Yeast src Homology Region 3 Domain-binding Proteins Involved in Bud Formation

Yasushi Matsui,* Rie Matsui,* Rinji Akada,† and Akio Toh-e*  
*Department of Biological Sciences, Graduate School of Science, The University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113, Japan; and †Department of Applied Chemistry and Chemical Engineering, Faculty of Engineering, Yamaguchi University, Tokiwadai, Ube 755, Japan

Abstract. The yeast protein Bemlp, which bears two src homology region 3 (SH3) domains, is involved in cell polarization. A Rho-type GTPase, Rho3p, is involved in the maintenance of cell polarity for bud formation, and the rho3 defect is suppressed by a high dose of BEM1. Mutational analysis revealed that the second SH3 domain from the NH2 terminus (SH3-2) of Bemlp is important for the functions of Bemlp in bud formation and in the suppression of the rho3 defect. Boi2p, which bound to SH3-2 of Bemlp, was identified using the two-hybrid system. Boi2p has a proline-rich sequence that is critical for displaying the Boi2p-Bemlp two-hybrid interaction, an SH3 domain in its NH2-terminal half, and a pleckstrin homology domain in its COOH-terminal half. A BOI2 homologue, BOII, was identified as a gene whose overexpression inhibited cell growth. Cells overexpressing either BOII or BOI2 were arrested as large, round, and unbudded cells, indicating that the Boi proteins affect cell polarization. Genetic analysis revealed that BOII and BOI2 are functionally redundant and important for cell growth. Δboii cells became large round cells or lysed with buds, displaying defects in bud formation and in the maintenance of cell polarity. Analysis using several truncated versions of BOI2 revealed that the COOH-terminal half, which contains the pleckstrin homology domain, is essential for the function of Boi2p in cell growth, while the NH2-terminal half is not, and the NH2-terminal half might be required for modulating the function of Bemlp. Overproduction of either Rho3p or the Rho3p-related GTPase Rho4p suppressed the boi defect. These results demonstrate that Rho3p GTPases and Boi proteins function in the maintenance of cell polarity for bud formation.

During bud formation in the yeast Saccharomyces cerevisiae, cell polarity is established for the initiation of bud emergence and it is maintained during bud growth. Patches of actin filaments become concentrated at the bud site, towards which the transport of secretory vesicles is directed for the construction of the daughter cell (Tkacz and Lampen, 1972; Field and Schekman, 1980; Pringle and Hartwell, 1981; Cabib et al., 1982; Adams and Pringle, 1984; Kilmartin and Adams, 1984; Novick and Botstein, 1985; Pringle et al., 1986; Drubin, 1991). The establishment and maintenance of cell polarity require the functions of Rho-type GTPases Cdc42p, Rho3p, and Rho4p, which belong to the Ras superfamily (Johnson and Pringle, 1990; Matsui and Toh-e, 1992a; Imai et al., 1996). GTPases of the Ras superfamily act as molecular switches through their conformational change between the GTP-bound active form and GDP-bound inactive form (Barbacid, 1987; Bourne et al., 1991; Boguski and McCormick, 1993). Defects in either CDC42 or CDC24, which encodes a GTP-GDP exchange factor for Cdc42p, disrupt the asymmetric localization of actin filaments and cause cells to become unbudded, large, and round, an indication that Cdc42p and Cdc24p are essential for the establishment of cell polarity (Sloat and Pringle, 1978; Sloat et al., 1981; Adams et al., 1990; Johnson and Pringle, 1990; Zheng et al., 1994).

Defects in RHO3 cause severe growth defects. Disruption of RHO4, which encodes a Rho3p-related GTPase, enhances the growth defect of Δrho3 cells (Matsui and Toh-e, 1992a). Temperature-sensitive rho3 mutant cells lose cell polarity at nonpermissive temperatures: the asymmetric localization of actin filaments is disrupted in the rho3 cells, and the rho3 cells are arrested as large, round cells, although, in contrast to cdc42 mutant cells, not all of these cells are arrested as unbudded cells (Imai et al., 1996). Depletion of both Rho3p and Rho4p results in lysis of cells that have small buds (Matsui and Toh-e, 1992b). These observations strongly suggest that Rho3p is required for the maintenance of cell polarity for bud growth. The rho3 defect is suppressed by the overexpression of BEM1, an indication that Bemlp has functions that affect...
the Rh3 pathway (Matsui and Toh-e, 1992b). **BEM1** encodes a protein with two copies of the src homology region 3 (SH3) domain (SH3-1 and SH3-2; see Fig. 1 A). Disruption of **BEM1** results in temperature-sensitive growth. At nonpermissive temperatures, **bem1** cells become unbudded, large, and round, with the loss of actin polarization, indicating that Bem1p is involved in cell polarization (Bender and Pringle, 1991; Chenevert et al., 1992). Bem1p can bind to Cdc24p independently of its SH3 domains (Peterson et al., 1994). Cdc24p binds to the Ras-type GTPase Rsr1p/Bud1p, which is required for the proper selection of bud sites (Chant and Herskowitz, 1991; Bender and Pringle, 1989; Zheng et al., 1995). These findings lead to the possibility that Bem1p is a component of the protein complex that is needed for bud emergence. Since SH3 domains mediate protein–protein interactions (e.g., Cicchetti et al., 1992), it is possible that Bem1p recruits another component to the complex via its SH3 domains. To clarify the role of the SH3 domains of Bem1p, we characterized several **bem1** mutants that were defective in these domains. Using the two-hybrid system, we identified Boi2p as a protein that bound to the SH3 domain and was important for the function of Bem1p. In addition, Boi1p, which was functionally redundant with Boi2p, was identified by its inhibitory effect on bud emergence. Our genetic and morphological studies indicate that the function of Boi protein is related to the Rh3 pathway and is important for bud growth. We present a model in which Boi proteins and Rh3p are involved in the modulation of the Bem1p-containing complex for bud growth.

**Materials and Methods**

**Microbiological Techniques**

Rich medium containing glucose (YPD), synthetic minimal medium (SD), and synthetic complete medium (SC) are as described (Sherman et al., 1986). YPGal and SCGal are YPD and SC, respectively, except that 2% glucose is replaced with 5% galactose and 0.3% sucrose. YPGalo1 is YPGal, except that the concentration of galactose is 0.1%. SC-U and SCGal-U are SC and SCGal, respectively, without uracil. SC-L and SCGal-L are SC and SCGal, respectively, without leucine. SC-UT and SCGal-UT are SC and SCGal, respectively, without uracil and tryptophan. Yeast transformations were performed by the method of Ito et al. (1983).

**Strains and Plasmids**

The yeast strains used are listed in Table I. Plasmid pBTM116 is a high copy number plasmid that harbors the 2-μm DNA origin TRP1 and the sequence for the lexA DNA-binding domain. pGAD424 is a high copy number plasmid that harbors the 2-μm DNA origin LEU2 and the sequence for the Gal4 trans-activation (Gal4 TA) domain (Fields and Sternglanz, 1994). Plasmid pRS316-RH03 carries a 1.8-kb KpnI-XhoI fragment that encompasses the coding region for RH03 gene on pRS316, a low copy number plasmid harboring URA3 (Sikorski and Hieter, 1989). Plasmid pKT10 is a high copy number plasmid that carries the 2-μm DNA origin URA3, the TDH3 promoter before a unique EcoRI site, termination codons downstream of the EcoRI site, and the TDH3 terminator (Tanaka et al., 1988). pORP3 and pORP1 are pKT10-based plasmids that carry the coding region of RH03 and the coding region of RHO1, respectively, under the control of the TDH3 promoter (Matsui and Toh-e, 1992b). Plasmid pORP4 is a high copy number plasmid that carries the coding region of RH04 under the control of the PYK1 promoter (Matsui and Toh-e, 1992a). Plasmid pKT10myc was constructed by inserting the sequence for the initiator methionine, a myc epitope tag (EQKLISEEDL), and a multicloning site into the EcoRI site of pKT10. The KpnI-KpnI, BamHI-KpnI, HpaI-KpnI, and KpnI-Dral fragments carrying the **BEM1** sequence (see Fig. 1 A) were inserted in frame into the multicloning site of pKT10myc to generate pBEM1KK, pBEM1A1, pBEM1ΔΔ2, and pBEM1AC, respectively. The 0.6-kb HindIII-Sall fragment carrying the sequence for SH3-2 was removed from pBEM1KK and pBEM1ΔΔ2 to create pBEM1ΔΔ2 and pBEM1ΔΔAC, respectively, and was religated in frame after blunting the overhangs. BEM1 in pBEM1KK lacked the sequence for the 45 NH2-terminal amino acids but was able to serve as a multiplicity suppressor of rho3 (see Fig. 1 B, sector 2). The DNA sequences of the fragments, which were derived from the PCR (Sanger et al., 1980) or oligonucleotide-directed mutagenesis and used for generating plasmids, were determined to confirm precise replication during each procedure. Nucleotide sequences were determined by the method of Sanger et al. (1977).

**Construction of bem1 Mutants with Mutations in the SH3 Domain**

The 2.3-kb fragment, derived from pSR01 (Matsui and Toh-e, 1992b), from the BamHI site in the **BEM1** coding region to the BamHI site in the 3' noncoding region, was inserted into a derivative of pBlueScriptII that had been constructed from pBlueScriptII KS+ (Strategene, La Jolla, CA) by removal of the sequence between the EcoRV and XhoI sites. The 1.1-kb HindIII-HindIII fragment carrying URA3 was inserted into the HindIII site (in the 3' noncoding region of **BEM1**) of the resultant plasmid to generate YipUBEM1. The 0.7-kb Sall-HindIII fragment, carrying the Smal site in the 5' noncoding region of **BEM1** to the SalI site in the sequence for SH3-2, was inserted into the Smal and SalI sites (in the sequence for SH3-2) of YipUBEM1 to generate YipUBEM1. Proline 123 in SH3-1 and proline 208 in SH3-2 were replaced with leucine to generate bem1L123 and bem1L208, respectively, with an oligonucleotide-directed in vitro mutagenesis system (Amersham Corp., Arlington Heights, IL) using the primers 5'-AAAATAGCTTAAAGCACAACC (for bem1L123), 5'-AAACCAAACTAGTACAGGCGG (for bem1L208), and YipUBEM1 as the template. For the construction of bem1LASH3s, a truncated version of bem1L without the sequence for both SH3-1 and SH3-2, the fragment from the Smal site in the 5' noncoding region to the KpnI site in the coding region was inserted in frame between the Smal and SalI sites of YipUBEM1 after blunting the overhangs to generate YipUBEM1ASH3s, which lacked the sequence between the KpnI and SalI sites of YipUBEM1. For the construction of bem1LΔAC, the 1.0-kb Sall-HindIII fragment from pBEM1AC, which contained the **BEM1** sequence between the SalI and DraI sites, the termination codons after the DraI cleavage site, and the TDH3 terminator, was inserted between the SalI and HpaI sites of YipUBEM1 after blunting the overhangs of the HindIII cleavage site. For the replacement of the wild-type **BEM1** allele with a mutant allele, each derivative of YipUBEM1, digested with Smal and XbaI, was introduced into the cells. Replacement was confirmed by PCR.

**Isolation of BOI2 and Assay of the Two-hybrid Interaction**

The construction of plasmids for the lexA DNA-binding domain fused with Bem1p (lexA-Bem1p) was performed as follows. An EcoRI site was introduced before the initiator methionine codon of **BEM1** by oligonucleotide-directed in vitro mutagenesis. The EcoRI-HpaI fragment of bem1 Leu123, which was transformed with plexBEM1 and then with a yeast genomic library in which yeast genomic DNA was expressed as fusion proteins with the Gal4 TA domain (Fields and Hieter, 1989), was identified by its inhibitory effect on bud emergence. Our genetic and morphological studies indicate that the function of Boi protein is related to the Rh3 pathway and is important for bud growth. We present a model in which Boi proteins and Rh3p are involved in the modulation of the Bem1p-containing complex for bud growth.

---

1. **Abbreviations used in this paper.** a.a., amino acid; GST, glutathione S-transferase; PH, pleckstrin homology; SC, synthetic complete medium; SD, synthetic minimal medium; SH3, src homology region 3; YPD, rich medium containing glucose.
lacZ expression was examined by measuring the activity of β-galactosidase in L40 cells with the method described (Miller, 1972). DNA clones carrying BOI2 were isolated from a yeast genomic library (Matsui and Toh-e, 1992a) in XZAPII (Stratagene) by hybridization using the ρsSKBOI2 as a probe. ρsSKBOI2 was a plasmid derived from the yeast genomic library and carried a 6-kb fragment encompassing BOI2 in pBlueScript SK− (Stratagene).

Construction of Plasmids for the Two-hybrid Interaction Assay

Plasmids for truncated versions of lexA-Bemlp were constructed as follows. plexASHs was created by removing the EcoRI-SalI (in the sequence Plasmids for truncated versions of lexA-Bemlp were constructed as follows. AatII site for the region (a.a. 436-464) that contained the proline-rich sequence) and the PstI site (in the multicloning site of pBTM116) by hybridization using the same method described (Matsui and Toh-e, 1992b). Toh-e, 1992a) in hZAPII (Stratagene) by hybridization using the same method described (Matsui and Toh-e, 1992b).

Interaction Assay

Toh-e, 1992a) in hZAPII (Stratagene) by hybridization using the same method described (Matsui and Toh-e, 1992b).

Yeast SH3-binding Proteins

In Vitro Binding Assay

The Nc1-EcoRI fragment encoding Boi2p (amino acids [a.a.] 67-545) was inserted into the multicloning site downstream of the sequence for glutathione S-transferase (GST) in pGEXKG. Boi2p (a.a. 67-545) fused with GST (GST-Boi2p) was produced by use of this construct and affinity purified with glutathione-agarose beads (Sigma Immunochemicals, St. Louis, MO) as described (Shirayama et al., 1994). About 10⁶ yeast cells (wild-type strain K12) producing myc-tagged Bemlp1 were washed twice in PBS. The cell pellet was disrupted by blending with glass beads in 400 μl of lysis buffer (50 mM Tris-HCl, pH 7.5, 5 mM EDTA, 0.5 mM PMSF) and was clarified by centrifugation at 14,000 g for 15 min at 4°C. 30 μl of the supernatant (as the total cell lysate) was resolved by SDS-PAGE, and 160 μl of the supernatant was mixed with 50 μl GSH-agarose beads with 2 μg immobilized GST-Boi2p (or GST for control) and incubated at 4°C for 3 h. The beads were then washed extensively in washing buffer (50 mM Tris-HCl, pH 7.5, 0.1% [vol/vol] Triton X-100, 10% [vol/vol] glycerol). Bound proteins were eluted with 100 μl elution buffer (50 mM Tris-HCl, pH 9.0, 20 mM glutathione) and 20 μl of the eluate were resolved by SDS-PAGE.

Isolation of BOI1

YEp51B, which carries LEU2, the 2-μm DNA origin, and the GAL10 promoter, was constructed by removing a 0.27-kb BamHI-Sall fragment adjacent to the GAL10 promoter from YEp51 (Broach et al., 1983). Diploid strain DRA4 was transformed with a yeast genomic DNA library, based on YEp51B, in which yeast genomic DNAs were expressed under the control of the GAL10 promoter. The resultant transformants that grew well on SC-L, but not on SGCαL (which contained 2% galactose, instead of 5% galactose and 0.3% sucrose), were selected at 28°C, and the plasmids were recovered (details of the screening will be described elsewhere). pGA58, one of the isolates, carried BOI1 downstream of the GAL10 promoter. The BOI1 gene in pGA58 was expressed from the internal methionine at position 378, as judged from a comparison of the nucleotide sequences of pGA68 and BOI1 (Bender et al., 1996).

BOI2 Expression Plasmids and Disruption of BOI1 and BOI2

YEpUGAL7 is a YEp plasmid that carries the GAL7 promoter. URA3, and the BamHI and Sall sites downstream of the GAL7 promoter (Matsui and...
A BamHI site was introduced before the initiator methionine codon of BOI2 by oligonucleotide-directed in vitro mutagenesis, and the BglII site in the 3' noncoding region of BOI2 was replaced with a Sall site by insertion of a Sall linker (Takara, Tokyo, Japan). The BamHI-Sall fragment carrying the complete BOI2 coding region was inserted between the BglII and Sall sites of YipUGAL7 to create YipUGAL7BOI2. In this context, the pGAL7:BOI2AC, pGAL7:BOI2B, and pGAL7:BOI2C were expressed under the control of the GAL7 promoter. YipUGAL7BOI2 was digested with BglII and religated after blunting the overhangs to create YipUGAL7BOI2AC. From this construct, designated pGAL7:BOI2AC, Boi2p without its COOH-terminal half was produced under the control of the GAL7 promoter. Synthetic oligonucleotides containing the initiating methionine codon and the Xhol and BglII sites were inserted into the BglII site of YipUGAL7 to create YipUGAL7f. The 2.1-kb AatII-SalI fragment containing the sequence for the COOH-terminal half of Boi2p (a.a. 466–end) was inserted between the Xhol and Sall sites of YipUGAL7f after blunting the overhangs of the Xhol and AatII cleavage sites to create YipUGAL7BOI2F. From this construct, designated pGAL7:BOI2F, Boi2p without its NH2-terminal half was produced under the control of the GAL7 promoter. An ~6-kb Sall Slee-Sall fragment carrying BOI2 from pSKBOI2 was inserted into pYO324, a high copy number plasmid that carries 2-µm DNA origin and TRP1 (Ohya et al., 1991), and into pRS314, a low copy number plasmid that carries CEN6 and TRP1 (Sikorski and Hieter, 1989), to create pYO324-BOI2 and pRS314-BOI2, respectively. An AatII site was introduced downstream of the 9th codon of BOI2 in pSKBOI2 by oligonucleotide-directed in vitro mutagenesis, and the resultant plasmid was digested with AatII and religated in frame to create pSKBOI2AN. This construct (BOI2AN) encodes Boi2p without its NH2-terminal half (a.a. 10–464). An ~5-kb Sall Slee-Sall fragment carrying BOI2AN was inserted into pYO324 and pRS306, a Yip plasmid carrying URIA3 (Sikorski and Hieter, 1989), to create pYO324-BOI2AN and pRS306-BOI2AN, respectively. pYO324-BOI2 and pRS304-BOI2 were digested with BglII and religated after blunting the overhangs to create pYO324-BOI2AC and pRS314-BOI2AC, respectively. From these constructs, designated BOI2AC, Boi2p without its COOH-terminal half (a.a. 697–end) was produced. The YipUGAL7l and pRS306-derived plasmids were digested with StuI and then introduced into cells for targeted integration at the ura3 locus.

The BOI2 sequence between the Pmll site (in the 5' noncoding region) and the Sall site of pSKBOI2 was replaced with the 1.1-kb URIA3 fragment to create pBOI2Δ. In this construct, the sequence from the position 443 bp upstream of the initiation codon to the 829th codon of BOI2 was deleted. The plasmid for the disruption of BOI1 was constructed as follows. The 2.8-kb Sall HindIII fragment from the insert of pGA68 was inserted into pBluescript KS to create pSKBOI1. To create pRS306-BOI1, the 2.2-kb BamHI-BamHI fragment that carried the 3' half of the BOI1 gene from pGA68 was inserted into the BamHI site of pRS306. To clone the 3' half of BOI1, the yeast genomic DNA in which pRS306-BOI1 had been integrated at the BOI1 locus was digested with EcoRV, religated, and introduced into Escherichia coli. With the recovered plasmid as a template, a 860-bp fragment carrying the 5' noncoding region of BOI1 and the sequence for the NH2-terminal portion (a.a. 1–87) of Boi1p was amplified by PCR. In the PCR, the primer was designed to replace the 87th codon of BOI1 in the amplified fragment with a stop codon, TAG. The amplified fragment was inserted into the BamHI site of pKSBOI1 to create pKSBOI1-2, which carried a 3.3-kb insert encompassing a version of BOI1 in which the sequences for a.a. 91–374 were deleted. A 1.8-kb BamHI fragment carrying HIS3 was inserted into the BglII site in the BglII coding region of pKSBOI2-2 to create pBOI1Δ. In this construct, the BOI1 gene was disrupted with the termination codon at the 87th codon, with the deletion of the sequence for a.a. 91–374 and with the insertion of HIS3 pBOI1Δ and pBOI1Δ were digested with PvuII and introduced into the cells by replacement transformation to disrupt BOI1 and BOI2, respectively. These replacements did not disrupt any open reading frames other than BOI1 and BOI2, as judging from nucleotide sequences. The disruptions were confirmed by Southern analysis and PCR.

**Cell Lysis Assay**

Cell lysis was assayed by monitoring the leakage of alkaline phosphatase, a yeast intracellular protein, into the culture medium by the method described (Paravicini et al., 1992). Cells were streaked on YPD plates. After incubation, the plates were overlaid with a solution of BCIP (10 mM 5-bromo-4-chloro-3-indolyl phosphate [Sigma], 0.1 M Tris-HCl, pH 9.5, and 1% agar) and incubated for 1 h at 37°C.

**Detection of myc Epitope-tagged Protein**

Proteins containing the myc epitope tag were detected by Western blotting analysis with anti-myc antibodies as described (Yoko-o et al., 1995).

**Morphological Observations**

Cells were stained with rhodamine-phalloidin to reveal actin filaments as described (Pringle et al., 1989). Cells were fixed with 5% formaldehyde for 10 min, washed with PBS, stained with 1:50 diluted rhodamine-phalloidin solution (Molecular Probes, Inc., Eugene, OR) for 2 h, and washed five times with PBS. The sample was then mounted in p-phenylene-diamine (1 mg/ml in 90% glycerol) and observed under an epifluorophotomicroscope (BH-2, Olympus, Tokyo, Japan). Dead cells were stained with methylene blue (Rose, 1975). A solution of methylene blue (0.02% methylene blue, 2% sodium citrate) was mixed with an equal volume of cell culture, and the cells were observed immediately with Nomarski optics.

**Results**

**Bem1p Domains Required for Suppression of the Δrho3 Defect**

Overexpression of BEM1 suppresses the growth defect caused by the disruption of RHO3 (Matsui and Toh-e, 1992b; Fig. 1 B, sector 2). To examine whether the SH3 domains of Bem1p are necessary for the suppression or not, we constructed truncated BEM1 sequences (shown diagrammatically in Fig. 1 A) and analyzed the suppression activities of the various constructs. Δrho3 cells that overexpress BEM1 without the sequence for SH3-1 grew as well as Δrho3 cells that overexpressed BEM1 (Fig. 1 B, right, sectors 2 and 3), indicating that SH3-1 is not required for the ability of BEM1 to serve as a multicopy suppressor of rho3. However, Δrho3 cells that overexpressed BEM1 without the sequence for SH3-2 (Fig. 1 B, right, sectors 4, 5, and 7) or the COOH terminus of 35 a.a. (Fig. 1 B, right, sectors 6 and 7) formed colonies as tiny as those of Δrho3 cells that harbored a control plasmid (Fig. 1 B, right, sector 8), indicating that these truncated versions of BEM1 did not suppress the Δrho3 defect. The deletion of SH3-2 or of the COOH-terminal sequence did not reduce the amount of the myc-tagged Bem1p, as judged by Western analysis (data not shown). These results indicate that SH3-2 and the COOH-terminal sequence are critical if BEM1 is to function as a multicopy suppressor of rho3.

**The Effects of Substitutions in the SH3 Domains and of Deletion of the COOH-terminal Region of Bem1p on Cell Growth**

The replacement of the conserved proline residue by leucine destroys the function of an SH3 domain in the serine/threonine protein kinase SH3-2 (Zakeri and Hieter, 1992). We introduced the corresponding mutation into SH3-1 (bem1 Leu123) and SH3-2 (bem1 Leu208). The bem1 Leu208 cells and bem1 Leu123, Leu208 cells displayed temperature-sensitive growth (Fig. 1 C, sectors 3 and 4). At 37°C, the cells were arrested as large, round, unbudded cells, and the asymmetric organization of actin filaments was disrupted in these cells (data not shown). These phenotypes are similar to those of the Δbem1 cells. By contrast, bem1 Leu123 cells grew as well as wild-type cells, even at elevated temperatures (Fig. 1 C, sector 2). These results indicate that SH3-2 is important for the function of Bem1p in bud emergence.
The COOH-terminal 35 a.a. of Bem1p is essential for the suppression of the Δrho3 defect (Fig. 1 B). We examined whether the COOH-terminal 35 a.a. is also important for the function of Bem1p in cell growth or not. We replaced the wild-type BEM1 allele with a truncated allele that lacked the sequence for the COOH-terminal 35 a.a. (bem1-AC). The bem1-AC cells displayed temperature-sensitive growth (Fig. 1 C, sector 5) and were arrested as large, round, unbudded cells at 37°C (data not shown). These results indicate that the COOH-terminal 35 a.a. is important for the function of Bem1p in bud emergence.

It has been reported that the COOH-terminal half of Bem1p interacts with the COOH-terminal half of Cdc24p (Peterson et al., 1994). We examined whether the COOH-terminal 35 a.a. of Bem1p is required for the Cdc24p-Bem1p interaction using two-hybrid system (Fields and Stern, 1994). The lexA-Bem1p fusion (from plexBEM1) increased the level of expression of lacZ in L40 cells (lexA-lacZ strain), with the increase depending on the presence of the Ga14TA-Cdc24p fusion from pGADCDC24. The lexA-fused Bem1p without the COOH terminus of 35 a.a. (from plexBEM1ΔC), however, did not (Table II). These results indicate that the
COOH-terminal 35 a.a. of Bemlp is required for the two-hybrid interaction between Cdc24p and Bemlp.

**Isolation of BO12**

A screening was made for genes that encode the Bemlp-binding protein using the two-hybrid system. Plasmid plexBEM1, carrying the sequence encoding the lexA-Bemlp fusion and the yeast *S. cerevisiae* genomic library expressed as fusion proteins with the Gal4 TA domain (Chien et al., 1991), were introduced into L40 cells that carried *lexA-lacZ* and *lexA-HIS3*. The plasmids were then recovered from the colonies that formed on selective plates (SD+3AT). Among the plasmids recovered from the His+ transformants, only plasmid pGADBO12 increased the level of expression of both *HIS3* and *lacZ* in L40 cells in a plexBEM1-dependent manner. DNA clones were isolated from the yeast genomic library (Matsui and Toh-e, 1992a) with a fragment from pGADBO12 as a probe. The nucleotide sequence of the clones revealed a gene that encoded a 1,040-a.a. protein with an SH3 domain, a proline-rich sequence, and a pleckstrin homology (PH) domain (Muscachio et al., 1993; Fig. 2). A homology search of the Genbank database using the FASTA program revealed a homologue with 38% identity. This homologous gene was identified on the basis of its two-hybrid interaction with Bemlp1 (Bender et al., 1996). Both groups designated these genes BO11 and BO12 (as bem one interacting), and the gene on pGADBO12 was designated BO12. Four domains of Boi2p, namely, domain I (residues 39–113) including an SH3 domain (residues 50–102), domain II (residues 266–331), domain III (residues 393–462) including a proline-rich sequence (residues 438–458), and domain IV (residues 731–943) including a PH domain (residues 767–891), were highly homologous to those of Boi1p (Fig. 2 B), with the extent of identity being 71, 65, 78, and 69%, respectively.

**Domains That Are Required for the Two-hybrid Interaction between Bemlp1 and Boi2p**

To identify the sequence responsible for the two-hybrid interaction between Bemlp1 and Boi2p, several constructs that encoded lexA-Bemlp1 fused proteins with truncation and/or mutations (shown diagrammatically in Fig. 3 A) were introduced into L40 cells that harbored pGADBO12, and the two-hybrid interaction was examined. The BEM1 sequence for the NH2-terminal half (a.a. 1–325), which contained both SH3 domains, was sufficient for the interaction (Fig. 3 A, plexΔC). Introduction of the bem1Leu208 mutation into this BEM1 sequence, however, abolished the increase in the expression level of the reporter genes (Fig. 3 A, plex-s2 and plex-s12). By contrast, the plasmid carrying only the bem1Leu123 mutation increased the expression (Fig. 3 A, plex-s1). These results indicate that SH3-2 is essential for the Bemlp1–Boi2p interaction.

To identify the Boi2p domain that participates in the two-hybrid interaction, we constructed several plasmids that encoded Gal4TA–Boi2p fused proteins, as shown diagrammatically in Fig. 3 B. In the original isolate, pGADBO12, the BO12 sequence was ligated with the sequence for Gal4TA at the Sau3AI site that was located in the sequence for the SH3 domain of Boi2p, and the SH3 domain in the fusion was disrupted (see Figs. 2 A and 3 B). Thus, the SH3 domain of Boi2p was dispensable for the Boi2p–Bemlp1 interaction. The deletion of the COOH-terminal sequence of Boi2p (threonine 465–end) did not reduce the interaction (Fig. 3 B, pGAD△A1), an indication that the NH2-terminal half of Boi2p is sufficient for the interaction. More extensive deletion of the COOH-terminal sequence (alanine 442–end including the proline-rich sequence), however, abolished the interaction (Fig. 3 B, pGAD△A2). The deletion of the sequence for a.a. 436–464, including the proline-rich sequence, also abolished the interaction (Fig. 3 B, pGADAPRO). Proline-rich sequences are reported to be the motif of SH3 domain–binding sites (e.g., Cicchetti et al., 1992). These results suggest that the interaction between Boi2p and Bemlp1 is mediated by the interaction between the proline-rich sequence of Boi2p and SH3-2 of Bemlp. Deletion of the NH2-terminal sequence (a.a. 1–266), however, also abolished the interaction (Fig. 3 B, pGAE2A2). It is likely that the NH2-terminal sequence affects the conformation of Boi2p, allowing the proline-rich sequence to bind efficiently or, alternatively, the NH2-terminal sequence might stabilize the Gal4–Boi2p fusion protein.

**The Interaction of Bemlp1 and Boi2p In Vitro**

The interaction of Boi2p with Bemlp1 was examined in vitro with purified GST-Boi2p. Yeast cells were transformed with the plasmids for myc-tagged Bemlp1, shown diagrammatically in Fig. 1 A. The myc-tagged Bemlp1 in the lysates was detected with anti-myc antibodies (Fig. 3 C, left). The lysates containing myc-tagged Bemlp1 were incubated with GST-Boi2p that had been bound to GSH beads. The versions of Bemlp1 that possessed SH3-2 were coprecipitated with GST-Boi2p (Fig. 3 C, right, lanes 1, 2, and 5), whereas the versions of Bemlp1 that lacked SH3-2 only coprecipitated at very low levels, if at all, with GST-Boi2p (Fig. 3 C, right, lanes 3, 4, and 6). Both the two-hybrid experiments and the in vitro binding assays indicate that SH3-2 is critical for the Boi2p–Bemlp1 interaction and that SH3-1 is not essential for this interaction.

**Phenotypes of Cells that Overexpressed BO11 and BO12**

**BO11** was identified during a screening for genes whose expression, under the control of the GAL10 promoter, was lethal to yeast cells. The isolated BO11 was fused to the GAL10 promoter at the Sau3AI site located in the sequence for codons 374–375 of BO11. In this fused gene, the 378th methionine codon is expected to be the initiation codon. Cells that carried the multicopy plasmid that harbored the **BO11** sequence under the control of the **GAL10** promoter did not grow on SCGal-L, a galactose-containing selective medium, and they were arrested as large.

---

**Table II. Assay of Two-hybrid Interaction between Cdc24p and Bemlp**

| Plasmid       | pGAD424 | pGADCDC24 |
|---------------|---------|-----------|
| pBTM116       | 0.4     | 0.7       |
| plexBEM1      | 7       | 54        |
| plexBEMΔC     | 4       | 0.5       |

β-Galactosidase activities (units) of L40 cells that harbored plasmids in the indicated combinations are shown.
Figure 2. Amino acid sequence of Boi2p. (A) The nucleotide and deduced amino acid sequences of Boi2p. The nucleotide sequence of Boi2p has been deposited in DDBJ/EMBL/Genbank under the accession No. D38310. The Sau3A1 restriction site, namely, the site of the junction with the sequence for Gal4 TA in pGADBOI2, is indicated by a box. The restriction sites for BstEII, XbaI, HindIII, BanII, AatII, EcoRI, and BglII, which were used for the construction of truncated versions of Boi2p (shown schematically in Fig. 3 B) are underlined. (B) Comparison of the amino acid sequences of Boi2p and Boilp. Identical residues are boxed in black. Domains I, II, III, and IV are underlined.
The overproduction of Boi2p without its NH\textsubscript{2}-terminal domain, and unbudded cells (Fig. 4 Ab). Overexpression of Boi2 from the GAL7 promoter (pGAL7:BOI2) also inhibited cell growth (Fig. 4 B, sector 4), and cells overexpressing Boi2 were arrested as large, round, unbudded cells (Fig. 4 Ab). The asymmetric organization of actin filaments was disrupted in cells that overexpressed Boi1 and in cells that overexpressed Boi2 (data not shown). These results indicate that overexpression of Boi1 and Boi2 inhibits bud emergence, and that the Boi proteins affect cell polarization.

To identify the domain that is important for the inhibitory effect, we constructed two truncated versions of Boi2, namely pGAL7:BOI2\textsubscript{AN} and pGAL7:BOI2\textsubscript{AC}. The overproduction of Boi2p without its NH\textsubscript{2}-terminal half from pGAL7:BOI2\textsubscript{AN} inhibited cell growth (Fig. 4 B, sector 3) and cells expressing pGAL7:BOI2\textsubscript{AN} were arrested as large, round, unbudded cells (data not shown). The overproduction of Boi2p without its COOH-terminal half from pGAL7:BOI2\textsubscript{AC}, however, did not have any inhibitory effect on cell growth (Fig. 4 B, sector 2). These results indicate that the COOH-terminal half that contains domain IV is important for inhibition of bud emergence, while the NH\textsubscript{2}-terminal half that contains domains I, II, and III is not essential for the inhibition.

The finding that the NH\textsubscript{2}-terminal half is not essential for the inhibition suggests that the Boi1p–Boi2p interaction is not critical for the inhibitory effect. To clarify this, we introduced both constructs for the overexpression of Boi1 genes (pGA68 and pGAL7:BOI2) into bem1 mutant cells in which bem1 lacked the sequence for both SH3 domains (bem1-\textsubscript{ASH3}s). The overexpression of either Boi1 or Boi2 inhibited the growth of the bem1-\textsubscript{ASH3}s cells (data not shown). These results indicate that the protein–protein interaction between Boi1p and the Boi proteins is not critical for the inhibitory activity of the overexpressed Boi1 gene.
**Figure 4.** Overexpression of *BOI*. (A) The morphology of *BOI*-overexpressing cells (*a* and *b*). (*a*) Cells (strain W303-1A) carrying pGA68 and cultured in SC-L were shifted to SCGal-L, and (*b*) cells (strain W303-1A) carrying pGAL7:BOI2 and cultured in YPD were shifted to YPGal. Cells were harvested 8 h after the shift. (B) Cells (strain W303-1A) carrying a dummy plasmid YlpUGAL7 (sector 1), pGAL7:BOI2ΔC (sector 2), pGAL7:BOI2ΔN (sector 3), or pGAL7:BOI2 (sector 4) were streaked on an SCGal-U plate and incubated at 30°C for 2 d.

**Phenotypes of the boi-disrupted Cells**

Disruption of neither *BOI1* nor *BOI2* alone resulted in a cell growth defect (Fig. 5 A, sectors 2 and 3). However, all but one of ~200 Δboi1 Δboi2 spores isolated from Δboi1+/Δboi2/+ heterozygous diploid cells failed to form colonies. The single viable Δboi1 Δboi2 spore formed a tiny colony that grew very poorly. Δboi1 Δboi2 cells with *BOI2* under the control of the *GAL7* promoter (pGAL7:BOI2) grew on medium that contained 0.1% galactose, which induced the expression of pGAL7:BOI2 with low efficiency. Δboi1 Δboi2 pGAL7:BOI2 cells dramatically reduced the growth rate 24 h after a shift to glucose-containing medium, which repressed the expression of pGAL7:BOI2 and then grew very poorly (Fig. 5 A, sector 4). Δboi1 Δboi2 pGAL7:BOI2 cells grew very poorly on glucose-containing medium at all temperatures tested (20, 25, 30, and 37°C). These results indicate that *BOI1* and *BOI2* are functionally redundant, and that the Boi proteins are important for cell growth.

About 24 h after the shift to glucose-containing medium, Δboi1 Δboi2 pGAL7:BOI2 cells began to stop growing and lysed, as judged from the leakage of alkaline phosphatase into the medium (Fig. 5 B). From the staining of
dead cells with methylene blue (Rose, 1975), it appeared that ~30% of all the cells in the culture 24 h after the shift were dead, and ~95% of these dead cells were budded (Fig. 6 a). Within 48 h after the shift, the fraction of dead budded cells increased to ~60% of the total cells. The remaining cells were large and round (Fig. 6 a), and in these large round cells, the asymmetric organization of actin filament (as observed in wild-type cells; Fig. 6 b) was disrupted (Fig. 6 d). A majority of ∆boi1 ∆boi2 cells lysed with buds, and the death of these ∆boi1 ∆boi2 cells was not suppressed by the addition of an osmotic stabilizer into the medium (data not shown). In the presence of an osmotic stabilizer that partially prevented cell lysis, however, most of the ∆boi1 ∆boi2 cells were uniformly observed as large and round cells in the glucose-containing medium rather than as dead budded cells. Furthermore, the asymmetric organization of actin filaments was disrupted in these large round cells (Fig. 6, e and f), indicating that these ∆boi1 ∆boi2 cells have lost cell polarity. These morphological observations strongly suggest that cells without the Boi function failed to maintain cell polarity, and that the loss of cell polarity caused a defect in bud growth and subsequent cell lysis.

The Boi2p Domain That Is Required for Cell Growth

To identify the domain that is critical for the Boi function, we constructed two truncated versions of BOI2: BOI2AN, which lacked the sequence for domains I, II, and III; and BOI2AC, which lacked the sequence for domain IV. ∆boi1 ∆boi2 cells carrying BOI2AN grew as well as wild-type cells (Fig. 5 A, sector 5), but ∆boi1 ∆boi2 cells carrying BOI2AC on either a low copy number plasmid or a high copy number plasmid grew as poorly as ∆boi1 ∆boi2 cells (Fig. 5 A, sectors 6 and 7). These results indicate that the COOH-terminal half is essential and sufficient for cell growth while the NH2-terminal half is dispensable.

The Effect of BOI2 on ∆rho3 Cells

Boi2p interacted with SH3-2, which was required for suppression by Bem1p of the ∆rho3 defect. This result may suggest the involvement of Boi2p in the suppression. We examined whether or not multiple copies of BOI2 could suppress the ∆rho3 defect. Although the overexpression of BOI2 from pGAL7:BOI2 inhibited cell growth, the introduction of pYO324-BOI2, a high copy number plasmid that harbored BOI2, into cells did not inhibit the growth of wild-type cells or ∆rho3 cells that had been rescued by overexpression of ∆rho3 cells that carried pYO324-BOI2 was as poor as that of ∆rho3 cells without pYO324-BOI2 (Fig. 7, right, sectors 1 and 2), indicating that BOI2 can not serve as a multicopy suppressor of the ∆rho3 defect. Conversely, multiple copies of BOI2 inhibited the growth of ∆rho3 cells that overexpressed BEM1. The growth defect of ∆rho3 cells was rescued by overexpression of BEM1 (Fig. 7, right, sector 3), but in the presence of pYO324-BOI2, the growth of the ∆rho3 cells that overexpressed BEM1 was as poor as that of ∆rho3 cells without the BEM1 overexpression plasmid (Fig. 7, right, sector 4). However, neither BOI2AN nor BOI2AC on a high copy number plasmid did not show the inhibitory effect (Fig. 7, right, sectors

Figure 5. Disruption of BOI. (A) Strains to be tested were streaked on a YPD plate and incubated at 30°C for 2 d as follows. Wild-type strain W303-1A (sector 1); ∆boi1 strain YMB101 (sector 2); ∆boi2 strain YMB202 (sector 3); ∆boi1 ∆boi2 pGAL7:BOI2 strain YMB1207 (sector 4); ∆boi1 ∆boi2 BOI2AN strain YMB1202 (sector 5); ∆boi1 ∆boi2 pGAL7:BOI2 strain YMB1207 carrying pRS314-BOI2AC (sector 6); and ∆boi1 ∆boi2 pGAL7:BOI2 strain YMB1207 carrying pYO324-BOI2AC (sector 7). (B) Wild-type strain W303-1A (1), ∆boi1 ∆boi2 pGAL7:BOI2 strain YMB1207 (2), and ∆rho3 pGAL7:RHO4 strain YMR505 (3) were streaked on a YPD plate, incubated for 40 h at 30°C, and overlaid with a solution of BCIP. Black regions in the photograph were blue and indicated cell lysis.
5 and 6). These results indicate that the NH₂-terminal half and the COOH-terminal half of Boi2p is critical for the inhibitory activity. It might be possible that the BEM1-overexpressing Δrho3 cells are hypersensitive to the inhibitory effect of BOI2 on bud emergence, which was observed in case of pGAL7:BOI2. Such was not the case, however, since in addition to the difference in required domains, the BEM1-overexpressing Δrho3 cells that harbored pYO324-BOI2 showed morphology similar to that of Δrho3 cells without the BEM1 overexpression plasmid (showing an increase of the proportion of lysed cells with buds) instead of becoming large, round, unbudded cells (data not shown). These results suggest that Boi2p inhibits the function of Bem1p in the suppression of the Δrho3 defect.

**Genetic Interactions among the BOI Genes, RHO3, and RHO4**

Since (a) Boi2p interacts with Bem1p, (b) BEM1 interacts genetically with RHO3, and (c) the morphological pheno-
Figure 7. Effect of multiple copies of BOI2 on the growth of ∆rho3 cells. ∆rho3 pGAL7:RH04 strain YMR505, carrying the indicated plasmids, was incubated on SCGal-UT (left) and SC-UT (right) plates at 30°C for 4 d. The ∆rho3 strain YMR505 grew very poorly on glucose-containing medium but grew well on galactose-containing medium as a result of the overexpression of RH04 from pGAL7:RH04 (Matsui and Toh-e, 1992b). Plasmids were as follows: pYO324 and pKT10, dummy plasmids (sector 1); pYO324-BOI2, a high copy number plasmid that harbored BOI2, and pKT10 (sector 2); pBEM1KK, a BEM1-overexpressing plasmid, and pYO324 (sector 3), pBEM1KK and pYO324-BOI2 (sector 4); pBEM1KK and pYO324-BOI2AN, a high copy number plasmid that harbored BOI2AN (sector 5); pBEM1KK and pYO324-BOI2AC, a high copy number plasmid that harbored BOI2AC (sector 6).

(type of the ∆boi1 ∆boi2 cells is similar to that of ∆rho3 cells (Matsui and Toh-e, 1992b), we examined whether BEM1, RH03, or RH03-related genes could serve as multicopy suppressors of the boi defect. Plasmids were introduced into ∆boi1+/∆boi2+/ heterozygous diploid cells, and the transformants were sporulated and dissected. After incubation at 25°C for 3 d, ∆boi1 ∆boi2 segregants carrying plasmids that overexpressed either RH03 or RH04 (i.e., plasmids pOPR3 or pOPR4) formed visible colonies. They grew as well as wild-type cells and much better than ∆boi1 ∆boi2 cells without pOPR3 and pOPR4 (Fig. 8). These results indicate that both RH03 and RH04 can serve as a multicopy suppressor of boi. Plasmids overexpressing either BEM1 or RHO1, encoding a Rho-type GTPase, (i.e., pBEM1KK or pOPR1) did not rescue the growth defect of ∆boi1 ∆boi2 segregants (data not shown).

Discussion

Functions of the Boi Proteins

The phenotypes of ∆boi1 ∆boi2 cells resemble those of cells depleted of both Rho3p and Rho4p. ∆rho3 ∆rho4 cells carrying pGAL7:RH04, RH04 under the control of the GAL7 promoter cease to grow in a glucose-containing medium, and the Rho3p- and Rho4p-depleted cells lyse with buds (Matsui and Toh-e, 1992b; Fig. 5 B). In the presence of an osmotic stabilizer, Rho3p- and Rho4p-depleted cells become large and round, and the asymmetric organization of actin filaments is disrupted (Matsui and Toh-e, 1992b). The phenotypes of the Boi protein-depleted cells (Figs. 5 B and 6) were quite similar to those of Rho3p- and Rho4p-depleted cells, and they strongly suggest that these cells are defective in the maintenance of cell polarity for bud growth. Moreover, both RH03 and RH04 can serve as a multicopy suppressor of the boi defect. Both the strong genetic interactions and the phenotypes of mutants strongly suggest that the Boi proteins, Rho3p, and Rho4p are all involved in the same process that maintains cell polarity for bud growth.

Domains of Boi Proteins

Boi2p possesses four domains that are highly conserved in Boi1p (Fig. 2 B), and the high degree of conservation suggests that the domains play an important role in the function of the Boi proteins. Domain III contains a proline-
rich sequence that is required for displaying the two-hybrid interaction with SH3-2 of Bem1p (Fig. 3). Domain I contains an SH3 domain that can interact with an SH3 domain–binding protein. Domain IV contains a PH domain. We analyzed the role of the domains using truncated versions of Boi2p, and the results are summarized in Fig. 9. BOI2ΔN, which lacked the sequence for domains I-III, complemented ∆boi1 ∆boi2, while BOI2ΔC, which lacked domain IV, did not, an indication that the COOH-terminal half, including domain IV, of Boi2p is essential and sufficient for the function of Boi2p in cell growth, while the NH2-terminal half is dispensable. The role of a PH domain is still obscure, but it has been reported that PH domains participate in interactions with lipid moieties and proteins (Musacchio et al., 1993; Harlan et al., 1994). It is possible that Boi proteins interact with proteins other than Boi1p via the PH domain. In this context, it is of interest to recall that the overexpression of BOI genes under the control of the galactose-dependent promoter inhibited bud emergence (Fig. 4). As in the case of the function of Boi2p in cell growth, the COOH-terminal half of Boi2p (from pGAL7:BOI2ΔN) is essential and sufficient for the inhibitory effect, and the interaction of Boi1p and the Boi proteins is not required. These findings suggest that the COOH-terminal half of Boi2p interacts with a factor(s) involved in bud emergence. It might be possible that the interaction between the COOH-terminal half and the component(s) for bud emergence can replace, in part, the role of the Cdc24p–Boi2p interaction and can allow cells to grow well without the NH2-terminal half of the Boi proteins.

**The Role of the Bem1p–Boi2p Interaction**

Although the NH2-terminal half of Boi2p is not essential for either cell growth or inhibition of bud emergence, the NH2-terminal half of Boi2p might be required to inhibit the suppression of the growth defect of Arho3 cells by Bem1p. These findings strongly suggest that the Boi proteins possess the potential to modulate the function of Bem1p and that the NH2- and COOH-terminal portions of the Bem proteins play a critical role in modulating the function of Bem1p. The NH2-terminal portion of Boi2p interacts with SH3-2 of Bem1p, and SH3-2 is critical for suppression of the Arho3 defect. Thus, it is likely that the Bem1p–Boi2p interaction plays a role in the modulating activity.

The role of the Bem1p–Boi2p interaction in the suppression of the Arho3 defect is suggested to be negative; multiple copies of BOI2 did not suppress the Arho3 defect, but they inhibited the growth of ∆rho3 cells that had been rescued by the overproduction of Bem1p (Fig. 7). From these results, we cannot exclude the possibility that the intrinsic amount of Boi2p might play a positive role in the suppression of the ∆rho3 defect. It is possible, however, that another protein that interacts with SH3-2 might play an important role in suppressing the Arho3 defect, acting in concert with or independently of Boi2p, and that Boi2p might modulate the function of Bem1p by interacting competitively with SH3-2. It was reported very recently that Bem1p interacts with Ste20p and Ste5p, two components of the pheromone-responsive mitogen-activated protein kinase cascade, as well as with actin. The Ste20p protein kinase requires both SH3-2 and the COOH-terminal half of Bem1p for the Ste20p–Bem1p interaction (Lecuew et al., 1995). Ste20p is therefore a candidate for a protein that is critical for the suppression of the Arho3 defect. At this time, however, we have no experimental evidence to suggest the involvement of Ste20p.

**Protein Complex for Bud Formation**

The COOH-terminal 35 a.a. of Bem1p is required for bud emergence at elevated temperatures and for the suppression of the rho3 defect (Fig. 1). Thus, it appears possible that protein–protein interaction at the COOH terminus of Bem1p is critical for the functions of Bem1p. One of the candidates for an interacting protein is Cdc24p (Peterson et al., 1994). Indeed, the COOH-terminal 35 a.a. was required for the two-hybrid interaction between Bem1p and Cdc24p (Table II). Cdc24p also interacts with Cdc42p as a GTP–GDP exchange factor and with Rsr1p/Bud1p, which is needed for determination of the bud site (Bender and Pringle, 1989; Chant and Herskowitz, 1991; Zheng et al., 1994, 1995). The finding that Bem1p required the Cdc24p–interacting COOH terminus for suppression of the Arho3 defect suggests the possibility that a protein complex containing Bem1p and Cdc24p might play a role in the process that involves Rhop3. It is plausible that the protein complex for bud–site selection is developed by the association of Cdc42p, Cdc24p, and Bem1p for the polarization of cells. After the initiation of bud emergence, the protein complex for cell polarization should be developed and/or rearranged to terminate the process of initiation of bud emergence and for the continuation of bud growth. In this context, it is of great interest that factors involved in bud growth exhibit genetic and protein–protein interactions with factors that are involved in bud emergence and, moreover, that Boi2p might be able to modulate the function of Bem1p and to interact with a factor(s) other than Bem1p that is involved in cell polarization. Our present working hypothesis is that for the regulation of cell mor-
phogenesis, Rho3p and Boi proteins control the development of the protein complex for bud growth, and the Boi proteins mediate the connections between the protein complex for cell polarization and the machinery for bud growth via its affinities for Bem1p and for the factor(s) involved in cell polarization.

We thank A. Bender for communicating information before publication, S. Fields and R. Stern glanz for the plasmids pBTM116 and pGAD424 and the yeast genomic library, and Y. Ohy a for the plasmid carrying CLS4/CDC24.

Part of this work was supported by a grant for scientific research from Monbusho, Japan.

Received for publication 8 June 1995 and in revised form 22 February 1996.

References

Adams, A.E.M., D.J. Johnson, R.M. Longnecker, B.F. Sloat, and J.R. Pringle. 1990. CDC42 and CDC43, two additional genes involved in budding and the establishment of cell polarity in the yeast Saccharomyces cerevisiae. J. Cell Biol. 111:131-142.

Adams, A.E.M., and J.R. Pringle. 1994. Relationship of actin and tubulin distribution to bud growth in wild-type and morphogenetic mutant Saccharomyces cerevisiae. J. Cell Biol. 98:934-945.

Barbacid, M. 1987. ras genes. Annu. Rev. Biochem. 56:779-827.

Bender, A., and J.R. Pringle. 1989. Suppression of the cdg2 budding defect in yeast by CDC42 and three newly identified genes including the rad-related RSI1 gene. Proc. Natl. Acad. Sci. USA. 86:9976-9980.

Bender, A., and J.R. Pringle. 1991. Use of a screen for synthetic lethal and multicopy suppressor mutants to identify two new genes involved in morphogenesis in Saccharomyces cerevisiae. Mol. Cell. Biol. 11:1295-1305.

Bender, L., H.S. Lo, H. Lee, V. Koj plan, J. Peterson, and A. Bender. 1996. As sociations among PH and SH3 domain-containing proteins and Rho-type GTPases in yeast. J. Cell Biol. 133:879-894.

Boguski, M.S., and F. McCormick. 1993. Proteins regulating Ras and its relatives. Nature (Lond.). 366:643-654.

Bourne, H.R., D.A. Sanders, and F. McCormick. 1991. The GTPase superfamily: conserved structure and molecular mechanism. Nature (Lond.). 349:137-122.

Broach, J.R., Y. Li, L. Wu, and M. Jayaram. 1983. Vectors for high level inducible expression of cloned genes in yeast. J. Cell. Biot. 109:1093-1096.

Field, C., and R. Schekman. 1980. Localized secretion of acid phosphatase reflects the pathway of cell surface growth in Saccharomyces cerevisiae. J. Biol. Chem. 255:123-128.

Chianti, J., and I. Herskowitz. 1991. Genetic control of bud site selection in yeast by a set of genes products that constitute a morphogenetic pathway. Cell. 65:1093-1096.

Field, C., and R. Schekman. 1980. Localized secretion of acid phosphatase reflects the pattern of cell surface growth in Saccharomyces cerevisiae. J. Cell Biol. 86:123-128.

Field, S., and R. Stern glanz. 1994. The two-hybrid system: a method to identify and clone genes for proteins that interact with a protein of interest. Proc. Natl. Acad. Sci. USA. 81:5645-5649.

Chien, C.T., P.L. Bartel, R. Sternglanz, and S. Fields. 1991. The two-hybrid system: a method to identify and clone genes for proteins that interact with a protein of interest. Proc. Natl. Acad. Sci. USA. 88:5645-5649.

Cicchetti, P., B.I. Mayer, G. Thiel, and D. Baltimore. 1992. Identification of a protein that binds to the SH3 region of Abi and is similar to Bcr and GAP rho. Science (Wash. DC). 257:803-806.

Drubin, D.G. 1991. Development of cell polarity in budding yeast. Cell. 65:1093-1096.

Harlan, J.E., P.J. Hajduk, H.S. Yoon, and S.W. Fesik. 1994. Pleckstrin homology domains bind to phosphatidylinositol 4,5-bisphosphate. Nature (Lond.). 371:160-170.

Imai, J., A. Toh-e, and Y. Matsui. 1996. Genetic analysis of the Saccharomyces cerevisiae RHO3 gene, encoding a Rho small protein GTPase, provides evidence for a role in bud formation. Genetics. 142:359-369.

Ito, H., Y. Fukuda, K. Murata, and A. Kinura. 1983. Transformation of intact cells treated with alkali cations. J. Gen. Microbiol. 153:163-168.

Johnson, D.I., and J.R. Pringle. 1990. Molecular characterization of CDC42, a Saccharomyces cerevisiae gene involved in the development of cell polarity. J. Cell Biol. 111:143-152.