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Isolation and Characterization of Bud6p, an Actin Interacting Protein, from *Yarrowia lipolytica*

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The identification of genes involved in true hypha formation is important in the study of mechanisms underlying the morphogenetic switch in yeast. We isolated a gene responsible for the morphogenetic switch in *Yarrowia lipolytica*, which forms true hyphae in response to serum or N-acetylglucosamine. The isolated gene, encoding 847 amino acids, had sequence identities of 27% and 25% with the Bud6 (Aip3) proteins of *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, respectively. Disruption of this gene, designated *YlBUD6*, in haploid and diploid strains significantly reduced the ability of *Y. lipolytica* to switch from the yeast form to the hyphal form in hypha-inducing media. It was also found that *YlBud6Δ* mutants were rounder than the wild type when grown in the yeast form. These results indicate that the YlBud6 protein is necessary for hyphal growth and cell polarity in both haploid and diploid *Y. lipolytica* cells.

Key words: *Yarrowia lipolytica, BUD6,* yeast, hyphae, cell polarity

Dimorphic yeasts can switch from a budding yeast form to a pseudohyphal or true hyphal form in response to diverse environmental conditions. Since the yeast-to-hypha morphological transition was reported to be associated with the pathogenesis of *Candida albicans*, studies have concentrated on the dimorphic transition of *C. albicans* (Gow, 1997; Brown and Gow, 1999; Calderone and Fonzi, 2001; Liu, 2001; Sudbery, 2001; Michel *et al.*, 2002). In fact, the deletion of the genes involved in the dimorphic transition significantly reduced the virulence of *C. albicans,* regardless of signal transduction pathways in the morphological processes (Leberer *et al.*, 1997; Lo *et al.*, 1997; Stoldt *et al.*, 1997; Calera and Calderone, 1999; Calera *et al.*, 2000; Bensen *et al.*, 2002; Kim *et al.*, 2002; McNemar and Fonzi, 2002; Oberholzer *et al.*, 2002). Therefore, the yeast-to-hypha transition is considered one of the several virulence attributes that enable *C. albicans* to invade human tissues.

Saccharomyces cerevisiae, some diploid strains of which can undergo the transition to pseudohyphal growth in response to nitrogen starvation (Gimeno and Fink, 1994), is the most well used model organism in the study of morphological transition. Moreover, information obtained during research into the morphogenesis of *S. cerevisiae* have guided studies on dimorphism in *C. albicans,* because conventional genetic approaches are not easily applied to *C. albicans* due to its diploidy and apparent lack of a sexual cycle (Brown and Gow, 1999; Magee and Magee, 2000; Nakayama *et al.*, 2000; Navarro-Garcia *et al.*, 2001). Although the pathways controlling the morphological switches in *S. cerevisiae* and *C. albicans* do not strictly parallel each other (Kadosh and Johnson, 2001), it has been found that many homologous gene products function in the hyphal growth of *C. albicans* and the pseudohyphal growth of *S. cerevisiae* (Stoldt *et al.*, 1997; Schweizer *et al.*, 2000; Gancedo, 2001; Liu, 2001). However, since differences exist, at least in the cell cycles, of hyphae and pseudohyphae formation in yeast, many gene products contributing to the hyphal growth of *C. albicans* may neither be present nor have a similar function in *S. cerevisiae* (Gow, 1997; Brown and Gow, 1999).

Yarrowia lipolytica, which is a heterothallic dimorphic yeast, is able to form pseudohyphae and true hyphae depending on environmental conditions. Interestingly, *Y. lipolytica*, like *C. albicans*, forms true hyphae when serum is added to culture medium, although *Y. lipolytica* is not a pathogenic yeast (Kim *et al.*, 2000). Furthermore, *Y. lipolytica* is amenable to most of the sophisticated molecular genetic techniques available for *S. cerevisiae,* and has been used to isolate genes involved in the dimorphism of *Y. lipolytica* (Torres-Guzman and Dominguez, 1997; Hurtado *et al.*, 2000; Richard *et al.*, 2001; Hurtado and Rachubinski, 2002; Kim *et al.*, 2002). Therefore, *Y. lipolytica* is potentially a good model organism for the investigation of signaling pathways leading to hyphal development in yeast, especially in response to serum.

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Bud6p has been reported to direct cell polarity in the budding yeast *S. cerevisiae* and in the fission yeast *Schizosaccharomyces pombe* (Amberg *et al.*, 1997; Sheu *et al.*, 1998; Glynn *et al.*, 2001; Jin and Amberg, 2001; Segal *et al.*, 2002). Moreover, the deletion of *BUD6* in a diploid *S. cerevisiae* strain caused severe defects in cell polarity, including depolarization of actin cortical patches, disorganization or loss of actin cables, inefficient nuclear migration and nuclear division, and defects in septation (Amberg *et al.*, 1997). And, in *S. pombe* bud6p functions downstream of tea1p, a microtubule end-associated factor, and thus helps microtubule plus ends function to polarize the actin cytoskeleton (Glynn *et al.*, 2001).

A number of morphologically defective mutants of *Y. lipolytica* were isolated in our lab. One of the clones that conferred on morphological mutants the ability to form hyphae under hypha-inducing conditions was found to be a *BUD6* homologue. Although *Y. lipolytica BUD6* (*Yl-BUD6*) was reported to be involved in the hyphal growth of *Y. lipolytica* (Richard *et al.*, 2001), the full gene has not yet been cloned and its function was tested only in the haploid strain. The hyphal growth of *Y. lipolytica*, regardless of the degree of ploidy, generally begins at the pole distal from the previous division site (Herrero *et al.*, 1999), thus we thought it would be interesting to investigate the effects of the *Ylbud6* null mutation on the relationship between the bipolar budding pattern and dimorphic transition in both haploid and diploid *Y. lipolytica* strains.

Materials and Methods

Strains and growth conditions

The *Y. lipolytca* strains used in this study are listed in Table 1. The *Y. lipolytica* morphological mutant strain, YKS-73, was derived from *Y. lipolytica* SMS397A by UV mutagenesis. *Escherichia coli* used for DNA manipulation was DH5α (FΦ80d *lacZ* DM15Δ (*lacZYA-argF*) U169 *hsdR17* (rk-mk$^+$) *deoR recA1 supE44λ-thi1-gyrA96 relA1*).

The compositions of the media used were as follows: YPD-1% yeast extract, 2% bacto-peptone and 2% glucose; synthetic complete (SC) medium-0.67% yeast nitrogen base without amino acids (Difco), 2% glucose and drop-out amino acid mixture including all amino acids required; synthetic minimal (SM) medium-0.67% yeast nitrogen base, 2% glucose, and 30 mg/l adenine, 90 mg/l leucine, 45 mg/l tryptophan and 30 mg/l uracil as required; N-acetylglucosamine induction medium (SGN)-0.67% yeast nitrogen base, 1% N-acetylglucosamine, 30 mg/l adenine, 50 mg/l uracil, 90 mg/l leucine and 45 mg/l tryptophan; serum induction medium (SSE)-0.67% yeast nitrogen base without amino acids (Difco), 10% bovine calf serum (Sigma, USA), 30 mg/l adenine, 50 mg/l uracil, 90 mg/l leucine and 45 mg/l tryptophan; Mating medium (YM)-0.3% yeast extract, 0.3% malt extract, and 0.5% bacto-peptone; sporulation medium (CSM)-0.67% yeast nitrogen base and 1.5% sodium citrate. All *Y. lipolytica* cells were grown at 28°C.

Mating was carried out on solid YM medium (Barth and Gaillardin, 1996). Sporulation of the diploid strain was induced in liquid CSM at 23°C. To induce the hyphal growth of *Y. lipolytica*, cells were grown to OD_{600}=1.0 at 28°C in YPD medium, harvested, washed three times at room temperature, resuspended at 10^6 cells per ml in *N*-acetylglucosamine or serum induction medium, and incubated at 28°C.

DNA manipulations and recombinant DNA techniques

DNA manipulations, enzymatic reactions, and Southern

Table 1. *Y. lipolytica* strains used in this study

Strains	Relevant Genotype	Source
SMS397A	*MATA ade1 ura3 xpr2*	Kim *et al.*, 2000
CX39-74B	*MATB trp1*	ATCC 32339
SMS397A /CX39-74B	*MATA/MATB*	This study
YKS-73	A morphological mutant of SMS397A	This study
YlBI-1	*MATA ade1 ura3 xpr2 bud6::tc-URA3-tc*	This study
YlBI-2	*MATA ade1 ura3 xpr2 bud6::tc*	This study
YlBI-3	*MATA ade1 ura3 xpr2 bud6::tc leu2::URA3*	This study
YlBI-3[*YlBUD6*]	YlBI-3 harboring complementing plasmid	This study
YlBU	*MATB ura3 trp1 bud6::tc*	This study
YlBU-1	*MATB ura3 trp1 bud6::tc leu2::URA3*	This study
YlBU-1[*YlBUD6*]	YlBU-1 harboring complementing plasmid	This study
SMS397A /YlBU	*MATA/ MATB ura3/ura3 BUD6/bud6*	This study
YlBI-2/ CX39-74B	*MATA/MATB bud6/BUD6*	This study
YlBI-2/YlBU	*MATA/ MATB ura3/ura3 bud6/bud6*	This study
YlBI-3/YlBU-1[*YlBUD6*]	YlBI-3/YlBU-1 harboring complementing plasmid	This study

hybridizations were essentially performed using standard procedures (Sambrook and Russell, 2001). The DNA probes were labeled with digoxigenin using DIG labeling kit (Boehringer Mannheim, Germany). The polymerase chain reactions were performed with rTaq or ExTaq polymerase (Takara, Japan) using Gene Amp PCR system 2400 (Perkin-Elmer, USA). Genetic analysis and transformation of *Y. lipolytica* were carried out using the procedures described by Barth and Gaillardin (1996).

DNA sequence analysis

Sequencing was carried out on an ABI Model 373A automated DNA sequencer (Applied Biosystems, USA). The DNA sequence data of the *YlBUD6* gene presented in this paper was submitted to GenBank under Accession Number (AY237652). Nucleotide and amino acid sequences were analyzed using Vector NTI® Suite 6.0 (InforMax, USA). Homology searches of the GenBank database were done using the BLAST algorithm. Multiple sequence alignment was performed using ClustalW1.8 (Thompson *et al.*, 1994).

Calcofluor white staining

Calcofluor white staining was carried out to analyze the bud site selection pattern. Cells growing exponentially in liquid YPD medium were harvested, washed with 1 M phosphate-buffered saline (PBS) solution, resuspended in the same PBS solution, and stained with calcofluor white (100 μg/ml) for 10 min in the dark at room temperature.

Invasive growth test

To assess the ability to grow invasively into agar, 10 μl of cell suspension (10^6 cells/ml) was dropped onto YPD plates. The plates were then incubated at 28°C for 2 days. The plates were then photographed, washed under a gentle stream of running water, and immediately re-photographed.

Results

Cloning and analysis of Y. lipolytica BUD6.

A number of morphologically defective mutants (derived from strain SMS397A; *MATA ade1 ura3 xpr2*), which were unable to form hyphae on medium containing N-acetylglucosamine or serum after 3 days of incubation at 28°C, were isolated. A mutant strain, YKS-73, which formed smooth surfaced colonies on both complex and minimal media, and which grew as a budding yeast form in hypha-inducing media, was chosen for further analyses. Mating the mutant with a strain of the opposite mating type, CX39-74B (*MATB trp1*), and random spore analysis of the resulting diploid strain indicated that the mutation was recessive and occurred at a single allele. The mutant could not grow at 33°C, which permitted the growth of the wild type strain, indicating that the mutant had a temperature sensitive phenotype.

Since a genomic library constructed in the replicative *E. coli* shuttle vector pINA445 had the *YlLEU2* gene as a genetic selection marker (a gift from C. Gaillardin), a Leu⁻ strain of YKS-73 was needed to use the library. Therefore, we constructed pLPUT vector carrying the *YlLEU2* disruption cassette (3.0 kb), where the *YlURA3* gene was flanked by the *YlLEU2* promoter (645 bp) and terminator (693 bp) regions. The disruption cassette, which was amplified by PCR using the primers P_1Leu (5'-ACCCGTTGCTATCTCCACAC-3') and T_1Leu (5'-CTGTTCGGAAATCAACGGA-3'), was introduced into the mutant. The *LEU2* disrupted strain, YKS-73L (*MATA ade1 ura3 xpr2 leu2::URA3*), was isolated from the colonies growing on synthetic complete medium without uracil (SC-Ura) but not on the SC-Leu medium. The *leu2* disruption was confirmed by Southern blot analysis (data not shown). The YKS-73L cells were transformed with the genomic DNA library and plated onto the serum medium. Several candidate transformants were isolated after screening more than 8,000 transformants for their ability to form hyphae. The plasmids rescued from the hypha-forming transformants were reintroduced into the YKS-73L strain and one of these was found to be able to recover the ability of the mutant to form hyphae. Further analysis of the plasmid showed that the size of the insert DNA in the plasmid was about 6.0 kb. Sequencing of the fragments revealed that there was an open reading frame consisting of 2,541 bp, the deduced amino acids of which showed sequence identities of 27% and 25% with *S. cerevisiae* and *S. pombe* Bud6 proteins, respectively (Fig. 1A). Therefore, the cloned gene was designated *Y. lipolytica BUD6* (*YlBUD6*). ScBud6p has a coiled-coil region near its C terminus that may mediate its interaction with the actin cytoskeleton or homo-oligomerization. Interestingly, it was found that YlBud6p contained three predicted coiled-coil regions in the C-terminal domain (Fig. 1B) when analyzed using an ISREC program (URL: http://searchlauncher.bcm.tmc.edu/seq-serch/).

Functional analysis of the YlBUD6 gene.

In order to investigate whether the *YlBUD6* gene is actually involved in the hyphal growth of *Y. lipolytica*, haploid and diploid *Ylbud6Δ* mutants were constructed and their phenotypes were examined under hypha-inducing conditions. To construct a *YlBUD6* disruption cassette, 5' and 3' regions (0.5 kb and 0.4 kb) of the *YlBUD6* gene were amplified using the two primer sets, Sb-X (5'-GCTCTAGATGCAGCAGAAGCCAAA-3') and S-b1 (5'-CCATGGATCCGTAGATCTAAGCACAGCTGGCTGTTA-3'), and Bf-X (5'-GCTCTAGATTTAGCGAGTGTTTCGG-3') and B-f1 (5'-AGATCTACGGATCCATGGTGGCCTTGACATGTTCCAA-3'). The *URA3* blaster (*Tc::YlURA3-::Tc*, 2.7 kb), which allows the recycling of the *YlURA3* marker, thereby permitting the sequential disruption of target genes within the same strain, was inserted between the amplified *YlBUD6* gene fragments, resulting in a *YlBUD6* disruption

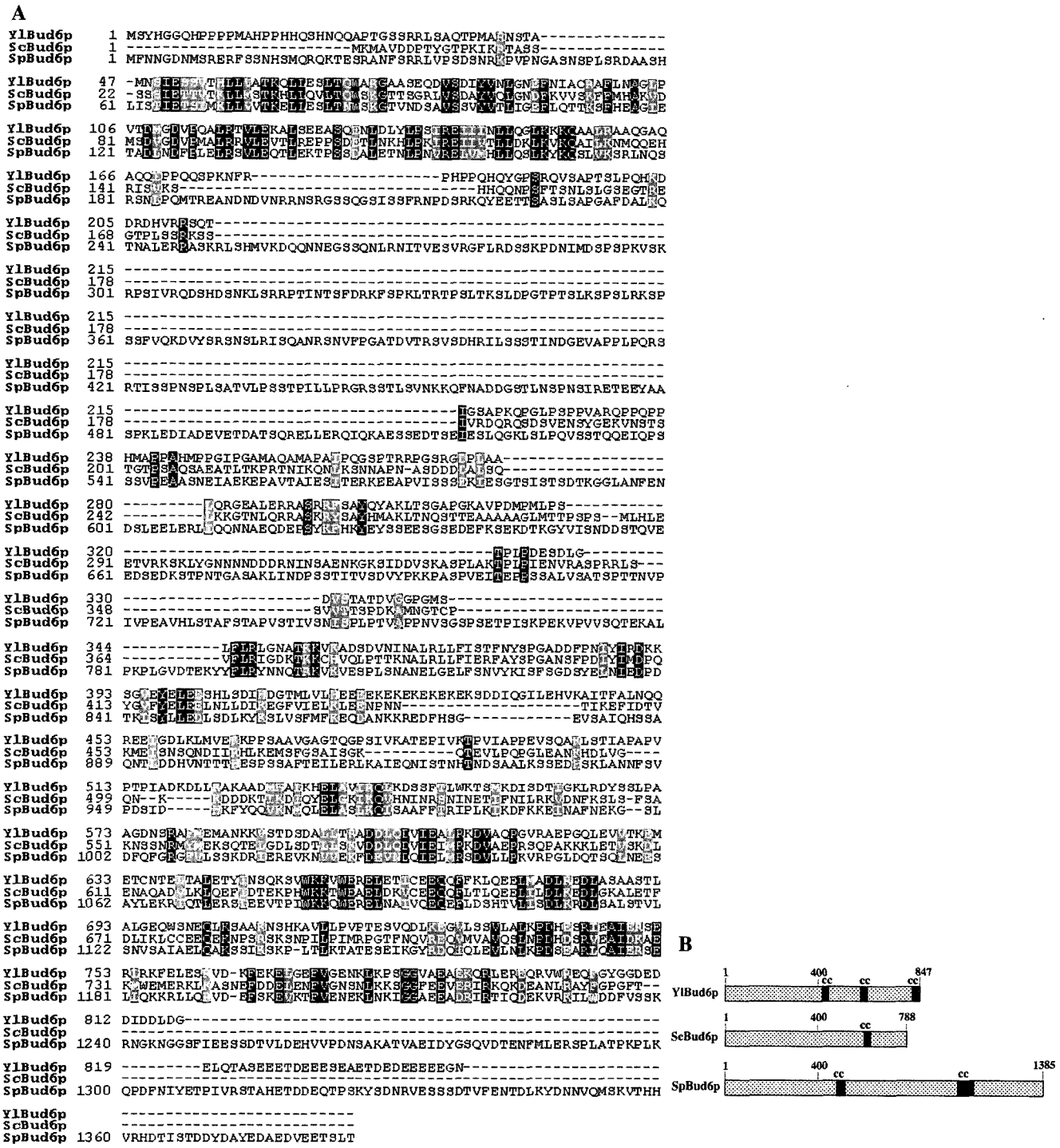

Fig. 1. (A) Multiple sequence alignment of the *Yarrowia lipoytica* Bud6 protein (YlBud6p; GenBank Accession No. AY237652), the *Saccharomyces cerevisiae* Bud6 protein (ScBud6p; GenBank Accession No. U35668) and the *Schizosaccharomyces pombe* Bud6 protein (SpBud6p; the sequence was available from *S. pombe* genome-sequencing project). The alignment was performed with ClustalW1.8 (Thompson *et al.*, 1994), and shaded using the Boxshade 3.21 program. Identical residues are shown as white letters in black boxes, and similar residues are shaded in gray. (B) The domain structure of Bud6 proteins. Black boxes represent coiled-coil regions (cc), as predicted by the ISREC program.

cassette (3.6 kb) (Fig. 2A). The wild type strain of the mating type A, SMS397A (*MATA ade1 ura3 xpr2*), was then transformed with the *YlBUD6* disruption cassette. Cells forming round and smooth colonies on SC-Ura medium were selected and subjected to Southern blot analysis to check for *YlBUD6* gene disruption. One of the selected *Ylbud6Δ* mutants, YlBI-1 (*MATA ade1 ura3 xpr2 bud6::Tc-YlURA3-Tc*), was spread onto 5-fluoroorotic acid (5-FOA,

1 mg/ml) medium and a strain with the *YlURA3* gene popped-out (YlBI-2; *MATA adel ura3 xpr2 bud6::Tc*) was obtained (Fig. 2B). YlBI-2 was mated with a strain of the opposite mating type (CX39-74B; *MATB trp1*) and the resultant diploid strain, YlBI-2/U, was sporulated on YPD medium. Colonies showing smooth surfaced morphology were selected and tested for mating type and for *YlBUD6* disruption by PCR. As a result, a *YlBud6Δ* strain of the mat-

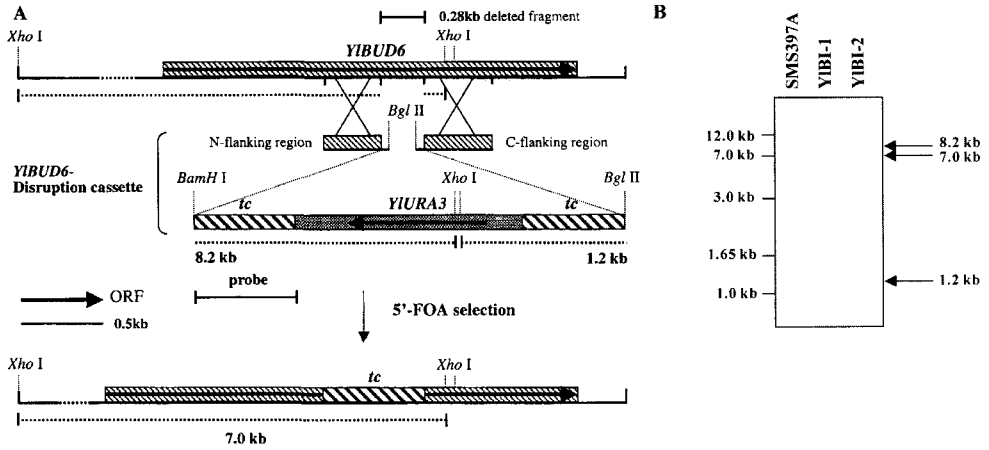

Fig. 2. Disruption of the *YlBUD6* gene. (A) Schematic representation of the disruption of the *YlBUD6* gene. (B) Southern blot analysis of wild type (SMS397A) and *Ylbud6Δ* (YlBI-1 and YlBI-2) strains. YlBI-2 is the same as YlBI-1 except for the *URA3* gene, which was popped-out when grown on 5-FOA medium. Genomic DNAs were completely digested by *Xho*I and hybridized with the 0.6 kb *tc* DNA fragment.

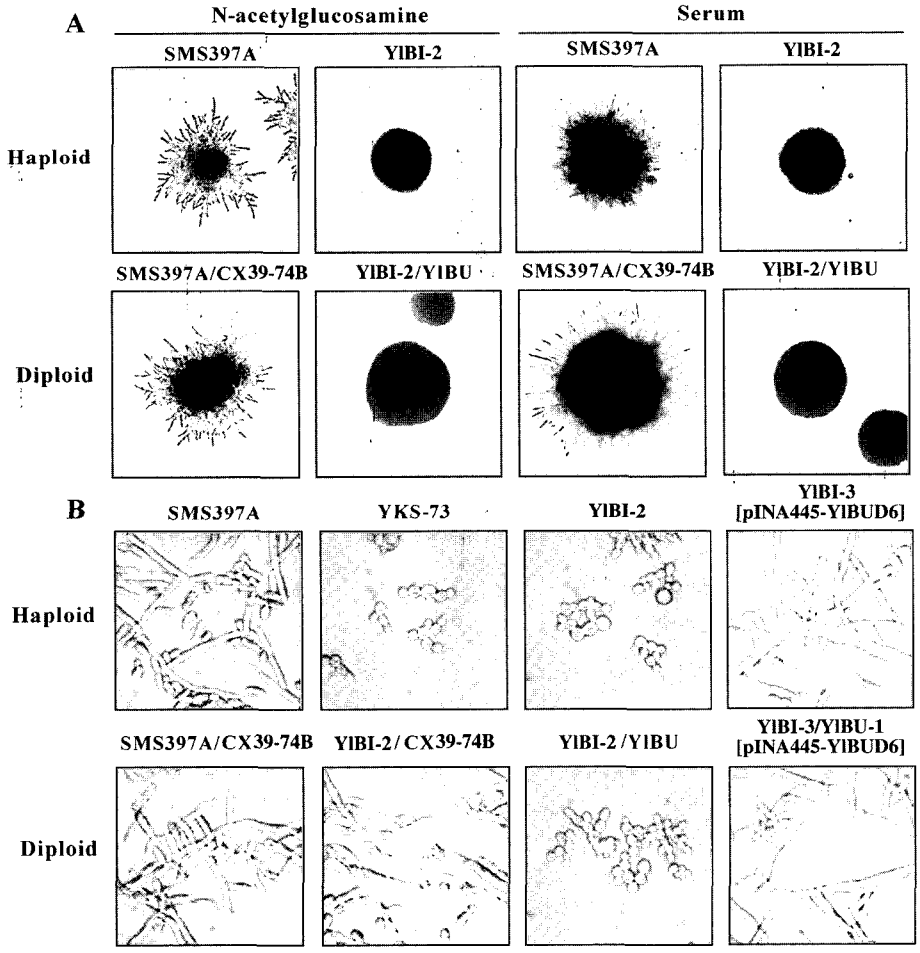

Fig. 3. Colony and cell morphologies of wild type and *Ylbud6Δ* strains. (A) Colonies were grown on solid *N*-acetyl-D-glucosamine or serum induction medium at 28°C for 3 days. They were then photographed under a light microscope (40X). (B) Cells were grown at 28 °C for 20 h in liquid medium containing 10% bovine calf serum, and photographed using DIC microscopy (400X).

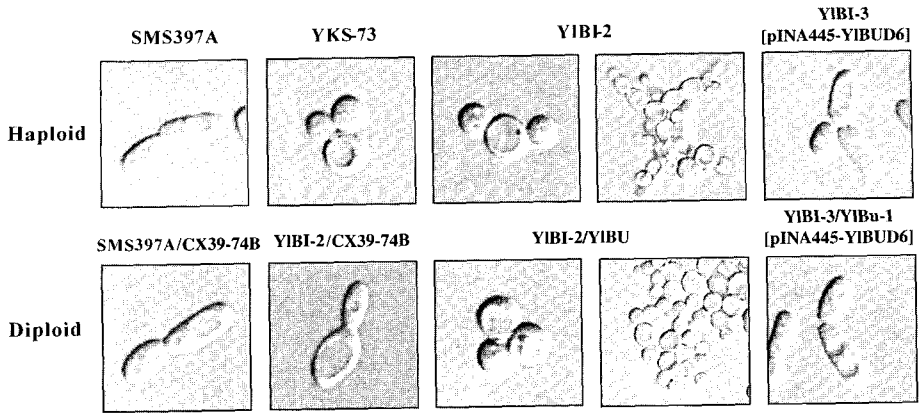

Fig. 4. Loss of cell polarity in *Ylbud6Δ* strains. Cells were grown at 28°C for 1 day in liquid YPD medium. They were visualized using DIC microscopy at a magnification of 600X.

ing type B, YlBU (*MATB ura3 trp1 bud6::Tc*) was obtained. The YlBU strain was mated with the YlBI-2 strain, generating the diploid *YlBud6Δ* mutant (*MATA/MATB ade1/ADE1 ura3/ura3 xpr2/XPR2 TRP1/ trp1 bud6/bud6*).

In order to study the function of *YlBUD6* in *Y. lipolytica*, *Ylbud6Δ* haploid and diploid mutant strains were grown in media containing 10% serum or 1% N-acetyl-D-glucosamine. As shown in Fig. 3, the deletion of *YlBUD6* caused a profound morphological defect in the strains; the mutant strains could not form hyphae either in liquid media or on solid media. In addition, the *YlBud6Δ* mutant strains had a round yeast form compared with the ellipsoidal form of the wild type strain in YPD medium, which is indicative of a loss of cell polarity in the *YlBud6Δ* mutants (Fig. 4). In addition, the mutants frequently formed clusters or chained cells, suggesting a delay in cytokinesis or cell separation. These morphological abnormalities were corrected by introducing into the mutants the wild type *YlBUD6* gene on a low copy number plasmid (pINA445-YlBUD6) (Fig. 3 and 4), thus demonstrating that *YlBUD6* is responsible for the defects in the hyphal growth and cell polarity as well as the cytokinesis of haploid and diploid *Y. lipolytica* cells.

Y. lipolytica cells are able to penetrate the agar surface. To test whether *YlBUD6* is responsible for the agar invasion property of *Y. lipolytica*, the wild type and *YlBud6Δ* cells were spotted onto solid YPD medium and incubated at 28°C for 5 days. The *YlBud6Δ* strains completely washed off the agar surface, whereas the wild type cells remained in the agar after the cells had been washed with water (Fig. 5). This result indicates that *YlBUD6* also plays a role in the agar invasiveness of *Y. lipolytica*.

To investigate whether YlBud6p is involved in bud site selection, the bud scars of exponentially growing *Y. lipolytica* cells were stained with calcofluor white, a chitin-specific dye, and viewed by fluorescence microscopy. Bud scars are chitin-rich ring structures that mark the region where a mother cell separated from its daughter cell. Cells having scars at both poles, but not in the middle, were regarded as bipolar, and cells having at least one scar not at either pole were regarded as random. As shown in Fig. 6, bipolar budding pattern was observed with the wild type strain but not with the *Ylbud6Δ* strains. Therefore, it seems that YlBud6p is involved in bud site selection for bipolar budding.

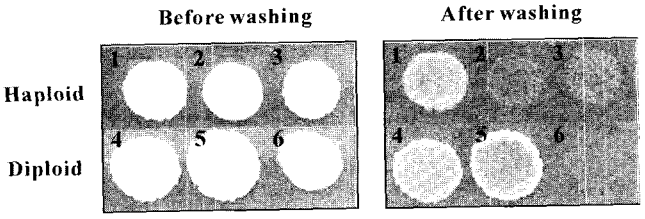

Fig. 5. Invasive growth phenotype of wild type and *Ylbud6Δ* strains. The yeast strains spotted onto a solid YPD medium were grown at 28°C for 2 days and washed with water. 1, SMS397A; 2, YKS-73; 3, YlBI-2; 4, SMS397A/CX39-74B; 5, YlBI-2/CX39-74B; 6, YlBI-2/YlBU.

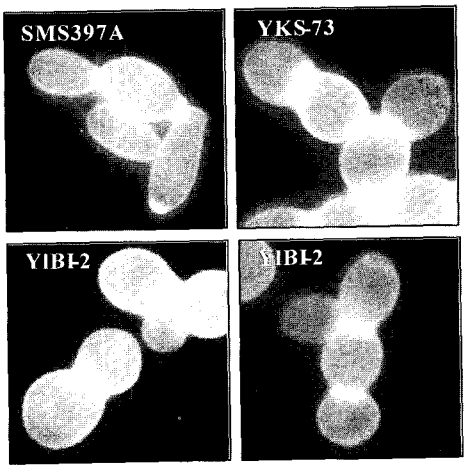

Fig. 6. Budding patterns of wild type and *Ylbud6Δ* strains. Cells were stained for chitin with Calcofluor White and visualized by fluorescence microscopy (600X).

Discussion

Searching for genes involved in the morphological

changes of *Y. lipolytica*, we isolated the *YlBUD6* gene. The complete sequence of the *YlBUD6* gene was determined and analyzed in this study. The amino acid sequence analysis of YlBud6p displayed 27% identity to that of *S. cerevisiae* Bud6p, an actin-interacting protein required for bipolar budding, and predicted to have three conserved coiled-coil regions within the C-terminal domain. In *S. cerevisiae,* the predicted coiled-coil region near the C-terminus of Bud6p is thought to mediate the homo-oligomerization of the protein (Amberg *et al.*, 1997). Accordingly, the coiled-coil regions in YlBud6p are presumed to mediate the interaction of YlBud6p with actin or with itself.

S. cerevisiae Bud6 protein has been implicated in cell polarity, including bud site determination, and actin organization in diploid cells (Amberg *et al.*, 1997; Sheu *et al.*, 1998). The localization of Bud6p revealed that the protein functions to select a bipolar bud site in diploid *S. cerevisiae* cells, but not in haploid cells, which bud in an axial budding pattern (Amberg *et al.*, 1997). In the fission yeast *S. pombe*, the deletion of the *bud6*$^{+}$ gene caused a specific defect in the establishment of cell polarity at the previous cell division site; after cell division, wild type *S. pombe* initially grew only at the previous end (the "old" end), and in the G2 phase, initiates growth at the previous cell division site (the "new" end), but *S. pombe bud6* mutant cells failed to grow at the new end (Glynn *et al.*, 2001). However, *S. pombe bud6* mutant cells exhibited normal rod cell shapes and cell division patterns and had a fairly normal actin cytoskeleton, unlike *S. cerevisiae bud6* mutants that had defects in actin organization (Glynn *et al.*, 2001; Jin and Amberg, 2001). Thus, it is conceivable that *bud6* mutants of different yeast species may show their own specific phenotypic characteristics, although the *in vivo* functions of Bud6 protein are essentially related with cell polarity and morphogenesis.

In this study, the Bud6 protein of *Y. lipolytica* was found to be related not only with cell polarity but also with the ability to form hyphae in hypha-inducing media. Since Bud6p is a component necessary for establishing spatial cues to position the site for bipolar budding in diploid *S. cerevisiae* and to direct bipolar growth in *S. pombe* (Sheu *et al.*, 1998; Glynn *et al.*, 2001), it may be reasoned that deletion of *YlBUD6* results in a loss of cell polarity in *Y. lipolytica*, which buds in a bipolar manner. The defective hyphal growth of *Ylbud6* mutant cells may result from a failure to organize the normal actin cytoskeleton structures, which are needed for continued polarized growth (Evangelista *et al.*, 1997; Freedman *et al.*, 2000; Jaquenoud and Peter, 2000; Ho and Bretscher, 2001). However, in the study of a homozygous *bud6/bud6* mutant *of C. albicans*, which had lost cell polarity, we found that the mutant strain was able to form hyphae in liquid hypha-inducing medium but not on solid hypha-inducing medium (Song and Kim, unpublished data), indicating that Bud6 protein is required only under certain conditions for the hyphal growth of *C. albicans*. Therefore, it would be of interest to investigate the mechanism by which YlBud6p functions to form hyphae in *Y. lipolytica* and *C. albicans*, and how *C. albicans* can overcome the morphological defect caused by the disruption of the *BUD6* gene in liquid hypha-inducing medium. These studies are currently under way in our lab.

Acknowledgments

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References

Amberg, D.C., J.E. Zahner, J.W. Mulholland, J.R. Pringle, and D. Botstein. 1997. Aip3p/Bud6p, a yeast actin-interacting protein that is involved in morphogenesis and the selection of bipolar budding sites. *Mol. Biol. Cell* 8, 729-753.

Barth, G. and C. Gaillardin. 1996. *Yarrowia lipolytica*, p. 313-388. *In* K. Wolf (ed.), Nonconventional yeasts in biotechnology. Springer-Verlag, Berlin Heidelberg.

Bensen, E.S., S.G. Filler, and J. Berman. 2002. A forkhead transcription factor is important for true hyphal as well as yeast morphogenesis in *Candida albicans*. *Eukaryot. Cell* 1, 787-798.

Brown, A.J. and N.A. Gow. 1999. Regulatory networks controlling *Candida albicans* morphogenesis. *Trends Microbiol.* 7, 333-338.

Calderone, R.A. and W.A. Fonzi. 2001. Virulence factors *of Candida albicans*. *Trends Microbiol.* 9, 327-335.

Calera, J.A. and R. Calderone. 1999. Histidine kinase, two-component signal transduction proteins of *Candida albicans* and the pathogenesis of candidosis. *Mycoses* 42 Suppl 2, 49-53.

Calera, J.A., X.J. Zhao, and R. Calderone. 2000. Defective hyphal development and avirulence caused by a deletion of the *SSK1* response regulator gene in *Candida albicans*. *Infect. Immun.* 68, 518-525.

Evangelista, M., K. Blundell, M.S. Longtine, C.J. Chow, N. Adames, J.R. Pringle, M. Peter, and C. Boone. 1997. Bni1p, a yeast formin linking cdc42p and the actin cytoskeleton during polarized morphogenesis. *Science* 276, 118-122.

Freedman, T., A. Porter, and B. Haarer. 2000. Mutational and hyperexpression-induced disruption of bipolar budding in yeast. *Microbiology* 146 (Pt 11), 2833-2843.

Gancedo, J.M. 2001. Control of pseudohyphae formation in *Saccharomyces cerevisiae*. *FEMS Microbiol. Rev.* 25, 107-123.

Gimeno, C.J. and G.R. Fink. 1994. Induction of pseudohyphal growth by overexpression of *PHD1*, a *Saccharomyces cerevisiae* gene related to transcriptional regulators of fungal development. *Mol. Cell. Biol.* 14, 2100-2112.

Glynn, J.M., R.J. Lustig, A. Berlin, and F. Chang. 2001. Role of bud6p and tea1p in the interaction between actin and microtubules for the establishment of cell polarity in fission yeast.

Curr. Biol. 11, 836-845.

Gow, N.A. 1997. Germ tube growth of *Candida albicans*. *Curr. Top. Med. Mycol.* 8, 43-55.

Herrero, A.B., M.C. Lopez, L.F. Lago, and A. Dominguez. 1999. *Candida albicans* and *Yarrowia lipolytica* as alternative models for analysing budding patterns and germ tube formation in dimorphic fungi. *Microbiology* 145 (Pt 10), 2727-2737.

Ho, J. and A. Bretscher. 2001. Ras regulates the polarity of the yeast actin cytoskeleton through the stress response pathway. *Mol. Biol. Cell* 12, 1541-1555.

Hurtado, C.A., J.M. Beckerich, C. Gaillardin, and R.A. Rachubinski. 2000. A rac homolog is required for induction of hyphal growth in the dimorphic yeast *Yarrowia lipolytica*. *J. Bacteriol.* 182, 2376-2386.

Hurtado, C.A. and R.A. Rachubinski. 2002. Isolation and characterization of *YlBEM1*, a gene required for cell polarization and differentiation in the dimorphic yeast *Yarrowia lipolytica*. *Eukaryot. Cell* 1, 526-537.

Jaquenoud, M. and M. Peter. 2000. Gic2p may link activated Cdc42p to components involved in actin polarization, including Bni1p and Bud6p (Aip3p). *Mol. Cell. Biol.* 20, 6244-6258.

Jin, H. and D.C. Amberg. 2001. Fission yeast Aip3p (spAip3p) is required for an alternative actin-directed polarity program. *Mol. Biol. Cell* 12, 1275-1291.

Kadosh, D. and A.D. Johnson. 2001. Rfg1, a protein related to the *Saccharomyces cerevisiae* hypoxic regulator Rox1, controls filamentous growth and virulence in *Candida albicans*. *Mol. Cell. Biol.* 21, 2496-2505.

Kim, J., S.A. Cheon, S. Park, Y. Song, and J.-Y. Kim. 2000. Serum-induced hypha formation in the dimorphic yeast *Yarrowia lipolytica*. *FEMS Microbiol. Lett.* 190, 9-12.

Kim, J., S.A. Cheon, Y. Song, and J.-Y. Kim. 2002. Cloning and characterization of the multiprotein bridging factor 1 (*YlMBF1*) gene from the dimorphic yeast *Yarrowia lipolytica*. *J. Microbiol.* 40, 173-177.

Leberer, E., K. Ziegelbauer, A. Schmidt, D. Harcus, D. Dignard, J. Ash, L. Johnson and D.Y. Thomas. 1997. Virulence and hyphal formation of *Candida albicans* require the Ste20p-like protein kinase CaCla4p. *Curr. Biol.* 7, 539-546.

Liu, H. 2001. Transcriptional control of dimorphism in *Candida albicans*. *Curr. Opin. Microbiol.* 4, 728-735.

Lo, H.J., J.R. Kohler, B. DiDomenico, D. Loebenberg, A. Cacciapuoti, and G.R. Fink. 1997. Nonfilamentous *C. albicans* mutants are avirulent. *Cell* 90, 939-949.

Magee, B.B. and P.T. Magee. 2000. Induction of mating in *Candida albicans* by construction of MTLa and MTLalpha strains. *Science* 289, 310-313.

McNemar, M.D. and W.A. Fonzi. 2002. Conserved serine/threonine kinase encoded by *CBK1* regulates expression of several hypha-associated transcripts and genes encoding cell wall proteins in *Candida albicans*. *J. Bacteriol.* 184, 2058-2061.

Michel, S., S. Ushinsky, B. Klebl, E. Leberer, D. Thomas, M. Whiteway, and J. Morschhauser. 2002. Generation of conditional lethal *Candida albicans* mutants by inducible deletion of essential genes. *Mol. Microbiol.* 46, 269-280.

Nakayama, H., T. Mio, S. Nagahashi, M. Kokado, M. Arisawa, and Y. Aoki. 2000. Tetracycline-regulatable system to tightly control gene expression in the pathogenic fungus *Candida albicans*. *Infect. Immun.* 68, 6712-6719.

Navarro-Garcia, F., M. Sanchez, C. Nombela, and J. Pla. 2001. Virulence genes in the pathogenic yeast *Candida albicans*. *FEMS Microbiol. Rev.* 25, 245-268.

Oberholzer, U., A. Marcil, E. Leberer, D.Y. Thomas, and M. Whiteway. 2002. Myosin I is required for hypha formation in *Candida albicans*. *Eukaryot. Cell* 1, 213-228.

Richard, M., R.R. Quijano, S. Bezzate, F. Bordon-Pallier, and C. Gaillardin. 2001. Tagging morphogenetic genes by insertional mutagenesis in the yeast *Yarrowia lipolytica*. *J. Bacteriol.* 183, 3098-3107.

Sambrook, J. and D.W. Russell. 2001. Molecular Cloning: A Laboratory Manual, 3rd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Schweizer, A., S. Rupp, B.N. Taylor, M. Rollinghoff, and K. Schroppel. 2000. The TEA/ATTS transcription factor CaTec1p regulates hyphal development and virulence in *Candida albicans*. *Mol. Microbiol.* 38, 435-445.

Segal, M., K. Bloom, and S.I. Reed. 2002. Kar9p-independent microtubule capture at Bud6p cortical sites primes spindle polarity before bud emergence in *Saccharomyces cerevisiae*. *Mol. Biol. Cell.* 13, 4141-4155.

Sheu, Y.J., B. Santos, N. Fortin, C. Costigan, and M. Snyder. 1998. Spa2p interacts with cell polarity proteins and signaling components involved in yeast cell morphogenesis. *Mol. Cell. Biol.* 18, 4053-4069.

Stoldt, V.R., A. Sonneborn, C.E. Leuker, and J.F. Ernst. 1997. Efg1p, an essential regulator of morphogenesis of the human pathogen *Candida albicans*, is a member of a conserved class of bHLH proteins regulating morphogenetic processes in fungi. *EMBO J.* 16, 1982-1991.

Sudbery, P.E. 2001. The germ tubes of *Candida albicans* hyphae and pseudohyphae show different patterns of septin ring localization. *Mol. Microbiol.* 41, 19-31.

Thompson, J.D., D.G. Higgins, and T.J. Gibson. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* 22, 4673-4680.

Torres-Guzman, J.C. and A. Dominguez. 1997. *HOY1*, a homeo gene required for hyphal formation in *Yarrowia lipolytica*. *Mol. Cell. Biol.* 17, 6283-6293.