, 1991). The first class is specifically required for the axial pattern. Mutations in genes encoding any of these proteins result in bipolar budding in haploid cells, but they do not disrupt the diploid budding pattern. It is thought that these genes are important for tagging the axial pole and/or recognizing the axial tag (Chant and Herskowitz, 1991; Flescher et al., 1993; Fugita et al., 1994; Chant et al., 1995; Halme et al., 1996; Roemer et al., 1996a; Sanders and Herskowitz, 1996). The second class is specifically required for the bipolar pattern. It is likely that these proteins are involved in marking the poles of diploid cells at sites for budding or helping direct those tags to their appropriate location (Snyder, 1989; Bauer et al., 1993; Zahner et al., 1996; Chen et al., 2000; Sheu et al., 2000). The third class is required for both the axial and bipolar patterns. These gene products are believed to recognize the axial or bipolar tags provided by the first two classes of gene products and convey this information to the machinery involved in establishing cell growth (Bender and Pringle, 1989; Chant and Herskowitz, 1991; Chant et al., 1991; Park et al., 1993). Although much is known about the molecular mechanisms that help mediate the axial pattern of budding, much less is known concerning the mechanisms of the bipolar pattern.

A number of components important for bipolar bud site selection have been identified and characterized (Herskowitz et al., 1995; Pringle et al., 1995; Drubin and Nelson, 1996; Costigan and Snyder, 1998;). Three genes, BUD8, BUD9, and STE20, when mutated, cause diploid cells to form buds at one pole (Zahner et al., 1996; Sheu et al., 2000). bud8Δ/bud8Δ and ste20Δ/ste20Δ cells bud at the proximal pole of the daughter cell, whereas bud9Δ/bud9Δ cells bud at the distal pole. Bud8 and Bud9 have been proposed to act as bipolar landmarks or tags that recruit components involved in bud formation (Zahner et al., 1996). Consistent with this hypothesis, Bud8 localizes at the distal pole; two different localizations of Bud9 have been reported, one at the distal pole (where it would most likely be a repressor of bud formation) and the other at the proximal poles (where it might serve as a tag) (Taheri et al., 2000; Harkins et al., 2001). Ste20 is a PAK protein kinase homologue that has been shown to lie in the same genetic pathway as Bud8 (Sheu et al., 2000). How tags are produced, processed, and localized to the poles has not been investigated.

The actin cytoskeleton is essential for both polarized growth and bipolar bud site selection and is thought to mediate directional transport of secretory vesicles (Adams and Pringle, 1984; Kilmartin and Adams, 1984; Novick and Botstein, 1985; Mulholland et al., 1994; Drubin and Nelson,

1996; Pruyne et al., 1998). Both cortical actin patches and components of the secretory apparatus localize to sites of growth (Adams and Pringle, 1984; Kilmartin and Adams, 1984; Finger and Novick, 1998). Mutations in genes encoding actin, several actin-associated proteins, or proteins involved in actin organization cause defects in bipolar budding similar to those of act1 cells (Drubin et al., 1990; Adams et al., 1991; Crouzet et al., 1991; Vojtek et al., 1991; Bauer et al., 1993; Holtzman et al., 1993; Sivadon et al., 1995; Zahner et al., 1996).

A variety of other nonessential polarity proteins are also required for bipolar bud site selection. Deletion of SPA2, PEA2, BUD6, or BNI1 cause a random budding defect. Spa2, Pea2, Bud6, and Bni1 form a complex, the 12S polarisome, that localizes to sites of polarized growth (Fujiwara et al., 1998; Sheu et al., 1998); these proteins are present as a patch at the incipient bud site, at the tip of the growing bud, and at the mother-bud neck region before cytokinesis (Snyder, 1989; Gehrung and Snyder, 1990; Snyder et al., 1991; Kohno et al., 1996; Valtz and Herskowitz, 1996; Zahner et al., 1996; Amberg et al., 1997; Evangelista et al., 1997). Spa2p, Pea2p, Bud6p, and Bni1p are each required for apical growth (Sheu et al., 2000), which is the initial phase of bud growth in which cells grow at the bud tip (Lew and Reed, 1993). It has been suggested that these proteins are required for the polarized deposition of the distal tag during this period (Sheu et al., 2000).

Understanding bipolar bud site selection and identifying the molecules that participate in this process will provide insight into the molecular mechanisms of how this process is carried out and coordinated with other cellular events. Recently, a set of diploid strains containing homozygous deletions of nonessential yeast open reading frames (ORFs) was generated; this set allows a genome-wide investigation into the bipolar bud site selection (Winzeler et al., 1999). Here we report a genome-wide systematic screening of 4168 homozygous diploid mutants (affecting 84% of the total nonessential annotated ORFs) for those defective in the bipolar budding pattern. These studies led to the identification of 127 genes involved in this process, of which only 16 have been reported to affect bipolar budding previously. Based on the classes of mutants identified and the subsequent analysis of these mutants, we have identified a variety of cellular processes involved in the bipolar pattern and present a global model of how this process occurs.

MATERIALS AND METHODS

Yeast Strains and Media

All strains used in this study are congenic derivatives of FY2, which is itself a direct descendant of S288C (Brachmann et al., 1998). Most of the strains contain precise deletions in which the entire ORF after the ATG is replaced with a kanMX4 marker. Two independent transformants were prepared and MAT**a** and MATα segregants were recovered from each transformant and mated to form the homozygous diploid strains (Winzeler et al., 1999). The different strains are available from Research Genetics (Huntsville, AL). Growth media and genetic manipulation have been described previously (Sherman et al., 1986; Guthrie and Fink, 1991).

Budding Pattern Analysis

Mutant strains were grown individually in 0.8 ml of liquid YPAD in 96-well format boxes (Dot Scientific, Burton, MI) and incubated at

30°C with shaking. Each well of the box contained one 3.5-mm glass bead to facilitate mixing. Cells were first grown to stationary phase and then diluted into another 96-well box with glass beads; cells were grown for at least six generations until mid-log-phase. Mid-log–phase cells were then fixed by the addition of formaldehyde to a final concentration of 3.7%. Cells were subsequently incubated for 1 h at 30°C, washed with phosphate-buffered saline, and resuspended in phosphate-buffered saline. Calcofluor White (Sigma, St. Louis, MO) was added to a final concentration of 2 μg/ml, bud scars and birth scars were visualized by fluorescence microscopy. Birth scars are chitin-depleted structures that mark the region where mother cells separated from their daughter cells. A bud site is defined as proximal, distal, or medial depending on whether the bud or bud scar resides in the one-third portion of the cell closest to the birth scar, the one-third of the cell opposite the birth scar, or the middle-third of the cell, respectively (Flescher et al., 1993). Bud site selection mutants were identified and retested. All of the unipolar and axial-like mutants were tested for mating type and appropriate auxotrophic markers and examined for cell size to ascertain whether they are diploid or haploid. Twenty-eight strains were found to be haploids. Only mutants that reproducibly exhibited a budding pattern defect and were not haploids were chosen for further analysis.

To quantify the fidelity of bipolar bud site selection, only cells that had experienced at least three budding events were examined. Cells with bud scars exclusively at one pole were classified as unipolar; cells with medial bud scars were scored as random budding. The percentage of random budding cells was then determined for each sample. For each sample, 100 cells were counted from at least four independent fields. For some mutant strains, bud site selection was scored for the first, second, and third bud divisions with the use of the criteria described (Flescher et al., 1993; Zahner et al., 1996). For these analyses a total of 200–600 cells from each sample were scored for each division type.

To determine whether the genes required for the bipolar pattern are also important for the haploid axial pattern, 104 *MATa* deletion strains were examined by Calcofluor staining. For the genes whose loss in diploids caused a unipolar pattern, both *MATa* and *MATα* strains were examined.

Examination of Cell Morphology

The cells used for morphological analysis were grown as described above. Cells were examined by phase-contrast and differential interference contrast microscopy, and mutants were classified as described in RESULTS.

Construction of GFP-tagged Yeast Strain

GFP-tagged fusion proteins were constructed in *MATa* and *MATα* haploid wild-type yeast strains with the use of the strategy described by Schneider et al. (1995). Primers were designed with the use of the N-terminal coding sequence of the gene of interest and used to amplify a region of the plasmid pMPY-GFP (a generous gift from Jun-Yi Leu). The resulting ~2.0-kb polymerase chain reaction (PCR) product contains the complete *URA3* gene flanked by direct repeats encoding the GFP gene; the entire cassette is flanked by 45 bp of the gene sequence. These DNA fragments were transformed into haploid yeast, and transformants that had integrated the fragment correctly at the chromosomal locus were identified by PCR analysis. The integrants were allowed to grow overnight in YPAD and plated onto medium containing 5-fluoroorotic acid; resistant colonies that had lost the *URA3* gene through homologous recombination between the two GFP-coding sequences were identified by PCR. This recombination event leaves a single in-frame GFP-coding sequence after the ATG codon. *MATa* and *MATα* strains containing the in-frame GFP cassette were then mated, and homozygous tagged diploid strains were analyzed to determined whether the GFP allele was functional. Homozygous ORF-GFP/ORF-GFP dip-

loid strains were examined for growth rate and bipolar budding pattern defects.

GFP-Fluorescence Microscopy

Yeast strains harboring the GFP-Bud8 plasmid or strains with GFP-tagged Bud proteins were grown in synthetic complete medium lacking particular nutrients or synthetic complete medium to mid-log–phase. Cells from 3-ml cultures were harvested and immediately viewed with the use of a fluorescence microscope (Leica, Deerfield, IL) equipped with a GFP filter set (41017, Endow GFP, Chroma Technology, Brattleboro, VT). For similar experiments, all images were processed identically with the use of Photoshop 5.0 (Adobe Systems, Mountain View, CA).

RESULTS

Identification of Genes Important for Bipolar Budding in Yeast

To identify mutants that specifically affect the bipolar budding pattern, we examined 4168 homozygous diploid deletion strains disrupted in different nonessential ORFs by staining with Calcofluor White. Calcofluor binds the chitin-rich bud scars that remain on the cell surface after cytokinesis and thus serve as markers of sites of previous cell divisions (Hayashibe and Katohda, 1973). Yeast cells were grown to mid-log phase, fixed, and stained with Calcofluor, and the budding pattern and cell morphology of each strain were examined. Wild-type diploid yeast cells undergo bipolar budding and bud scars are usually found at both poles of the cell (Figure 1). We identified 127 mutants that reproducibly displayed altered budding patterns; these mutants fell into three categories: unipolar, axial-like, and random (Table 1). In the unipolar mutants, bud scars lay at either the proximal or the distal pole of the cell. In the axial-like mutants, cells displayed chains of adjacent bud scars reminiscent of those observed for haploid cells; the bud scars in these classes usually initiated at the proximal pole but sometimes occurred in the equatorial region or at the distal pole. In the random mutants, bud scars appeared distributed over the entire surface of the cells. Of the 127 mutants identified, 105 are disrupted for genes that have been previously characterized; 22 affected uncharacterized ORFs. Twenty of the new genes were named *BUD13-BUD32* (Tables 2-4).

Unipolar Mutants

Ten mutants were found that exhibited a unipolar budding pattern (Table 2). Seven of the mutants have a strong phenotype in which >70% of the cells exhibited a unipolar budding pattern when cells with more than three bud scars were scored. Three exhibit a partial phenotype in which 50–70% of the cells exhibit the defect (Table 2).

BUD8, BUD9, and STE20. Of the 10 mutants three, *bud8Δ/bud8Δ*, *bud9Δ/bud9Δ*, and *ste20/ste20Δ*, were known previously to exhibit a unipolar budding pattern (Zahner et al., 1996; Sheu et al., 2000; see INTRODUCTION). *bud8Δ/bud8Δ* and *ste20Δ/ste20Δ* cells bud at the proximal pole, whereas *bud9Δ/bud9Δ* mutants bud at the distal pole.

IST3 and BUD13/YGL174w. *IST3* is a gene that has been described to have a role in mediating sodium tolerance;
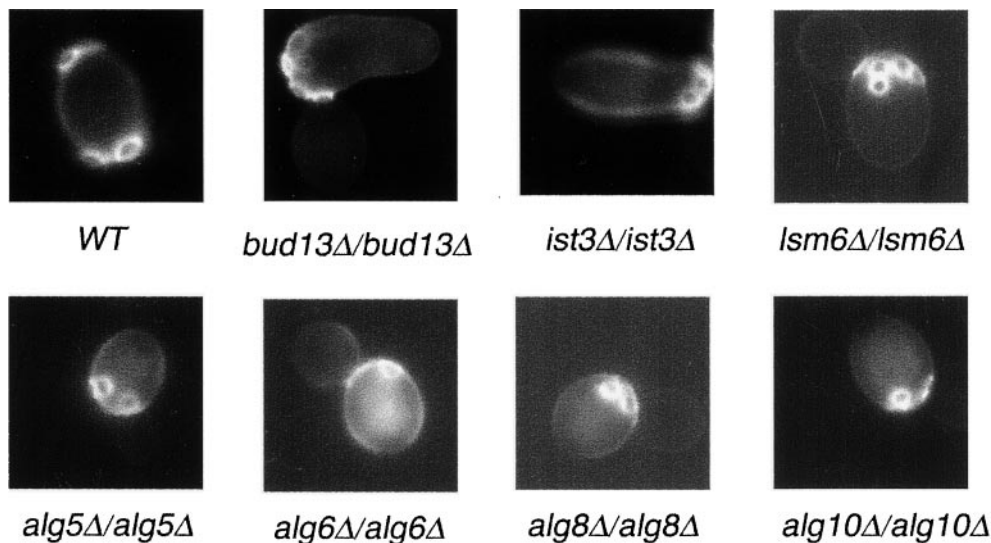
Figure 1. The budding pattern of diploid wild-type (*WT*) and unipolar mutant strains. Cells of the indicated strains were stained with Calcofluor to visualize bud scars.

however, its molecular role is not known (Entian *et al.*, 1999). *YGL174w* is an unknown gene that we named *BUD13*. These two proteins were recently reported to interact in vivo by the yeast two-hybrid assay (Uetz *et al.*, 2000). Mutants lacking these genes display a unipolar budding pattern, with bud scars clustered adjacent to the birth scar at the proximal pole (Figure 1). In these mutants, individual bud sites can be observed adjacent to, overlapping, or within the birth scar and in the vicinity of other bud scars; however, these bud scars are not always immediately adjacent to the bud site of the previous cell cycle and are more loosely distributed in the polar region. This distribution is typical of the bipolar pattern at the proximal pole and is distinct from the axial budding pattern used by wild-type haploid cells. In addition, *ist3Δ/ist3Δ* and *ygl174wΔ/ygl174wΔ* exhibit elongated cell shapes; this phenotype is distinct from *bud8Δ/bud8Δ* and *ste20Δ/ste20Δ* mutants, which are round and exhibit an apical growth defect (Sheu *et al.*, 2000).

ALG5, ALG6, ALG8, and ALG10. *ALG5, ALG6, ALG8,* and *ALG10* are a group of genes whose products function early in the yeast dolichol pathway that synthesizes the dolichol-linked oligosaccharide precursor for *N*-linked protein glycosylation (Heesen *et al.*, 1994; Stagljar *et al.*, 1994; Reiss *et al.*, 1996; Burda and Aebi, 1998). *ALG5* encodes a dolichol-P-glucose synthetase, and *ALG6, ALG8,* and *ALG10* are genes encoding glucosyltransferases that, respectively, transfer the first, second, and third α-1,3-linked glucose to Dol-PP-GlcNac2Man9 in the final modification of outer chain elongation of *N*-linked oligosaccharides. Mutations of these genes block the addition of glucose residues to the lipid-linked oligosaccharide, but the nonglucosylated oligosaccharides are still transferred to proteins (Huffaker and Robbins, 1983). Homozygous deletion strains lacking *ALG5, ALG6, ALG8,* or *ALG10* have a round cell shape and a partial unipolar distal budding pattern (74, 85, 56, and 53%, respectively) (Table 2; Figure 1). These results indicate that *N*-linked glycosylation is required for proper bud site selection.

LSM6. Lsm6 is a member of the Sm-like group of proteins and has been implicated in mRNA decay and the function of the U6 snRNP (small nuclear ribonucleoprotein particles), which is involved in mRNA splicing (Tharun *et al.*, 2000). Deletion of *LSM6* causes most cells to undergo unipolar distal budding (69.5% distal budding; 23.4% bipolar budding; 7.1% random budding; Figure 1). Interestingly, deletion of *LSM1*, which only affects mRNA decay and not U6 function (Tharun *et al.*, 2000), causes a random budding phenotype (see below). Presumably, Lsm6 regulates the stability or splicing of an RNA transcript important for mediating the bipolar budding pattern, such as Bud8.

Axial-like Mutants

Five mutants were identified that exhibit an axial-like budding pattern (Table 3). This pattern differs from the unipolar pattern in that the cells had long chains of bud scars, very similar to those seen on axial budding cells (Chant and Pringle, 1995), indicating that new buds formed next to the previous site, rather than simply clustering at one end of the cell. However, in contrast to normal axially budding cells, these mutants produced chains of bud sites starting at either the distal pole or the proximal pole, as well as in the equatorial region, although there was a strong bias in most cases for one end of the chain to originate from the proximal pole. The bud scar chains can be very long and sometimes extend from one pole to another.

BUD7. *BUD7* was previously identified in a visual screen for mutants with a defect in the bipolar budding pattern; it was described as having a heterogeneous budding pattern phenotype (Zahner *et al.*, 1996). We found that *bud7Δ/bud7Δ* cells exhibit an axial-like phenotype and that 75% of the deletion mutants use the proximal pole for the first two divisions (Figure 2A).

RAX2. Chen *et al.* (2000) recently reported that *RAX2* is required for the maintenance of the bipolar budding pattern,

Table 1. Budding pattern mutants detected during this screen

Phenotype	No.	%
Unipolar	10	0.24
Axial-like	5	0.14
Random		
Strong	74	1.78
Mild	38	0.91
Total mutants	127	
Total strains	4168	3.07

Table 3. Homozygous diploid deletion strains with axial-like budding pattern

Gene (ORF)	First bud proximal (%)	Morphological phenotype	MAT**a** budding pattern
BUD7	93	WT	WT
RAX2	87	WT	WT
RAX1	80	WT	WT
ISY1	81	WT	WT
YOR300w[a]	97	WT	WT

[a] Overlaps with BUD7.

and their published data suggest a random budding pattern. We independently found that rax2Δ/rax2Δ mutants are defective in bipolar budding, but the divisions are usually in an axial-like budding pattern (Figure 2A). Furthermore, we find that 78% of the first three budding events occurred at the proximal pole (Ni and Snyder, unpublished result). Further analysis of rax2Δ mutants is described below.

RAX1. Previous researchers have found that mutation of RAX1 converts the bipolar budding pattern of axl1Δ haploids into an axial budding pattern (Fujita et al., 1994), but the phenotype of rax1Δ/rax1Δ cells has not been described. We found that homozygous rax1Δ/rax1Δ cells bud in an axial-like pattern (Figure 2A); in their first two divisions they exhibit a strong bias to use the proximal pole.

ISY1 and YOR300w. Two other proteins were identified that exhibit an axial budding pattern (Figure 2A). Isy1p is a pre-mRNA–splicing factor, required for optimal splicing in vivo (Dix et al., 1999). YOR300w is an unknown gene. Quantitative analysis of the first three budding events in these mutants reveals that each possesses a bias to use the proximal pole during the first several cell cycles. yor300wΔ/yor300wΔ cells exhibit the most severe defect of all of the axial-like mutants; the cells often contain bud scars that span from one pole to the other (Figure 2). The analysis of YOR300w is complicated by the fact that the ORF overlaps BUD7 by 100 bp and lies immediately upstream of RAX1 (within 100 bp). Thus, the deletion removes carboxy-termi-nal coding sequence from BUD7 and might affect the expression of RAX1, thereby affecting two genes involved in the bipolar pattern. This combined defect might account for a phenotype that is more severe than either the bud7Δ/bud7Δ or rax1Δ/rax1Δ single mutants.

Overexpression of Bud8p in each of the axial budding mutants, rax1Δ/rax1Δ, rax2Δ/rax2Δ, bud7Δ/bud7Δ, isy1Δ/isy1Δ, and yor300wΔ/yor300wΔ, induces many of the first buds to form distally, similarly to wild-type cells (Figure 2B). However, the degree of suppression varies with the individual mutants, and Bud8p overexpression usually causes subsequent budding events to occur more randomly, indicating that Bud8p cannot completely compensate for the bipolar budding defect of these mutant strains. Bud8p-GFP localization appears normal in these mutants.

Random Budding Mutants

Seventy-four mutants were found to display a strong phenotype in which >50% of the cells budded randomly. An additional 38 mutants were found to exhibit a mild defect in which <50% of the cells budded randomly or sometimes random plus unipolar. The genes mutated in these strains were grouped into 11 functional categories (Table 4).

Ribosomal Proteins. The first category is ribosomal proteins. The yeast genome is predicted to encode 32 different small subunit proteins and 46 large subunit proteins. Genes

Table 2. Homozygous diploid deletion strains with unipolar budding pattern

Gene (ORF)	Budding pattern	Morphological phenotype[a]	Haploid budding pattern	Bud8p localization
BUD8	Proximal	Round 2	WT	WT
BUD9	Distal	WT	WT	WT
STE20	Proximal	WT	WT	WT
IST3	Proximal	Elongated 3	WT	Slightly reduced
BUD13/YGL174w	Proximal	Elongated 4	WT	Slightly reduced
ALG5	74% distal	Round 3	WT	More neck
ALG6	85% distal	Round 3	WT	More neck
ALG8	56% distal	Round 3	WT	More neck
ALG10	53% distal	Round 2	WT	More neck
LSM6	69.5% distal	WT	WT	Not tested

[a] Cells were scored from 1–4: 1, wild type (WT); 2, weak; 3, moderate; 4, exhibited a severe defect.

A

WT

bud7Δ/bud7Δ

rax2Δ/rax2Δ

rax1Δ/rax1Δ
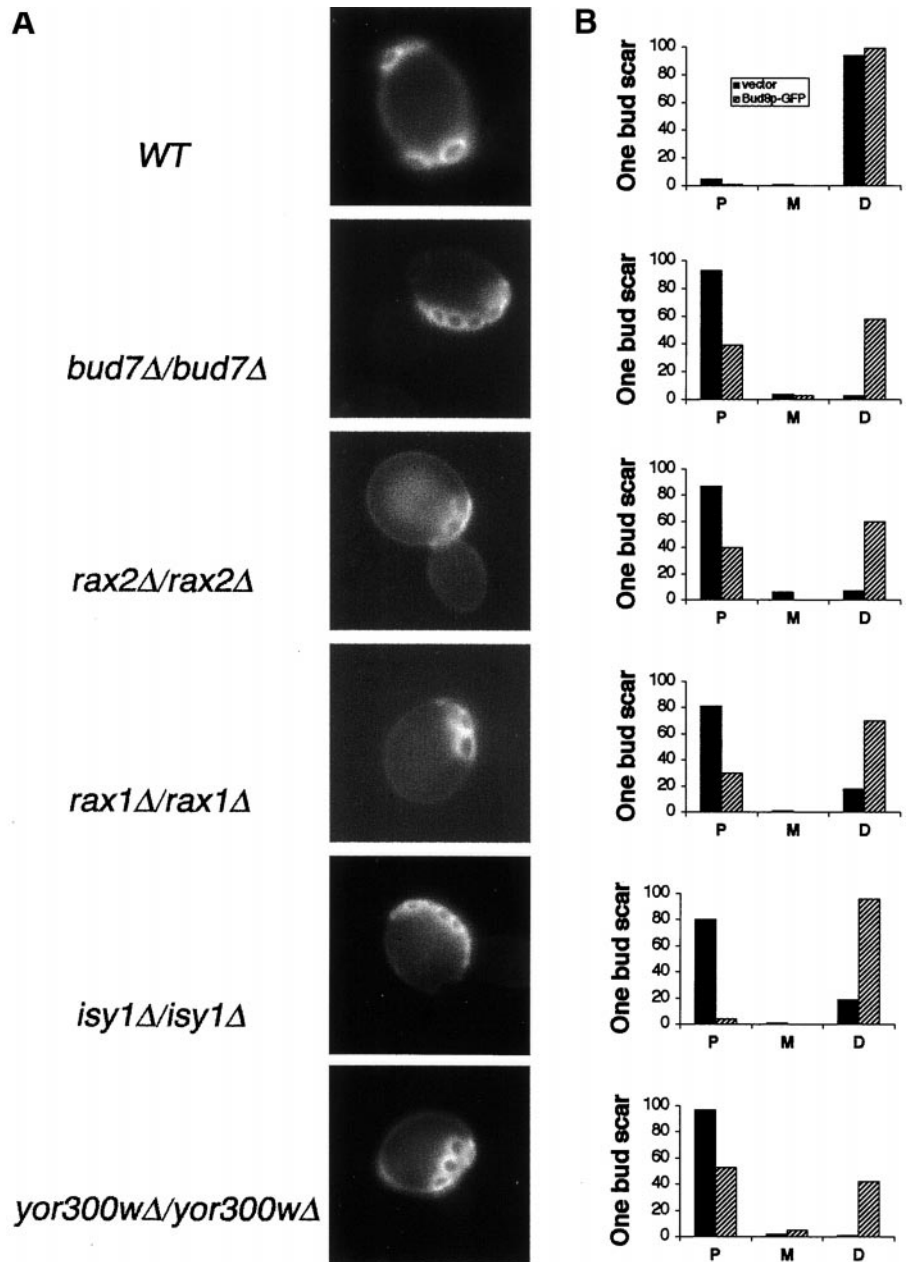
isy1Δ/isy1Δ

yor300wΔ/yor300wΔ

B

Figure 2. Budding pattern of axial-like diploid mutant strains. (A) Bud scar (Calcofluor) staining of wild-type (*WT*), *bud7Δ/ bud7Δ*, *rax2Δ/rax2Δ*, *rax1Δ/rax1Δ*, *isy1Δ/ isy1Δ*, and *yor300wΔ/yor300wΔ* cells. (B) Quantitative evaluation of first bud position in wild-type (WT) strains, axial-like mutants, and strains overexpressing Bud8. P,XXX; M, XXX; D, XXX. In all panels, >200 cells were counted.

for 59 of the ribosomal proteins are duplicated, resulting in 137 total ribosomal protein genes (Planta and Mager, 1998). We found that a number of the duplicated ribosomal protein genes are important for bud site selection (Table 4). *rps0bΔ/ rps0bΔ*, *rps7aΔ/rps7aΔ*, *rps27bΔ/rps27bΔ*, *rpl7aΔ/rpl7aΔ*, and *rpl22aΔ/rpl22aΔ* null mutants displayed a random budding pattern and reduced growth rate (Figure 3A). *rpl12bΔ/ rpl12bΔ*, *rps28bΔ/rps28bΔ*, *rps29aΔ/rps29aΔ*, *rps30aΔ/rps30aΔ*, *rps1bΔ/rps1bΔ, and rps18bΔ/rps18bΔ* strains exhibit a random budding pattern but grow similarly to wild-type cells; the percentage of cells exhibiting a random budding defect is reduced relative to the other strains. *rpl27aΔ/rpl27aΔ* has a reduced growth rate and also exhibits a weaker random

budding defect similar to the latter group of mutants. Interestingly, strains containing deletion mutations in the homologues of the affected genes (*rps0aΔ/rps0aΔ*, *rps7bΔ/rps7bΔ*, *rps27aΔ/rps27aΔ*, *rpl17bΔ/rpl17bΔ*, *rpl22bΔ/rpl22bΔ*, *rpl12aΔ/ rpl12aΔ*, *rps30bΔ/rps30bΔ*, *rps1aΔ/rps1aΔ*, *rps28aΔ/rps28aΔ*) have no obvious phenotype (i.e., budding pattern or growth rate) even though the affected proteins are often >90% identical. Thus, the duplicated copies of the ribosomal proteins are not equivalent; the copy affected in the first group is more important for the bud site selection than the copy mutated the latter group. These results suggest that inefficient translation of particular genes involved in bipolar budding affects bud site selection. Alternatively, because many

of the strains have a reduced growth rate, perhaps impaired growth of these particular strains results in a loss of bipolar budding.

Vesicular Transport Proteins. Mutants for 15 proteins involved in vesicular transport exhibited a random budding pattern (Table 4). Four proteins have been implicated in exocytosis. Ypt31 is a small GTPase that belongs to the Ypt/rab family that is involved in trans-Golgi vesicle formation and localizes to sites of polarized cell growth (Jedd *et al.*, 1997; Gerst, 1999). Bst1 was identified as a suppressor of the COPII gene *SEC13*, which is involved in transport from the endoplasmic reticulum to the Golgi (Elrod-Erickson and Kaiser, 1996). Sec22 and Snc2 are v-SNARE homologues involved in different steps of the secretory pathway; Sec22 is important for transport to the Golgi from the endoplasmic reticulum and Snc2 is important for late secretory steps (Woodman *et al.*, 1996; Spang and Schekman, 1998; Gerst, 1999). Loss of *SEC22* and *SNC2* in homozygous diploid deletion strains result in modest bud site selection defects. Yeast haploid strains deleted for any of these four genes are not affected in the axial pattern. These results indicate that Ypt31p and Bst1p are important for the transport of membrane-associated bipolar budding components. Possible candidates are Bud8, Bud9, and/or Rax2, which are predicted transmembrane proteins.

The End3 protein, which is required for endocytosis and actin organization, has been described previously to be involved in bipolar budding (Benedetti *et al.*, 1994). We also found that *end3Δ/end3Δ* mutants exhibit a random budding pattern. In addition, we found that Clc1 (Chu *et al.*, 1996), which is the clathrin light chain, is also important for bipolar budding: *clc1Δ/clc1Δ* mutants exhibit a random pattern (Figure 3B). These results indicate that endocytosis may play an important role in bud site selection. As described below, we speculate that endocytosis is important for maintaining the localization of bud site selection tags at the bud tip.

Finally, we found that mutations in a variety of other proteins involved in vacuolar targeting or function are also defective in bud site selection. These include the proteins Vps34, Vac7, Vam3, Vam8, Vma5, Luv1, Cos16, Vps45, and Cup5. Vam3 and Vam8 are t-SNAREs (Preston *et al.*, 1991; Rieder and Emr, 1997; Ungermann *et al.*, 1999); Vma5 and Cup5 are vacuolar ATPases (Nelson *et al.*, 1989; Bauerle *et al.*, 1993). Cos16 is a vacuolar associated protein that affects ion homeostasis (Paidhungat and Garrett, 1998). Vps45 is a protein of the Sec1p family essential for vacuolar protein sorting (Bryant *et al.*, 1998), and Vps34 is a phosphatidylinositol 3-kinase required for vacuolar protein sorting (De Camilli *et al.*, 1996). These proteins might affect late trans-Golgi network sorting and thereby affect bud site tag targeting. Consistent with this possibility, many of these mutants have been described previously to be defective in endocytosis and/or exocytosis (Gammie *et al.*, 1995; Rieder and Emr, 1997; Bryant *et al.*, 1998; Conboy and Cyert, 2000).

Actin Cytoskeleton. The actin cytoskeleton is essential for both polarized growth and bud site selection (Drubin, 1990; Adams and Pringle, 1991; Adams *et al.*, 1991; Crouzet *et al.*, 1991; Vojtek *et al.*, 1991; Bauer *et al.*, 1993; Holtzman *et al.*, 1993; Zahner *et al.*, 1996). We identified three mutants with defects in the actin cytoskeleton, *rvs167Δ/rvs167Δ*, *sla1Δ/*

sla1Δ, and *rvs161Δ/rvs161Δ*, that have defects in bipolar budding similar to several *act1* mutants; these had been known previously to be important for bud site selection. In addition to the cytoskeletal components that were previously shown to be important for bud site selection, we found that yeast strains mutant for two additional proteins involved in actin organization, Cap1 and Yke2, are defective in bud site selection in diploid cells (Figure 3C). Cap1 is the α subunit of actin-capping protein. It colocalizes with cortical actin patches at the site of bud emergence and at the tips of growing buds and shmoos; it does not colocalize with actin cables or with actin rings at the site of cytokinesis (Amatruda and Cooper, 1992). Careful analysis of budding patterns revealed that diploid *cap1Δ/cap1Δ* mutants bud randomly both in the first division and in subsequent divisions (first bud: distal 29.7%; media 32.4%; proximal 29.7%; n = 200), similar to yeast *bni1* mutants. Thus, in addition to its role in bipolar budding of mother cells, Cap1 might play an important role in the establishment of the distal bud tag in the daughter cell. Yke2 encodes prefoldin subunit 6, a component of the Gim protein complex that promotes formation of functional α-tubulin, γ-tubulin, and actin (Geissler *et al.*, 1998; Siegers *et al.*, 1999); mutation of *YKE2* causes random budding in diploids.

We also found that *mdm20Δ/dec1Δ* mutants exhibit a random budding defect. *mdm20Δ* mutants lack actin cables (Hermann *et al.*, 1997). They also are defective in mitochondrial movement and exhibit a reduced transfer of mitochondria into buds, which are actin-dependent processes (Boldogh *et al.*, 1998). Thus, the random budding defect of *mdm20Δ/mdm20Δ* cells is likely due to a defect in actin organization.

The random budding defect of the different mutants that affect bud site selection and actin organization indicates that the actin cytoskeleton plays an important role in this process. The actin cytoskeleton at the incipient bud site and at the neck might help to localize cortical bud site selection tags to proximal and distal sites and/or maintain them properly at those sites; alternatively, actin filaments might be important for transport of bud site selection or polarity establishment components to these tags.

Bud Site Selection and Cell Polarity Proteins. Seven deletion mutants affect bud site selection and cell polarity components (Table 4; Figure 3D). Five strains lack genes known to regulate bipolar budding pattern: *RSR1, BUD2, SPA2, BUD6, BNI1*; mutation of these genes cause random budding either in all cell divisions or after the first division (Snyder, 1989; Ruggieri *et al.*, 1992; Park *et al.*, 1993; Kohno *et al.*, 1996; Zahner *et al.*, 1996). *ROM2* and *BEM4* had not been reported previously to be involved in bipolar budding. Rom2 is a GDP-GTP exchange factor for Rho1p that is activated by cell wall defects; null mutations of *rom2* impair the redistribution of actin patches in response to cell wall stress (Ozaki *et al.*, 1996; Manning *et al.*, 1997). Similarly, Bem4 interacts with Rho-type GTPases that regulate actin cytoskeletal reorganization (Mack *et al.*, 1996; Hirano *et al.*, 1997). Thus, these results indicate that Rho protein regulators and interacting proteins are important for bud site selection in yeast.

Table 4. Homozygous diploid deletion strains with random budding pattern

Category	Gene (ORF)	Budding pattern[a]	Morphological phenotype	Mat a budding pattern	Bud8p localization
Ribosome proteins					
Strong phenotype	RPS27B	>50% R	Round 2	WT	
	RPL14A	>50% R	WT	WT	
	RPS0B	>50% R	Round 2	WT	
	RPL22A	>50% R	Round 3, clumpy 2	WT	
	RPS17A	>50% R	Small 2	WT	
	RPS7A	>50% R	Round 2+	WT	
	RPL7A	>50% R	WT	WT	
	RPL39	>50% R	Round 2, small 2	NT	
Weak phenotype	RPL12B	<30% R	Small 2	WT	
	RPL27A	<40% R	Round 2	WT	
	RPS28B	<50% R	Round 2	WT	
	RPS30A	<30% R	WT	WT	
	RPS29A	<30% R	WT	WT	
	RPS1B	<50% R	WT	WT	
	RPS18B	<30% R	Small 3	WT	
Vesicular transport proteins					
Strong phenotype	BST1	>50% R	Round 4	WT	WT
	CLC1	>50% R	Round 3, clumpy 3	WT	Weak or ND
	YPT31	>50%	Round 3	WT	More patch
	VMA5	>50% R	Round 2	WT	WT
	VAM8	>50% R	Large 2	WT	Diffuse at bud and more at neck
	VPS34	>50% R	Round 3, large 3	WT	WT
	VAM3	>50% R	Round 3	WT	Most or all of the bud periphery
	VAC7	>50% R	Large 2	NT	WT
	END3	>50% R	Small 3, round 2	NT	More concentrated at the tip
	COS16	>50% R	Rounds 2+	NT	NT
Weak phenotype	LUV1	<50% R	Round 2	WT	
	CUP5	<50% R	Round 3	WT	
	VPS45	<20% R, <20% U	Elongate 3, branch	NT	
	SEC22	15% R	Large 2	WT	
	SNC2	50% R	Round 3	WT	
Actin-Cytoskeleton					
Strong phenotype	SLA1	>50% R	Round 3	WT	
	RVS167	>50% R	Round 2, clumpy 3	WT	
	CAP1	>50% R	Large 3, round 3	WT	Diffuse
	YKE2	>50% R	Round 2	WT	
	MDM20	>50% R	Round 3	WT	
	RVS161	>50% R	Round 3	WT	
Bud site selection and cell polarity proteins					
Strong phenotype	RSR1	>50% R	Small 2, round 2	Random	
	BUD2	>50% R	WT	Random	
	SPA2	>50% R	Round 3	WT	Diffuse
	BUD6	>50% R	Round 3	WT	Diffuse
	ROM2	>50% R	Large 3, round 3	WT	
	BEM4	>50% R	Round 3	R; U; B	WT
	BNI1	>50% R	Round 3	WT	ND
Cell wall proteins					
Strong phenotype	CWH8	>50% R	Round 3, clumpy 2	Random	ND
	FKS1	>50% R	Small/round 3−	WT	WT
	GAS1	>50% R	Round 3, clumpy 3	WT	17% of the cells
Weak phenotype	SLG1	<20% R	Elongate 2	WT	
	KRE6	<50% R	Round 2, clumpy 2	WT	
	CCW12	40% R	Round 2	NT	
Lipid metabolism					
Strong phenotype	GUP1	>50% R	Round 2+, small 2+	WT	
	SUR4	>50% R	Round 2	B; U; R	
Weak phenotype	FEN1	23% U, 7% R	WT	NT	
	ERG4	30% R, 20% U	Round 3	WT	
	ERG3	40% U	Round 2	WT	
Protein modification					
Strong phenotype	PMT2	>50% R	Small 2, round 2	WT	
	MAP1	>50% R	Round 3	WT	
	OST3	>50% R	Small 3, round 3	WT	

(Continued)

Table 4. Continued

Category	Gene (ORF)	Budding pattern[a]	Morphological phenotype	Mat a budding pattern	Bud8p localization
	LAS21	>50% R	Round 4	WT	
	NAT3	>50% R	Round 3	WT	
	RAD6	>50% R	Large 3 or large/round 3	WT	
Weak phenotype	MNN2	30% R	Small 3, round 3	NT	
Transcriptional proteins					
Strong phenotype	CCR4	>50% R	Round 3	WT	Weak or ND
	NOT5	>50% R	Round 2, clumpy 2	WT	Weak or ND
	POP2	>50% R	Round 3	NT	Weak or ND
	RLR1	>50% R	Elongate 2	NT	
	CTK1	>50% R	Round 3	NT	
	SSN6	>50% R	Round 3, clumpy 3	NT	Weak or ND
	TUP1	>50% R	Round 3, clumpy 3	WT	Weak or ND
	HCR1	>50% R	Small 3	NT	
Weak phenotype	SIN4	50% U, 50% B	Clumpy 3+	WT	
	CTK3	20% R, few U	WT	WT	
	GCR3	43% U	WT	WT	
	RPB4	30% U, few R	Elongate 2	NT	
Nuclear proteins					
Strong phenotype	SPO7	>50% R	Round 3, large 2	WT	
	NPL3	>50% R	WT	WT	
	NSR1	>50% R	Football 2	WT	
	SFP1	>50% R	Small 2	WT	
	NEM1	>50% R	Round 2	WT	
	HMO1	>50% R	NT	NT	
	RAI1	>50% R	Round 2	NT	
	LSM1	>50% R	Small 3, round 3	WT	
	LOC1	>50% R	Round 3	WT	
Weak phenotype	TOP3	40% R	Large 2, elongate 2	WT	
	TRF4	20% U or R	Large 3	WT	
	ZUO1	<50% U	Round 2	NT	
Other proteins					
Strong phenotype	HEM14	>50% R	Round 3	WT	
	ATP14	>50% R	Small 2	WT	
	PRS3	>50% R	Round 3	WT	
	RIB4	>50% R	Round 3	WT	
Weak phenotype	YPS7	30% R	Small 3/round 3	WT	
	PRO1	50% R	Large 3, football 3	WT	
	ILM1	50% R	Round 3	NT	
Unknown protein					
Strong phenotype	BUD14/YAR014c	>50% R	Elongate 3	WT	
	BUD15/YBL047c	>50% R	WT	WT	
	BUD16/YEL029c	>50% R	Large 2, round 3	WT	
	BUD17/YNR027w	>50% R	Round 3	NT	
	BUD18/YER044c	>50% R	Small/round 3, clumpy 2	WT	
	YGR151c[a]	>50% R	Round 3	R	
	BUD19/YJL188c	>50% R	Round 3	WT	
	BUD20/YLR074c	>50% R	Small/round 3	WT	
	BUD21/YOR078w	>50% R	WT	WT	
	BUD22/YMR014w	>50% R	Small 2	NT	
	BUD23/YCR047c	>50% R	NT	NT	
Weak phenotype	BUD24/YDR320c	50% R	Small/round 2+	WT	
	BUD25/YER014c-a	<50% R	WT	WT	
	BUD26/YDR241w	40% R	Small 2	WT	
	BUD27/YFL023w	40% R	Football 3	WT	
	BUD28/YLR062c	<50% R, 15% U	Round 3	WT	
	BUD29/YOL072w	<40% R	Round 2	WT	
	BUD30/YDL151c	45% R, 10% U	Round 2	NT	
	BUD31/YCR063w	50% R, few U	Large 3, round 2	NT	
	BUD32/YGR262c	50% R	Large 2	NT	

WT, wild-type; ND, not detected; NT, not tested; R, random; U, unipolar; B, bipolar.
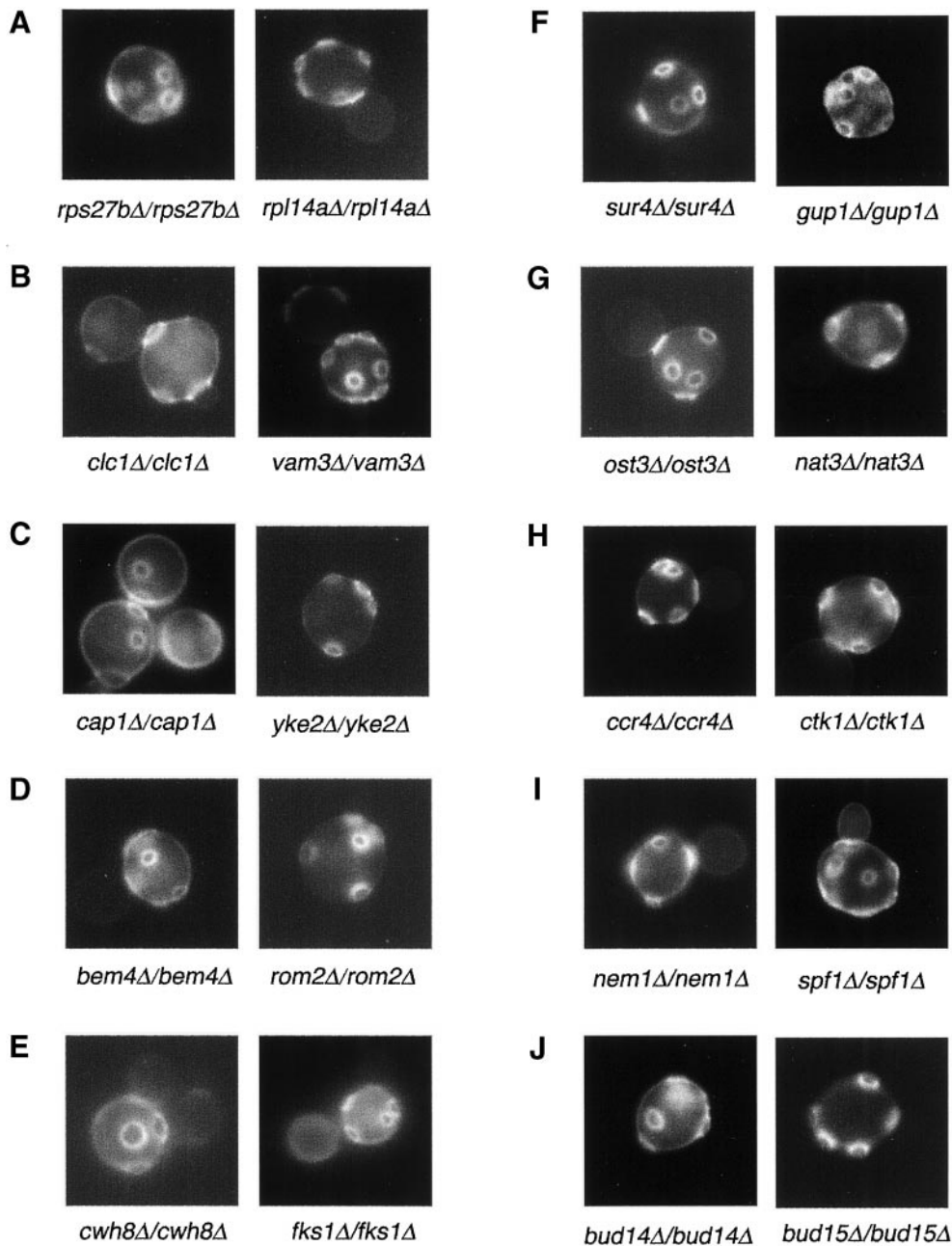[a] Overlaps with BUD1.

Figure 3. Micrographs of random budding mutants stained with Calcofluor. A–J are examples of 10 different functional classes. (A) Ribosomal protein mutants. (B) Vesicular transport mutants. (C) Actin-cytoskeleton mutants. (D) Bud site selection and cell polarity mutants. (E) Cell wall mutants. (F) Lipid metabolism mutants. (G) Protein modification mutants. (H) Transcription factor mutants. (I) Nuclear proteins mutants. (J) Uncharacterized proteins mutants.

Cell Wall Proteins. Six cell wall proteins were found to affect bipolar budding (Table 4). Three, *fks1Δ/fks1Δ*, *gas1Δ/gas1Δ*, and *cwh8Δ/cwh8Δ*, have a strong defect in which >50% of the cells bud randomly (Figure 3E). Fks1 is a component of β-1,3-glucan synthase; β-1,3-glucan is a major constituent of the yeast cell wall (Douglas *et al.*, 1994). Gas1 is a putative glycosidase important for cell wall maintenance; it is thought to direct β-1,6-glucans to cross-link to β-1,3-glucan rather than to chitin (Vai *et al.*, 1991). Cwh8 is a protein involved in the generation of the mannoprotein layer of the cell wall. Secreted proteins of *cwh8* mutants have abnormally low levels of *N*-glycosylation (van Berkel *et al.*,

1999). Interestingly, we found that *fks1Δ* and *gas1Δ* haploid cells do not have a defect in axial budding, whereas the *cwh8Δ* haploid strain exhibits a random budding defect.

We also found that mutations in three other components affecting cell wall integrity, *KRE6* (a glucan synthase subunit; Roemer and Bussey, 1991), *SLG1*, and *CCW12* (which are important for cell wall integrity) (Verna *et al.*, 1997; Mrsa *et al.*, 1999) exhibit a mild budding pattern defect in which <50% of the cells bud randomly. These results suggest that a proper cell wall is important for bud site selection in diploid cells. The cell wall might help anchor the tags and/or, in the case of Cwh8, might help produce a properly glycosylated tag.

Lipid Metabolism. We also identified a number of genes involved in lipid biosynthesis or metabolism, including *SUR4, FEN1, ERG3, ERG4,* and *GUP1* (Figure 3F). Sur4 and Fen1 are implicated in fatty acid synthesis and post-Golgi transport; mutations in these genes allow for the bypass of V-SNARE requirement in exocytosis (Oh *et al.*, 1997; David *et al.*, 1998). Erg3 and Erg4 are involved in ergosterol biosynthesis (Daum *et al.*, 1998). Gup1 is a diacylglycerol *O*-acyltransferase-related protein; *gup1Δ* mutants have reduced lipid synthesis and increased glycerol synthesis (Oelkers *et al.*, 2000). Mutants lacking these genes may have altered lipid compositions that might affect the secretion or function of bipolar markers and/or cell wall components important for the bipolar pattern.

Protein Modification. We identified seven bud site selection genes whose proteins participate in protein modification (Table 4; Figure 3G). Three are involved in glycosylation. Mnn2 and Pmt2p are mannosyltransferases involved in *N*-linked and *O*-linked glycosylation, respectively (Lussier *et al.*, 1995; Rayner and Munro, 1998). Ost3p is an oligosaccharyltransferase γ subunit, which is part of a complex that transfers core oligosaccharide from dolichol carrier to Asn-X-Ser/Thr motif, and helps position the OTase (oligosaccharyltransferase) complex for efficient *N*-glycosylation of secretory proteins (Knauer and Lehle, 1999). These results indicate that *N*-linked and *O*-linked glycosylation play a role in bipolar budding. It is likely that these proteins affect the modification of tags or cell wall components that anchor the tags.

Three other genes important for the bipolar pattern are involved in protein modification. Las21 is a protein required for addition of a side chain to the glycosylphospatidylinositol core structure (Hong *et al.*, 1999). Map1p is a methionine aminopeptidase that cleaves the N-terminal methionine if the second residue has a small side chain (Gly, Pro, Ser, Thr, Val; Walker and Bradshaw, 1999). Nat3p is a protein *N*-acetyltransferase (Polevoda *et al.*, 1999). Rad6 is a ubiquitin-conjugating enzyme involved in protein degradation (Jentsch *et al.*, 1987). These proteins presumably modify proteins (or for Rad6 affect the turnover of proteins) involved in the bipolar pattern.

Transcription Factors and Chromosomal Proteins. In this screen we identified 12 mutants lacking transcriptional factors that displayed a random budding pattern phenotype (Table 4; Figure 3H). Eight, *ccr4Δ/ccr4Δ*, *not5Δ/not5Δ*, *pop2Δ/pop2Δ*, *rlr1Δ/rlr1Δ*, *ctk1Δ/ctk1Δ*, *ssn6Δ/ssn6Δ*, *tup1Δ/tup1Δ*, and *hcr1Δ/hcr1Δ*, exhibit a strong defect, and four others, *sin4Δ/sin4Δ*, *ctk3Δ/ctk3Δ*, *gcr3Δ/gcr3Δ*, and *rpb4Δ/rpb4Δ*, displayed a weak defect. Ccr4, Not5, and Pop2 are components of the CCR4 transcriptional complex, which has positive and negative effects on transcription (Draper *et al.*, 1995; Liu *et al.*, 1998; Oberholzer and Collart, 1998). The CCR4 complex regulates transcription during late mitosis. It may function downstream of the Pkc1p-Mpk1p cascade to regulate the expression of a subset of yeast genes (Chang *et al.*, 1999). Ssn6 and Tup1 form a complex and are general repressors of RNA polymerase II transcription (Williams *et al.*, 1991). The Ssn6-Tup1 repressor has several distinguishing features, including its exceedingly efficient repression (>1000-fold for some target genes), the large number of genes repressed (as

many as 3% of the *S. cerevisiae* genes), and its versatility with respect to the nature and number of activators it can prevail against (reviewed by Smith and Johnson, 2000). Because Tup1p-Ssn6 is required for the repression of many genes (including haploid-specific genes; Fujita *et al.*, 1992; Keleher *et al.*, 1992), deletion of Tup1-Ssn6 may induce expression of genes that affect the budding pattern. These results suggest that these transcription factors and chromosomal proteins regulate the expression of genes involved in the bipolar pattern. Ctk1 and Ctk3 are subunits of a protein kinase that phosphorylates the C-terminal domain of the large subunit of RNA polymerase II (Lee and Greenleaf, 1997). Mutation of *CTK1* causes defects in both transcriptional repression and activation (Kuchin and Carlson, 1998). This kinase, which affects lexA-Tup1, might exert its activity by regulating gene expression (perhaps through Tup1) or by directly acting on components important for bud site selection. Altogether, our results indicate that gene regulation plays a very important role in bipolar bud site selection.

Nuclear Proteins. A variety of other genes involved in nuclear function or whose encoded proteins localize to the nucleus have been identified (Table 4; Figure 3I). These include *SPO7, NPL3, NSR1, SFP1, NEM1, HMO1, RAI1, LSM1, TOP3, ZUO1,* and *TRF4*. These gene products are involved in nuclear transport (Npl3, Sfp1; Blumberg and Silver, 1991; Bossie *et al.*, 1992), have RNA-binding motifs, are involved in RNA processing/modification (Rai1, Npl3, Lsm1, Nsr1, Zuo1, and Loc1; Kondo and Inouye, 1992; Kadowaki *et al.*, 1994; Yan *et al.*, 1998; Costanzo *et al.*, 2000; Tharun *et al.*, 2000; Xue *et al.*, 2000), are required for nuclear morphology and/or meiosis (Spo7, Nem1; Siniossoglou *et al.*, 1998), or are DNA-binding proteins and topoisomerase (or related) homologues (Top3, Trf4, Hom1; Kim and Wang, 1992; Castano *et al.*, 1996; Lu *et al.*, 1996). These gene products may be involved in the expression, processing, or transport of RNA transcripts for genes involved in bipolar budding. In addition, because some of these affect processing of rRNA (Npl3, Nsr1, Rai1; Kondo and Inouye, 1992; Russell and Tollervey, 1992; Xue *et al.*, 2000), they might function in bud site selection through ribosome biogenesis or translation.

Other Genes and Uncharacterized Genes. Other genes important for bipolar budding were also identified (Table 4; Figure 3J). These include seven known genes and 20 genes that have not been characterized previously. Deletion of 11 of the uncharacterized genes cause a strong random budding pattern, whereas the rest cause a weaker budding defect. We have named these genes *BUD13* through *BUD32*. Further characterization of some of these genes is described below.

Many Bud Site Selection Mutants Exhibit a Defect in Cell Morphology

In addition to screening for bud site selection defects, we also examined the diploid collection for morphological defects. Wild-type diploid cells are normally ellipsoid. Previous work has demonstrated that mutants defective in apical growth, which is critical for an elongated cell shape, exhibit random budding, presumably through defective localization of tags at the poles of the cell (Sheu *et al.*, 2000). By visually screening 3394 homozygous diploid deletion strains with the use of differential contrast interference microscopy of
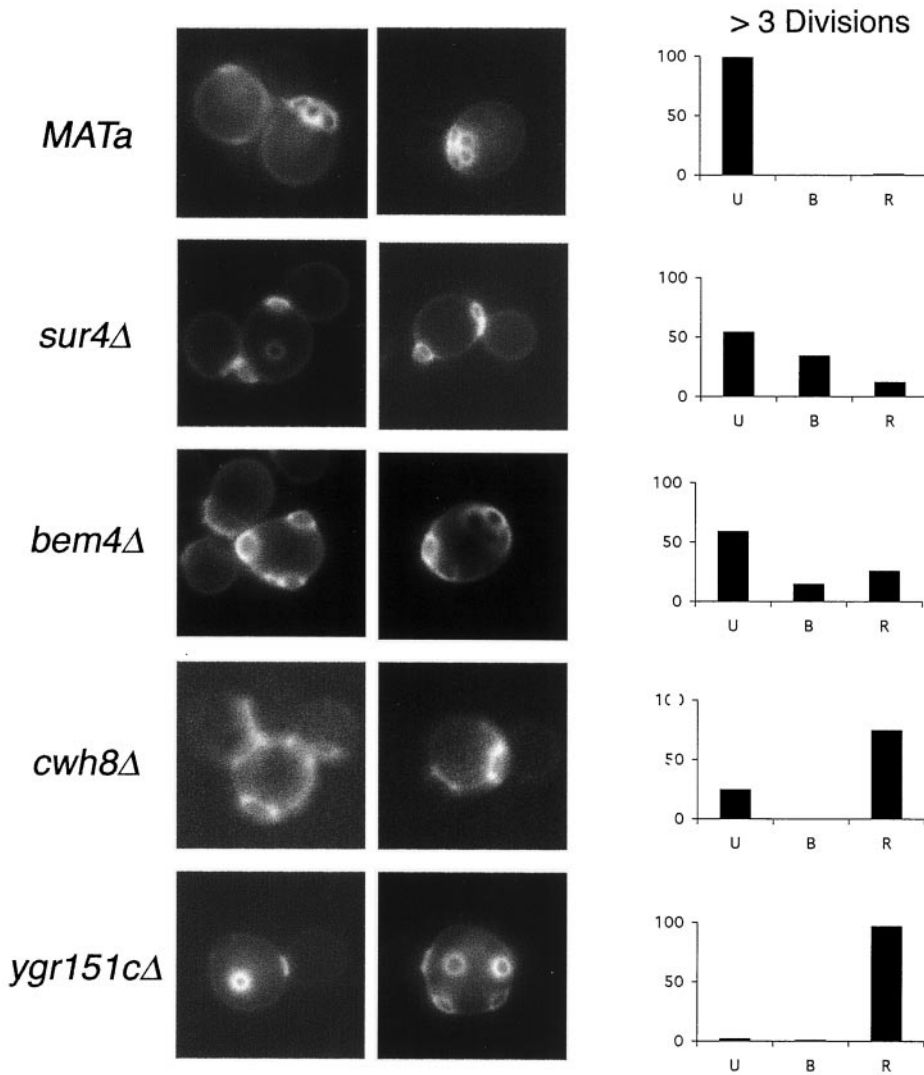
Figure 4. Budding patterns of haploid deletion mutants. Left, bud scar staining is presented for each sample. Right, cells with more than three bud scars were scored and classified as unipolar (U) if all bud scars were concentrated at one pole, bipolar (B) if one or more scars were at each pole, and random (R) if one of more scars were in the midsection of the cell (Flescher *et al.*, 1993). In all panels, >200 cells were counted.

fixed cells, we identified 459 strains exhibiting slight to strong morphological alterations. Details of the results will be published elsewhere. Some of these shapes are described in Tables 2–4. Of particular interest, >40% of unipolar *ist3Δ/ist3Δ* and *ygl174wΔ/ygl174wΔ* mutants exhibited enhanced growth at the end of the cell where budding occurred. These results suggest that both growth sites and division sites reside at one end of the cell in these strains.

We also noticed that 70% of our random budding pattern mutants display a round phenotype suggestive of an apical growth defect. This observation supports the hypothesis that apical growth is important for bipolar bud site selection, as suggested previously (Sheu *et al.*, 2000).

Several Genes Affect the Budding Pattern of Haploids

We expected that mutations affecting the diploid budding pattern would be of two types. The first type would be defective in both haploid and diploid budding patterns, such as *rsr1* or *bud2*; these mutants are thought to be defective in the general bud site selection machinery (Chant *et al.*, 1991; Ruggieri *et al.*, 1992; Park *et al.*, 1993). The second would be defective in functions specifically required for the bipolar budding pattern. To determine whether any of the mutations that affected the bipolar pattern also affected the axial pattern, we analyzed the budding patterns of 103 haploid *MATa* deletion strains with the use of Calcofluor staining.

Of the 103 mutants, 98 appeared to display normal or near normal axial budding, suggesting that these defects were indeed specific to the bipolar budding pattern. These include genes involved actin cytoskeleton organization, vesicular trafficking, ribosomal synthesis, transcriptional factors, and most cell polarity components (Tables 2–4). However, six haploid deletion mutants bud randomly; these are *rsr1Δ*, *bud2Δ*, *bem4Δ*, *cwh8Δ*, *sur4Δ*, and *ygr151cΔ* (Figure 4). Rsr1 and Bud2 were previously known to be important for the budding patterns of both haploid and diploid strains (Rug-

gieri *et al.*, 1992; Park *et al.*, 1993, 1999). ORF *YGR151c* partially overlaps Rsr1, and thus deletion of this ORF might affect Rsr1 function, thereby accounting for the random budding phenotype. *cwh8Δ*, *sur4Δ*, and *bem4Δ* have not been described previously to have a bud site selection defect in haploid cells. The Sur4, Cwh8, and Bem4 proteins might therefore function with either Rsr1, Bud2, or Bud5 to help select bud sites or with the polarity establishment proteins that are thought to be targeted to bud sites by the Rsr1 machinery.

Bud8-GFP Subcellular Localization in Diploid Wild-Type and Budding Mutant Strains

To further understand how the different bipolar bud site selection mutants function, we analyzed the localization of Bud8 in wild-type and many of the mutant strains. Bud8 has been proposed to act as a distal tag for bipolar bud site selection; it localizes to the bud tip (Taheri *et al.*, 2000; Harkins, *et al.*, 2001). As noted above, *bud8Δ/bud8Δ* mutants are unable to bud at the distal pole and instead produce a unipolar proximal budding pattern in diploids (Zahner *et al.*, 1996).

A high copy plasmid construct of the GFP:Bud8 gene (Harkins, *et al.*, 2001) was transformed in a *bud8Δ/bud8Δ* deletion strain and found to complement the *bud8Δ/ bud8Δ* mutations (Ni and Snyder, unpublished results). When transformed into a wild-type diploid strain, the cells budded in a bipolar pattern; however, there is an increase in use of distal bud sites. This bias is especially apparent in the later budding events. For example, we found that in vegetatively growing wild-type strains containing Bud8-GFP, 95% of the second buds, and 91% of third buds, formed at distal poles, compared with only 68% of the second buds and 40% of the third buds for wild-type cells with vector alone. These results indicate that Bud8 activity is increased when overexpressed.

We next examined the localization of Bud8-GFP in the diploid wild-type strain. The Bud8-GFP was observed in ~30% of the cells. As shown in Figure 5A, Bud8-GFP localized to the presumptive bud sites in unbudded cells, the distal tips of growing buds, and the proximal pole of some daughter cells, in agreement with previous results (Harkins, *et al.*, 2001). In addition Bud8-GFP is also localized to the mother-daughter neck in some cells and was biased toward the mother side of the neck. Double staining with Calcofluor White revealed that neck staining was observed in only the daughter cells undergoing their first division (Figure 5A). As described in DISCUSSION, we propose that Bud8 at the distal tip helps serve as a tag for the distal site, and Bud8 at the neck in new mothers might also help select these sites.

Bud8-GFP Localization in alg Mutants.
We next examined the Bud8-GFP localization in the *alg* mutants, which have reduced outer chain carbohydrate modification. In *alg5Δ/alg5Δ*, *alg6Δ/alg6Δ*, *alg8Δ/alg8Δ*, and *alg10Δ/alg10Δ* mutants, Bud8p-GFP localized to the bud tip and the mother-daughter neck in many of the cells. However, detailed quantitative analysis of the localization of the mother-daughter neck showed an important feature: more cells contained Bud8-GFP localization at the neck in *alg* mutants than in wild-type cells. In cells that showed any detectable

GFP signal, 48.6% of wild type have Bud8-GFP staining at the neck (n = 200); in contrast, for *alg5Δ/alg5Δ*, *alg6Δ/alg6Δ*, *alg8Δ/alg8Δ*, and *alg10Δ/alg10Δ* the percentage of cells with staining at the neck increases to 68.9, 65.8, 59.5, and 53.2% (n = 200), respectively. Thus, Bud8 outer chain modification might affect Bud8 targeting or degradation. For the latter possibility, in the absence of the outer chain modification Bud8 might be more stable and persist at the distal site longer. Alternatively, modification of other cell wall components might affect distal tag stability. Regardless, the increased presence of Bud8-GFP at the distal site (neck and bud tip) helps account for the partial unipolar budding phenotype of these cells; the cells might use the persistent Bud8 tag in multiple cell divisions.

Bud8-GFP Localization in Vesicular Transport Mutants.
Nine random budding mutants in the vesicular transport category were examined for Bud8-GFP localization. *bst1Δ/bst1Δ*, *vma5Δ/vma5Δ*, *vps34Δ/vps34Δ*, *vac7Δ/vac7Δ*, and *end3Δ/end3Δ* each localize Bud8-GFP at polarized sites (bud tip and neck) similar to wild-type cells. Four mutants were found to have abnormal Bud8-GFP localization patterns. Clc1, the clathrin light chain, plays a role in formation of clathrin coats and clathrin-coated vesicles (Chu *et al.*, 1996). Unlike a wild-type strain, in which >30% of the cells exhibit a polarized localization of Bud8-GFP, in *clc1Δ/clc1Δ* mutants >90% of the mutant cells lacked detectable Bud8-GFP localization; most of the remainder have a very weak Bud8-GFP signal, often visible as a tiny spot at the bud tip (Figure 5B). This result indicates that Bud8 undergoes intracellular trafficking through a clathrin-coated vesicle pathway or that disruption of that pathway indirectly alters Bud8 localization. In another mutant, *ypt31Δ/ypt31Δ*, which lacks a small GTPase involved in trans-Golgi vesicle formation, Bud8-GFP localized in a broader patch at the tip or/and neck (Figure 5B). Two other mutants that exhibit a defect in Bud8-GFP localization are in *vam3Δ/vam3Δ* and *vam8Δ/vam8Δ*; Vam3 and Vam8 encode syntaxin homologues (t-SNARE) thought to be important for vesicle docking and fusion reactions with the vacuole (Preston *et al.*, 1991; Rieder and Emr, 1997; Ungermann *et al.*, 1999). Bud8-GFP is not concentrated at the bud tip in *vam3Δ/vam3Δ* and *vam8Δ/vam8Δ* mutants. Instead, in *vam3Δ/vam3Δ* mutants it localizes throughout most or all of the bud periphery (Figure 5B). In *vam8Δ/vam8Δ* mutants, Bud8p is more diffuse at the tip and tends to form double rings at the neck (Figure 5B). These results indicate that Ypt 31, Vam3, and Vam8 may be involved in the targeting of Bud8p to the bud tip.

Bud8-GFP Localization in Cell Wall Mutants.
Bud8-GFP localization was also examined in three mutants that affect cell wall function. In *fks1Δ/fks1Δ*, Bud8-GFP localization appears normal. However, in *cwh8Δ/cwh8Δ*, Bud8-GFP cannot be detected, and in *gas1Δ/gas1Δ*, Bud8 localizes to polarized sites in only 17% of the cells and was not detectable in the remainder (n = 200; Figure 5C). These results indicate that Fks1 is not required for Bud8-GFP localization, whereas Cwh8 and Gas1 are required. Thus, specific components of the cell wall are important for Bud8 localization and/or Cwh8 is important for Bud8 modification.
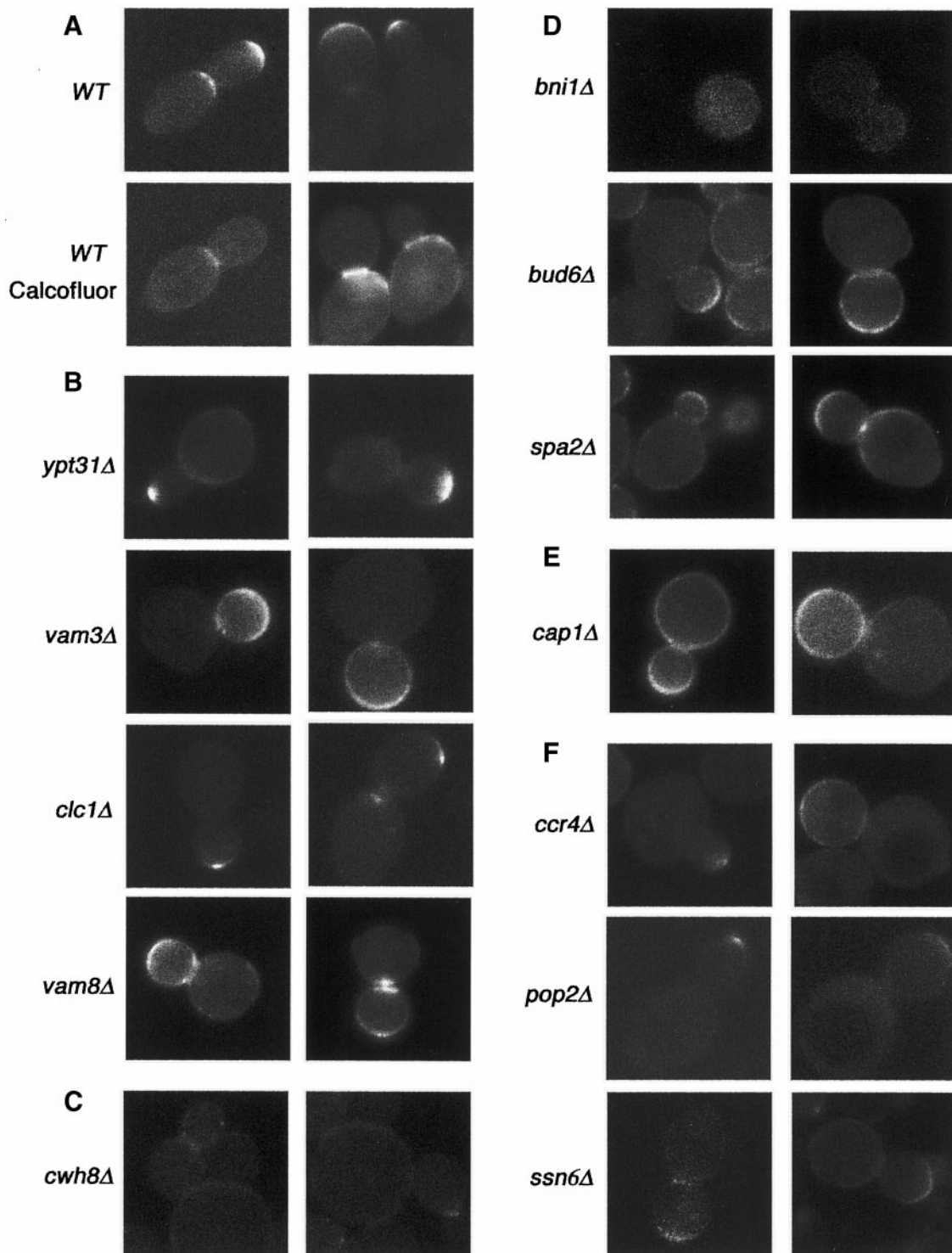
Figure 5. Localization of Bud8-GFP in diploid wild-type (*WT*) and mutant strains. Cells of the indicated strains carrying the plasmid Bud8-GFP were visualized by fluorescence microscopy. (A) Wild-type strains were viewed for both GFP and Calcofluor fluorescence (same cells). (B) Bud8-GFP in vesicular transport mutants. (C) Bud8-GFP in the *cwh8Δ/cwh8Δ* cell wall mutant. (D) Bud8-GFP in cell polarity mutants. (E) Bud8-GFP in the *cap1Δ/cap1Δ* actin-cytoskeleton mutant. (F) Bud8-GFP in transcription factor mutants.

Table 5. Localization of GFP fusion protein in tagged strains

Class	Gene name	ORF	GFP-protein localization	Budding pattern of the tagged strains
Unipolar	*IST3*	*YIR005w*	Nuclear	Bipolar
	BUD13	*YGL174w(C)*	Nuclear	Bipolar
Random	*BUD14*	*YAR014c*	Small bud tip, large bud neck	Bipolar
	BUD15	*YBL047c*	Small bud tip, large bud neck	Bipolar
	LOC1	*YFR001w*	Nuclear	Most bipolar, few random
	BUD18	*YJL188c*	ND	Random
	BUD20	*YLR074c*	Nuclear	Bipolar
	BUD21	*YOR078w*	Nuclear	Bipolar
	BUD22	*YMR014w*	Nuclear	Bipolar
		YGR151c[a]	ND	Random
	BUD18	*YJL188c*	ND, slow growth	Random

ND, not detected.

[a] Overlaps with RSR1.

Bud8-GFP Localization in Budding, Cell Polarity Mutants.

We also investigated Bud8-GFP localization in *spa2Δ/spa2Δ*, *bud6Δ/bud6Δ*, and *bni1Δ/bni1Δ* mutants. Spa2, Bni1, and Pea2 are important for apical growth and have been suggested to help concentrate polarized growth and secretion at bud tips and mother-bud necks (Sheu *et al.*, 2000). In *spa2Δ/spa2Δ* and *bud6Δ/bud6Δ* mutants, Bud8-GFP localization is more diffuse at the bud tip compared with wild-type cells (Figure 5D); the Bud8-GFP signal is weaker in *bud6Δ/bud6Δ* cells than in *spa2Δ/spa2Δ*. In *bni1Δ/bni1Δ*, Bud8-GFP localization is not detected, indicating that Bni1 is required for Bud8 localization (Figure 5D). The absence of Bud8-GFP in any cells is consistent with the observation that *bni1Δ/bni1Δ* mutants bud randomly both in the first division and in subsequent divisions. The diffuse localization of Bud8-GFP in *spa2Δ/spa2Δ* cells is consistent with the observation that the first bud does not always occur at the apex of the distal tip of the cell (Sheu *et al.*, 2000).

Bud8-GFP Localization in cap1Δ/cap1Δ Mutants.

Bud8-GFP localization was also investigated in *cap1Δ/cap1Δ* mutants, which lacks Cap1, an actin-capping protein α subunit. Bud8-GFP signal appeared more diffuse in this mutant, and the Bud8-GFP signal is diffuse not only in the bud but also in the mother, in contrast to the signal observed in the *spa2Δ/spa2Δ* and *bud6Δ/bud6Δ* mutants (Figure 5E). This result is consistent with the bud tip localization of Cap1 (Amatruda and Cooper, 1992) and implies that the actin cytoskeleton at the incipient bud site might help to localize cortical bud site selection tags to distal sites and/or maintain them properly at those sites.

Bud8-GFP Localization in Transcription Factor Mutants.

Bud8-GFP localization was examined in five transcription factors mutants. Ccr4, Not5, and Pop2 are components of the CCR4 transcriptional complex, which has positive and negative effects on transcription (Draper *et al.*, 1995; Liu *et al.*, 1998). The localization signal of Bud8-GFP in these mutants is very faint; most cells lack a detectable signal and the remainder have only a weak signal at the bud tip (Figure 5F). We presume that these mutants may directly or indirectly affect Bud8p expression. We also analyzed Bud8-GFP localization in *ssn6Δ/ssn6Δ* and *tup1Δ/tup1Δ* mutants. Tup1 and Ssn6 form a complex that functions as a transcriptional repressor (Williams *et al.*, 1991). In both mutants, Bud8-GFP localizes to polarized sites in <10% of the cells, but the signal is very weak compared with wild-type cells (Figure 5 F). Thus, the Ssn6-Tup1 complex may also be involved in Bud8 regulation.

The altered localization and/or signal of Bud8 in many of these mutants demonstrate that the activity of Bud8 is influenced by many gene products and that Bud8 plays a critical role in bipolar budding. The mislocalization of Bud8 in many random budding mutants offers a clue as to why these mutants select nonpolar sites for bud formation; presumably other tags or localization components are also mislocalized, leading to further randomization of the budding pattern.

GFP Tagging of Uncharacterized Genes Required for Bipolar Budding

To further understand how the genes that were uncharacterized or had limited characterization function in bipolar bud site selection, subcellular localization studies were performed. We analyzed two proteins whose loss caused a unipolar budding phenotype (Ist3 and Bud13/Ygl174w) and seven uncharacterized proteins whose loss caused a random budding pattern (Bud14/Yar014c, Bud15/Ybl047c, Bud18/Yjl188c, Bud20/Ylr074c, Bud21/Yor078w, Bud22/Ymr014c, Loc1/Yfr001w, and Ygr151c; Table 5).

A GFP cassette was precisely inserted in the chromosome at the second codon after the initiator ATG for each of the genes, except for *BUD13/YGL174w*, in which GFP was placed immediately upstream of the translational stop codon. Thus, the different constructs express the GFP-ORF fusion protein from the endogenous promoter (see MATERIALS AND METHODS). Three of the tagged constructs exhibited defects in bipolar budding, and hence, insertion of the GFP cassettes inactivated the target gene; GFP localization was not detected for any of these strains (Table 5). One tagged strain, Loc1p/Yfr001w-GFP, which is a protein predicted to bind double-stranded RNA and is involved in localization of mRNA (Costanzo *et al.*, 2000), displayed a mild defect in bipolar budding and a slow growth pheno-
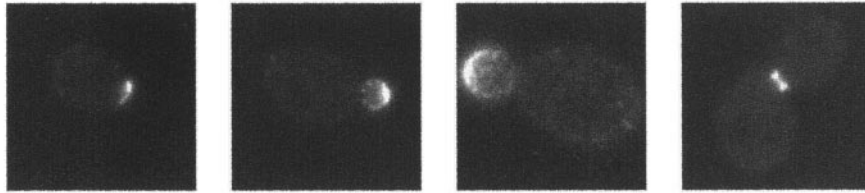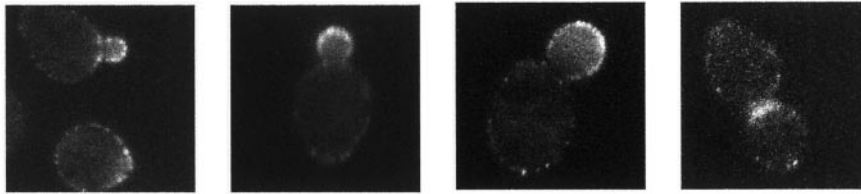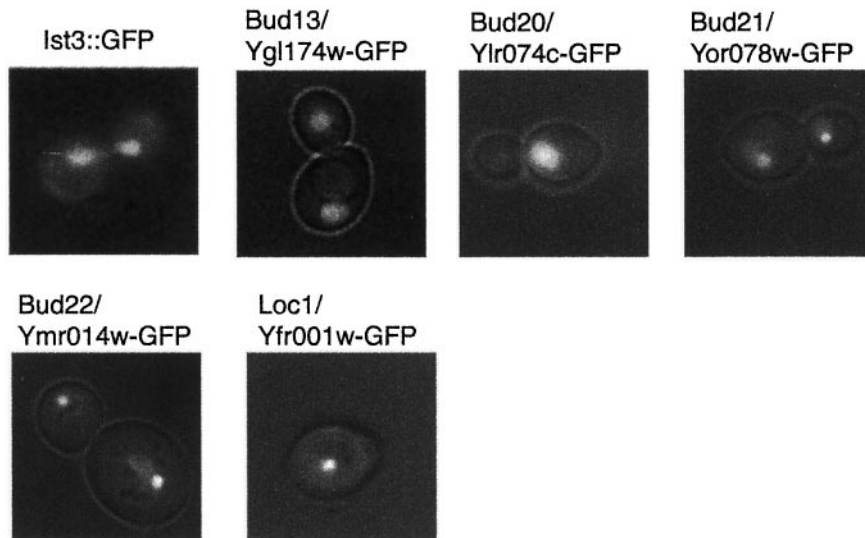
A Bud14/Yar014c-GFP

B Bud15/Ybl047c-GFP

C

Ist3::GFP

Bud13/
Ygl174w-GFP

Bud20/
Ylr074c-GFP

Bud21/
Yor078w-GFP

Bud22/
Ymr014w-GFP

Loc1/
Yfr001w-GFP

Figure 6. Localization of Bud-GFP proteins. (A) Bud14/Yar014c-GFP, which is localized as a patch at the presumptive bud sites in unbudded cells, the distal tips of growing buds and the mother-daughter bud neck in large-budded cells. (B) Bud15/Ybl047c-GFP, which at the bud tip in small and large budded cells and at the mother-daughter neck in cells undergoing cytokinesis. (C) Six other GFP fusion proteins localized to nucleus.

type indicative of a partial defect in gene function; the Loc1p/Yfr001w-GFP localized to the nucleus (Figure 6C). Seven tagged strains appeared normal in growth and bipolar budding pattern, indicating that these fusion proteins were functional; these strains were studied further for protein localization.

BUD14/YAR014c is an unknown ORF, which encodes a predicted SH3 domain protein. Bud14p/Yar014c-GFP was found to be presented at the presumptive bud sites in unbudded cells, the distal tips of growing buds, and the mother-daughter bud neck in large-budded cells (Figure 6A). This polarized localization of Bud14p/Yar014c and the random budding phenotype of *bud14Δ/bud14Δ* (*yar014cΔ/yar014cΔ*) are consistent with a role for Bud14p/Yar014 in polarized growth.

Bud15p/Ybl047c is an unknown protein with similarity to the cytoskeletal proteins Uso1p and Pan1p and contains three Eps homology domains in the N-terminal region,

which are often associated with regulation of protein transport, protein sorting, and membrane trafficking (Paoluzi *et al.*, 1998). Bud15p/Ybl047c-GFP localized as a patch concentrated in the developing bud tip in cells with small buds and at the mother-daughter neck in cells undergoing cytokinesis (Figure 6B). This cellular localization suggests that Bud15p/Ybl047c may affect bipolar budding through polarized secretion. During the preparation of this report, Bud15p/Ybl047c was reported to play a role in endocytosis and genetically interact with Rsp5, a ubiquitin ligase. These authors reported a localization pattern similar to the one that we describe (Gagny *et al.*, 2000).

GFP fusions for five other proteins, Ist3p, Bud13p/Ygl174w, Bud20/Ylr074c, Bud21/Yor078w, and Bud22/Ymr014w, localize to the nucleus. Three of these proteins, Ist3p, Bud13p/Ygl174w, and Bud20p/Ylr074c, localized throughout the nucleus (for Ist3p the signal was weak; Fig-
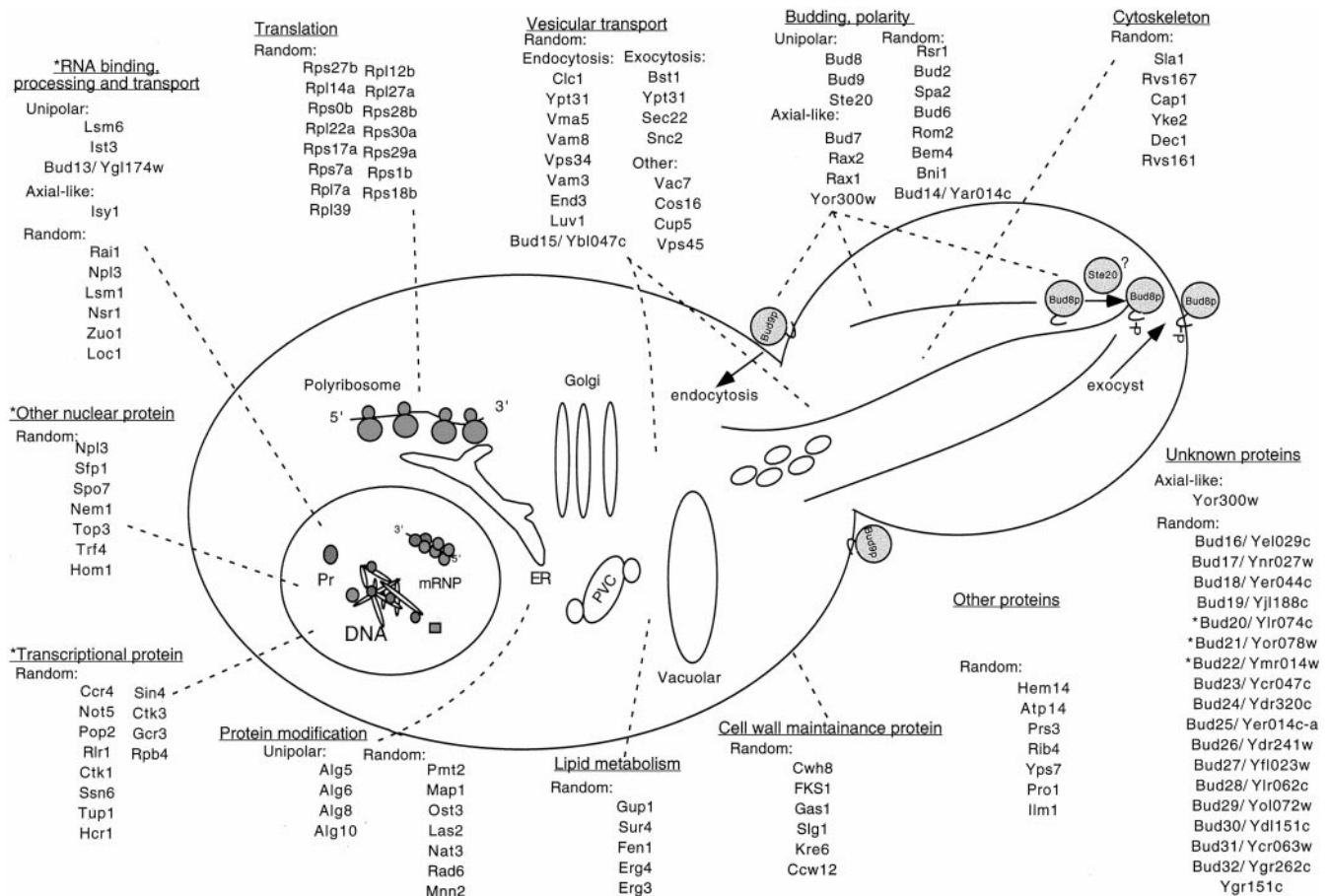
Translation
Random:
Rps27b Rpl12b
Rpl14a Rpl27a
Rps0b Rps28b
Rpl22a Rps30a
Rps17a Rps29a
Rps7a Rps1b
Rpl7a Rps18b
Rpl39

*RNA binding,
processing and transport
Unipolar:
Lsm6
Ist3
Bud13/Ygl174w
Axial-like:
Isy1
Random:
Rai1
Npl3
Lsm1
Nsr1
Zuo1
Loc1

Vesicular transport
Random:
Endocytosis: Exocytosis:
Clc1 Bst1
Ypt31 Ypt31
Vma5 Sec22
Vam8 Snc2
Vps34
Vam3 Other:
End3 Vac7
Luv1 Cos16
Bud15/Ybl047c Cup5
Vps45

Budding, polarity
Unipolar: Random:
Bud8 Rsr1
Bud9 Bud2
Ste20 Spa2
Axial-like: Bud6
Bud7 Rom2
Rax2 Bem4
Rax1 Bni1
Yor300w Bud14/Yar014c

Cytoskeleton
Random:
Sla1
Rvs167
Cap1
Yke2
Dec1
Rvs161

*Other nuclear protein
Random:
Npl3
Sfp1
Spo7
Nem1
Top3
Trf4
Hom1

Polyribosome
5' 3'
Golgi
endocytosis
exocyst
Ste20 ?
Bud8p Bud8p Bud8p
Bud8p
Bud8p

Unknown proteins
Axial-like:
Yor300w
Random:
Bud16/Yel029c
Bud17/Ynr027w
Bud18/Yer044c
Bud19/Yjl188c
*Bud20/Ylr074c
*Bud21/Yor078w
*Bud22/Ymr014w
Bud23/Ycr047c
Bud24/Ydr320c
Bud25/Yer014c-a
Bud26/Ydr241w
Bud27/Yfl023w
Bud28/Ylr062c
Bud29/Yol072w
Bud30/Ydl151c
Bud31/Ycr063w
Bud32/Ygr262c
Ygr151c

Pr mRNP
DNA
ER
PVC
Vacuolar

Other proteins
Random:
Hem14
Atp14
Prs3
Rib4
Yps7
Pro1
Ilm1

*Transcriptional protein
Random:
Ccr4 Sin4
Not5 Ctk3
Pop2 Gcr3
Rlr1 Rpb4
Ctk1
Ssn6
Tup1
Hcr1

Protein modification
Unipolar: Random:
Alg5 Pmt2
Alg6 Map1
Alg8 Ost3
Alg10 Las2
Nat3
Rad6
Mnn2

Lipid metabolism
Random:
Gup1
Sur4
Fen1
Erg4
Erg3

Cell wall maintainance protein
Random:
Cwh8
FKS1
Gas1
Slg1
Kre6
Ccw12

Figure 7. A model for bipolar bud site selection that integrates the results of this study. As described in the text the different bud site selection proteins are likely to act at the sites indicated in the diagram. The bud genes indicated with a asterisk might either participate in transcription or other nuclear processes. ER, endoplasmic reticulum. mRNP, mRNA-protein; Pr, proteins; PVC, prevacuolar compartment.

ure 6C). In contrast, two other proteins, Bud21p/Yor078w and Bud22p/Ymr014w, are highly concentrated in a single region of the nucleus (Figure 6C). In budded cells, the fluorescent signal was in the region of the nucleus closest to the bud. Fluorescence was observed in cells at all different phases of the cell cycle. These results suggest that these six proteins may function in the regulation of expression (transcription, processing, or transport, etc.) of bipolar budding components.

DISCUSSION

In this paper we report the screening of a large collection of homozygous diploid deletion mutants for defects in bipolar bud site selection. From a screen of 4168 mutant strains we found 127 bipolar budding mutants affecting both previously characterized genes as well as uncharacterized genes. The previously characterized genes include those involved in the actin cytoskeleton, bud site selection, cell polarity, exocytosis, and endocytosis, cell wall integrity, protein modification, lipid metabolism, translation, gene transcription, and nuclear functions. We also found a large number of new

genes, including several whose encoded proteins are highly polarized in the cell. Analysis of these different mutants generated a comprehensive list of nonessential genes important for bipolar bud site selection and therefore provides a framework for this process in yeast.

An Integrated View of Bud Site Selection in Yeast

A large number of different types of genes involved in the bipolar pattern were identified. These include those involved in transcription, translation, polarity, bud site selection, lipid metabolism, protein modification, vesicular trafficking, cytoskeleton, and others. Based on our identification of their bud site selection phenotypes, their effect on Bud8-GFP localization, and the localization of their gene products, a model for our genome-wide characterization of this process is presented in Figure 7.

We propose that the actin cytoskeleton is important for apical growth and perhaps the transport of the tags to cortical sites and/or their maintenance at those sites. Polarity proteins such as Spa2, Bud6, and Bni1 are required for the proper localization or maintenance of bipolar tags at their

proper site (Sheu *et al.*, 2000). Consistent with this interpretation, Bud8-GFP is not found at polarized sites in *bni1Δ/bni1Δ* strains and is more diffuse in *spa2Δ/spa2Δ* and *bud6Δ/bud6Δ* strains. These results account for the observations that *bni1Δ/bni1Δ* mutants bud randomly at each division, including the first one, whereas *spa2Δ/spa2Δ* and *bud6Δ/bud6Δ* mutants still bud at the distal pole at the first division (although not always immediately at the tip) but bud randomly in later divisions (Snyder, 1989; Zahner *et al.*, 1996; Sheu *et al.*, 2000).

One of the interesting classes of proteins is the cell wall components. Bud8 and Bud9 are predicted to be transmembrane proteins; anchoring in the cell wall may be important for forming permanent tags. Because Fks1 and Gas1 are important for only the bipolar pattern, they are likely to be important for anchoring the underlying cytoskeleton or for anchoring the cell wall tags (e.g., Bud8 and Bud9). In contrast, Cwh8 is important for both axial and bipolar budding; it may also serve to anchor Bud8 and Bud9, and perhaps Axl2, a transmembrane protein that has been proposed to be part of the axial tag (Halme *et al.*, 1996; Roemer *et al.*, 1996a).

Another interesting set of proteins is the membrane-trafficking components. Several of the proteins affecting the bipolar pattern are important for exocytosis, including Ypt31, Sec22, and Snc2 (Woodman *et al.*, 1996; Jedd *et al.*, 1997; Spang and Schekman, 1998; Gerst, 1999). We expect the putative membrane tags, such as Bud8, and components that might anchor the tag, such as cell wall components, would be delivered to the incipient bud site with the use of the secretory machinery. The finding that Bud8-GFP localizes diffusely (i.e., as a broader patch) in Ypt31 mutants suggests that Bud8 targeting is defective in this mutant.

Unexpectedly, several mutants that affect the bipolar pattern play a role in endocytosis, including the clathrin light chain and *end3* (Benedetti *et al.*, 1994; Chu *et al.*, 1996). This raises the possibility that endocytosis might play an important role in bud site selection. Perhaps recycling distal tags from the surface, especially those that are diffuse, and then redirecting them to distal sites during apical growth helps focus tags at the distal tip. Alternatively, some of these proteins might also play a role in exocytosis. Consistent with this latter possibility for Clc1, little Bud8-GFP is detected at polarized sites or the cell periphery in *clc1Δ/clc1Δ* mutants, suggesting that it does not get transported properly to the surface. Presumably, clathrin-mediated secretion is needed for targeting of bud site selection components. For *end3* mutants, Bud8-GFP may be more concentrated at the tip (data not shown); this result is expected for an endocytosis mutant but does not explain the random budding defect observed in this mutant. Presumably, other components of the tag or those factors that interact with or affect the regulation of tags are defective in these cells.

We also identified a number of components involved in lipid metabolism. These might function in one of several ways. As noted above, several of these mutants might affect the secretory pathway and thus affect the targeting of molecules important for bud site selection. Because deletion of *SUR4* affects both haploid and diploids, the Sur4 protein may be involved in delivery or anchoring of both axial and bipolar tags; Erg3 and Erg4 are involved in only the bipolar pattern and presumably only affect the bipolar tags. Interestingly, deletion of Fen1 (Oh *et al.*, 1997), which affects lipid

elongation at an earlier step in the lipid elongation than Sur4, affects only the bipolar pattern. Thus, the different lipids have different effects on the distinct budding patterns.

We also identified a number of components important for protein modification, including those involved in *N*- and *O*-linked glycosylation (Alg, Mnn2, Pmt2, Ost3), lipid modification (Las2), acetylation (Nat3), initiator methionine processing (Map1), and protein turnover (Rad6). Presumably, these different components function on the tags or components that transport or anchor the tags. Consistent with this interpretation, we note that Axl2 (Roemer *et al.*, 1996a), a component involved in axial bud site selection that may be part of the axial tag, is modified by *O*-linked glycosylation; *pmt4Δ* mutants fail in the initial step of *O*-linked modification and are defective in Axl2 modification and localization (Sanders *et al.*, 1999). Thus, bipolar, as well as distal, tags might require *N*-linked and *O*-linked glycosylation.

We identified several transcription factors in our screen. We propose that they are required for the proper expression of either tags (e.g., Bud8) or components responsible for the proper transport or modification of the tag. Several of the RNA-processing components (e.g., Loc1, Rai1, Npl3, Lsm1, Nsr1, Lsm6, and Zuo1; Kondo and Inouye, 1992; Kadowaki *et al.*, 1994; Yan *et al.*, 1998; Costanzo *et al.*, 2000; Tharun *et al.*, 2000; Xue *et al.*, 2000) might be important for the proper expression or transport of RNAs of such components as well. Those nuclear factors whose loss results in random budding are likely to be involved in the expression of multiple tags or their localization; they might affect apical growth or the actin cytoskeleton. Indeed, many of the mutants that exhibit random budding have a round cell shape, consistent with an apical growth defect.

Finally, we identified a number of ribosomal proteins that are important for bud site selection. The random budding of ribosomal protein mutants may be due to the inefficient translation of components important for the bipolar pattern; alternatively, it may be due to an overall growth defect. Consistent with the latter possibility, many of the mutants that exhibit a strong budding pattern defect also exhibit a strong growth defect. Because significant growth control occurs during G1, the coupling of translational efficiency and the onset of budding might be linked.

Novel Components Important for the Bipolar Pattern

In addition to known proteins, we also found a number of previously uncharacterized proteins that participate in bud site selection. Bud13p/Ygl174w and Ist3 localize to the nucleus and have a slightly reduced Bud8-GFP signal, suggesting that they might be important for the proper expression of Bud8 or other components that help localize Bud8. Bud14p/Yar014c and Bud15p/Ybl047c localize to sites of polarized cell growth. For Bud15p/Ybl047c the localization is more diffuse. Recently, another group identified Bud15/Ybl047c as an Eps homology-containing protein that is similar to Pan1 and End3, which are involved in endocytosis and actin organization (Gagny *et al.*, 2000). Perhaps Bud15/Ybl047c is important in localizing Bud8 and other proteins to their proper sites. Bud14p/Yar014c exhibits a highly polarized localization, similar to Spa2, Pea2, and Bni1. However, unlike *spa2Δ/spa2Δ*, *pea2Δ/pea2Δ*, and *bni1Δ/bni1Δ*, *bud14Δ/bud14Δ* cells are elongated, indicating that they do not have

an apical growth defect. We speculate that Bud14p/Yar014c may be important for the placement or recognition of proximal and distal bipolar tags.

The Unipolar, Random, and Axial-like Budding Patterns

The different mutants affecting the bipolar pattern generally exhibit three types of patterns: unipolar, random, or axial-like budding. As postulated previously, the unipolar patterns are most readily explained by a loss of a functional tag at the appropriate pole (Zahner *et al.*, 1996). Loss of functional Bud8, a component of the distal tag, yields a proximal budding pattern; loss of Bud9, a potential component of the proximal tag, produces a distal pattern. Random budding is most readily explained as either loss or mistargeting of functional tags for both poles.

The new category of mutants described in this study is the axial-like mutants. Previously this budding pattern has only been described for haploid cells (Fujita *et al.*, 1994; Chant and Pringle, 1995; Chant *et al.*, 1995; Halme *et al.*, 1996; Roemer *et al.*, 1996a; Sanders and Herskowitz, 1996). The fact that a significant number of our mutants exhibit this pattern indicates that the axial tags exist but are normally not recognized or are not functional in diploid cells. Consistent with this interpretation, most of the components required for axial budding (Bud3, Bud4, and Axl2/Bud10) are present in diploids (Chant *et al.*, 1995; Halme *et al.*, 1996; Sanders and Herskowitz, 1996). We speculate that in haploids these tags become the preferred site of budding either because the tags are modified to enhance their use or specific machinery that targets the axial sites is present in those cells. One candidate for an axial targeting or modification protein is Axl1 (Fujita *et al.*, 1994); Axl1 is specifically expressed in haploids and can help convert the bipolar pattern to an axial pattern in diploids.

Why do several of the diploid mutants bud axially whereas others bud randomly? We speculate that the random budding mutants either fail to localize bipolar and axial tags or do not recognize these tags. In contrast, the axially budding diploid mutants are specifically defective in forming or recognizing the bipolar tags.

Several Genes Important for Bipolar Budding Are Also Involved in the Axial Pattern

Most mutants that we identified were specific to the bipolar pattern. This includes those affecting the cytoskeleton and polarity, which are important for apical growth. Others components such as transcription factors and cell wall components might be important for helping express, transport, or anchor bipolar tags at their proper sites.

However six of the mutants affect the haploid axial pattern. Bud1 and Bud2 were known previously and are likely to be involved in directing polarity establishment components to the chosen site. As noted above, the coding sequence of Ygr151c partially overlaps with Bud1, and thus mutations in this ORF are likely to affect Bud1. The identification of three new mutants important for bipolar and axial budding doubles the number of gene products thought to be generally important for bud site selection in yeast. Bem4 interacts with several of polarity establishment components (e.g., Rho1 proteins) and may be one of the earliest compo-
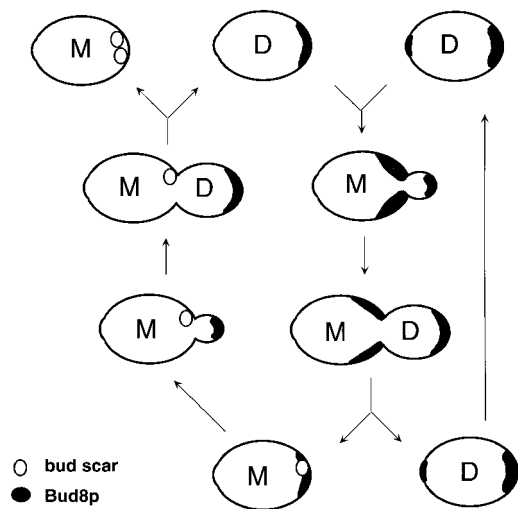

Figure 8. An improved model of Bud8 localization. Bud8, indicated by the gray-shaded areas, might help direct new growth sites at the distal tip during both the first and subsequent divisions. See DISCUSSION for more details. M, mother; D, daughter.

nents delivered to the tag; further characterization of this protein will be reported elsewhere. Cwh8 and Sur4, which have not been well characterized, might participate in anchoring, modifying, and/or transporting both axial and bipolar tags (see above).

It is interesting to note which mutants did not affect haploid axial budding. Several of the cell wall components, Fks1 and Gas1, and Erg3 and Erg4, which are involved in ergosterol biosynthesis, are imporant for only bipolar budding and not axial, suggesting that these constituents are specific for the bipolar tags. Likewise, the Pmt2 and Mnn2 proteins, which act later in the carbohydrate modification pathway, are involved in bipolar budding and not axial budding; whereas Pmt4, which acts at the initial step, does affect axial budding. These results indicate that early chain modification is required for the Axl2 tag and the axial pattern, whereas late chain modification is required for the bipolar pattern.

Bud8 as a Distal Tag in Both New Mother and Daughter Cells

The observation that Bud8 is found both at the tip of the bud and at the neck in new mother-daughter provides an improved model for bud site selection in diploids (Figure 8). As suggested previously (Zahner *et al.*, 1996; Taheri *et al.*, 2000), Bud8 at the tip might serve as part of a distal tag for marking bud formation at that site (alternatively, it might help establish the tag). The Bud8 that resides at the neck of the new mother might also serve as a labile tag and help mark the distal site for the first several divisions. This would account for the distal site bias of new mothers. Consistent with this hypothesis, an increase of Bud8p activity by overexpression of a Bud8-GFP construct results in an increase in distal budding through several generations. Thus, Bud8 may be part of the distal tag, as suggested previously (Zahner *et al.*, 1996; Taheri *et al.*, 2000).

Genome-wide Mutant Screens

This screen and that of Page, Beaulieu, Maynard, Azuma, Dijkgraaf, Li, Marcoux, Nguyen, Sdicu, and Bussey (unpublished results) are the first genome-wide screen of a defined null mutant collection. Previously, the largest collection of defined mutant alleles screened in yeast contained ~2000 genes mutated by transposon insertion; these transposon-mutagenized strains were screened for disruption phenotypes with the use of 20 different growth conditions (Ross-Macdonald *et al.*, 1999). The advantage of screening a bank of deletion strains is that nearly every nonessential gene may be investigated for its role in a given cellular process. Our screen for genes involved in bipolar bud site selection identified both previously characterized and novel genes.

At the time we carried out this screen, deletion strains for 4168 genes were available, which accounts for mutations in most annotated genes. We identified nearly all known nonessential genes (16 of 19) affecting bipolar bud site selection, including *RSR1/BUD1, BUD2, BUD6, BUD7, BUD8, BUD9, SPA2, BNI1,* and *SUR4* (Park *et al.*, 1993; Michelitch and Chant, 1996; Zahner *et al.*, 1996; Amberg *et al.*, 1997; Evangelista *et al.*, 1997; Sheu *et al.*, 2000; Taheri *et al.*, 2000). The only known mutants in nonessential genes that were not identified were *pea2Δ/pea2Δ*, which was not available at the time of the screen, and *bud5Δ/bud5Δ*, which is not in the deletion collection for technical reasons. In addition, a large number of new genes (113) important for the bipolar pattern were identified. Because many of these mutants (91) corresponded to previously characterized genes, it is possible to identify pathways important for the bipolar pattern. Such models have been valuable in dissecting the secretory pathway (Kaiser *et al.*, 1996). Indeed, based on the types of mutants identified in our screen we can present broad models for bipolar budding in yeast. Thus, one important advantage of screening a defined bank of mutants for which the function of many of the mutated genes is known is that it is possible to obtain a picture of the types of cellular processes that participate in the process of interest.

It is important to note that this collection does not identify all genes involved in a biological process such as bipolar budding. Mutations in essential genes and those that have not been annotated (either because they are <100 codons in length or because they overlap known genes) have not been screened. In addition, redundant genes for bipolar budding would not be identified in this mutation collection, because only one copy has been mutated.

In addition to previously known genes, we found that 22 of our mutants correspond to previously uncharacterized genes. One powerful advantage of screening a large defined collection of strains is that one can determine with certainty which mutants have been screened; thus, in addition to identifying genes that are important for a process, one can determine with certainty what genes are not required for a particular process. In addition, by screening a complete collection of mutants it is possible in principle to achieve saturation (or near saturation) from such an approach.

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