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Role of CaBud6p in the Polarized Growth of *Candida albicans*

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Bud6p is a component of a polarisome that controls cell polarity in *Saccharomyces cerevisiae*. In this study, we investigated the role of the *Candida albicans* Bud6 protein (CaBud6p) in cell polarity and hyphal development. CaBud6p, which consists of 703 amino acids, had 37% amino-acid sequence identity with the Bud6 protein of *S. cerevisiae*. The homozygous knock-out of *CaBUD6* resulted in several abnormal phenotypes, such as a round and enlarged cells, widened bud necks, and a random budding pattern. In hypha-inducing media, the mutant cells had markedly swollen tips and a reduced ability to switch from yeast to hypha. In addition, a yeast two-hybrid analysis showed a physical interaction between CaBud6p and CaAct1p, which suggests that CaBud6p may be involved in actin cable organization, like Bud6p in *S. cerevisiae*. Taken together, these results indicate that CaBud6 plays an important role in the polarized growth of *C. albicans*.

Keywords: *Candida albicans*, *BUD6*, polarisome, cell polarity, hypha

The establishment and maintenance of cell polarity are essential for the morphogenesis and development of many biological processes (Pruyne and Bretscher, 2000; Grebe *et al.*, 2001; Jan and Jan, 2001). Cell polarity involves complex processes requiring spatial and temporal coordination of protein localization and activation. In *Saccharomyces cerevisiae*, a protein complex called the polarisome localizes to growth sites in a cellcycle-dependent manner and affects polarized growth (Snyder, 1989; Flescher *et al.*, 1993; Valtz and Herskowitz, 1996; Fujiwara *et al.*, 1998; Sheu *et al.*, 2000; Ozaki-Kuroda *et al.*, 2001). The polarisome consists of Spa2p, Bni1p, Pea2p, and Bud6p, and perhaps additional proteins (Sheu *et al.*, 1998). Deletion of any component of the polarisome results in a defect in bipolar bud site selection and a moderate defect in polarized growth that results in rounder cells at all temperatures (Zahner *et al.*, 1996; Amberg *et al.*, 1997; Sheu *et al.*, 2000).

Spa2p interacts with Msb3p/Msb4p and Bni1p through distinct SHD domains and is considered a scaffold protein in the polarisome (Fujiwara *et al.*, 1998; Tcheperegine *et al.*, 2005). The formin Bni1p binds directly with Spa2p (Fujiwara *et al.*, 1998; Tcheperegine *et al.*, 2005) and several Rho GTPases controlling actin assembly and cell polarity (Evangelista *et al.*, 1997; Fujiwara *et al.*, 1998). Recently, Bni1p was found to nucleate actin filaments *in vitro* (Pruyne *et al.*, 2002; Sagot *et al.*, 2002a; Sagot *et al.*, 2002b). Bud6p is an actin monomer-binding protein that promotes Bni1p-stimulated actin assembly *in vitro* (Moseley *et al.*, 2004; Moseley and Goode, 2005). The biochemical function of Pea2p is not known.

Candida albicans is the most frequently encountered fungal pathogen in humans. It causes not only oral and vaginal thrush, but also systemic or life-threatening infections in immunocompromised hosts (Odds, 1987; Corner and Magee, 1997; Kim *et al.*, 2005). Morphological switching between yeast and hypha is believed to allow *C. albicans* to rapidly colonize and disseminate in host tissues, thereby facilitating the spread of infection (Braun *et al.*, 2001; Kadosh and Johnson, 2001). Based on the observation that *C. albicans* mutants that are unable to produce a hyphal form have exhibited a great reduction in virulence in a murine model, morphological switching is thought to contribute to the virulence of *C. albicans* (Lo *et al.*, 1997; Brown and Gow, 1999). For this reason, studies of the components and mechanisms underlying yeast hypha morphogenesis have been so numerous during the last decade (Liu, 2001; Berman and Sudbery, 2002).

Recently, the Spa2, Bni1, and Bud6 proteins in *C. albicans*, which are orthologs of the components of the *S. cerevisiae* polarisome, were found to localize

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predominantly to the tips of growing hyphae (Crampin *et al.*, 2005; Li *et al.*, 2005; Martin *et al.*, 2005). Deletion of *CaSPA2* caused the hyphae to thicken, but did not block overall hyphal initiation and growth, which suggests that CaSpa2p does not determine global polarity but modulates local polarized growth (Zheng *et al.*, 2003). Deletion of *CaBNI1* resulted in germ tube formation, but hyphal growth stopped at the step of polarity maintenance (Li *et al.*, 2005; Martin *et al.*, 2005).

Because the roles of CaBud6p in hypha formation of *C. albicans* have not been studied yet, we decided to investigate the importance of CaBud6p in modulating cell polarity in *C. albicans*. We searched the *C. albicans* genome database to identify an ortholog of the *S. cerevisiae BUD6* gene and constructed a homozygous *CaBUD6* null mutant to evaluate the *in vivo* function of the CaBud6 protein. In a hypha-inducing medium, the mutant cells demonstrated a severe defect in their ability to switch from yeast to hypha, which suggests that CaBud6p plays an important role in the polarized growth of *C. albicans*.

Materials and Methods

Strains, media, and growth conditions

All of the strains used in this work are described in Table 1. The *S. cerevisiae* and *C. albicans* strains were grown in YPD (1% yeast extract, 2% Bacto-peptone, and 2% glucose) medium with appropriate auxotrophic requirements. For synthetic complete (SC) media, SC-Ura (SC medium lacking uridine and uracil) was used. To select Ura^+ clones, the SC-Ura medium was supplemented with 60 μg/ml of uridine and 1 mg/ml of 5-fluoroorotic acid (FOA) (Fonzi and Irwin, 1993). To determine the ability of these organisms to undergo yeast-to-hypha transition, budding *C. albicans*cells were grown overnight at 28°C with vigorous shaking in YPD medium. Ten to fifty cells were then incubated per plate for the indicated times at 37°C on three kinds of hypha-inducing media: Solid spider medium, which contained 1% nutrient broth, 0.2% K_2HPO_4, 1.35% agar, and 1% mannitol as a carbon source (Liu *et al.*, 1994), Lee's medium, which was prepared as described by Lee *et al.* (1975) and serum medium, which was prepared by adding 10% (v/v) fetal calf serum (FCS) to YPD at 50°C after autoclaving.

Escherichia coli used for DNA manipulation was DH5 (FΦ80d *lacZ* DM15 Δ[*lacZYA-argF*] U169 *hsdR17* [rk^-mk^+] *deoR recA1 supE44λ-thi-1gyrA96 relA1*). *E. coli* cells were cultured in Luria-Bertani (LB) medium containing ampicillin, which was added to a concentration of 100 μg/ml.

Table 1. Yeast strains and plasmids used in this study

Strain or plasmid	Genotype or description	Original name/ Source
C. albicans strains		
SC5314	Clinical isolate (wild-type strain)	Fonzi and Irwin (1993)
CAI4	*ura3::imm434/ura3::imm434*	Fonzi and Irwin (1993)
CB6_1	as CAI4, but *CaBUD6/Cabud6*::3'*CaURA3-CaURA3*	This work
CB6_2	as CAI4, but *CaBUD6/Cabud6*::3'*CaURA3*	This work
CB6_3	as CAI4, but *Cabud6*::3'*CaURA3/ Cabud6*::3'*CaURA3-CaURA3*	This work
S. cerevisiae strains		
EGY48	*MAT his3 trp1 ura3 LexA*$_{op(x6)}$*-LEU2*	Estojak *et al.* (1995)
EGY48[p8opLacZ]	EGY48 with p8opLacZ	This work
EGY48[p8opLacZ]-CC	EGY48[p8opLacZ] with pB42AD and pLexA	This work
EGY48[p8opLacZ]-AB	EGY48[p8opLacZ] with pB42AD-CaACT1 and pLexA-CaBUD6	This work
Plasmids		
pCaBUD6D	*CaURA3*-marked vector with *CaBUD6* disruption cassette	This work
pB42AD	*TRP1* vector with P $_{GAL1}$-*B42-AD*	pJG4-5 Gyuris *et al.* (1993)
pLexA	*HIS3* vector with P $_{ADH1}$-*LexA-BD*	pEG202 Gyuris *et al.* (1993)
p8opLacZ	*URA3* vector with P $_{GAL1}$-*LexA*$_{op(x6)}$-*LacZ*	pSH18-34 Golemis *et al.* (1994) Estojak *et al.* (1995)
pB42AD-CaACT1	pB42AD containing *CaACT1*	This work
pLexA-CaBUD6	pLexA containing *CaBUD6*	This work

Disruption of CaBUD6

To construct a *CaBUD6* disruption cassette, 5' and 3' regions (628-bp and 641-bp, respectively) of the *CaBUD6* gene were amplified by polymerase chain reaction (PCR) from genomic DNA with two primer sets: CaBUD6-AF1 and -AB1 and CaBUD6-AF2 and -AB2 (Table 2). Both amplified products were PCR-fused using the linker sequence of the CaBUD6-AB1 and CaBUD6-AF2 primers, and the fused PCR product was cloned into pGEM-Teasy vector (Promega, USA). The *Bgl*II-digested 3'*CaURA3-CaURA3* blaster (1.6 kb) – which allows the *CaURA3* marker to be recycled, thereby permitting the sequential disruption of target genes within the same strain – was inserted into the *Bgl*II site between the amplified *CaBUD6* gene fragments, resulting in a plasmid (pCaBUD6D) containing the *CaBUD6* disruption cassette (2.9 kb).

Disruption of the two allelic copies of *CaBUD6* was performed using the two-step procedure described by Fonzi and Irwin (1993). The *Not*I fragment containing the *CaBUD6* disruption cassette was liberated from pCaBUD6D and used to transform the CAI4 strain (*ura3::imm434/ura3::imm434*) (Fonzi and Irwin, 1993) to uridine prototrophy using a lithium acetate-mediated procedure (Gietz *et al.*, 1992). Transformants were selected on SC medium without uridine (SC-Ura). One representative transformant, termed CB6_1 (*ura3::imm434/ura3::imm434 Cabud6::* 3'*Caura3-CaURA3/CaBUD6*), was plated on 5-FOA to select for intrachromosomal recombination between the original *CaURA3* gene and the duplicated 3' region of *CaURA3*, causing the loss of the *CaURA3* selectable marker. The resulting strain, CB6_2 (*ura3:: imm434/ura3::imm434 Cabud6*::3'*Caura3/CaBUD6*), was transformed with the disruption cassette to delete the second allele. A homozygous *CaBUD6* null mutant strain (*ura3::imm434/ura3::imm434 Cabud6::* 3'*Caura3/Cabud6::*3'*Caura3-CaURA3*) was obtained and named CB6_3. The strains obtained in each step were verified by PCR and Southern blot analysis.

Table 2. Primers used in this study

Primer	Sequence (5' to 3')
CaBUD6-AF1	CCTCATGAAGAAGCTGTTGC
CaBUD6-AB1	CCATGGATCCGTAGATCTGCGTGCCCACT GAGTTAAA
CaBUD6-AF2	AGATCTACGGATCCATGGACACCAAACCA TGAAAGTCG
CaBUD6-AB2	AAAGGAACTCCGGTCTGA
Linker sequence	AGATCTACGGATCCATGG
CaBUD6F	GGAATTCATGTCATCCAACAATTCCC
CaBUD6R	GGAATTCTATACCCCCAAACGAGC
CaACT1F	GGAATTCATGGACGGTGAAGAAGTTGCTG CTTTAA
CaACT1R	GGAATTCTTAGAAACCTTTGTGGTG

Two-hybrid analysis

The yeast two-hybrid system was carried out using the MATCHMAKER Two-Hybrid System (Clontech, USA) according to the manufacturer's protocol. To make the plasmids for the two-hybrid system, the complete *CaBUD6* and *CaACT1* genes were isolated from *C. albicans* genomic DNA using two primer sets: "CaBUD6F and CaBUD6R" and "CaACT1F and CaACT1R," respectively (Table 2). CaBud6p was fused with the LexA DNA-binding domain (BD) and CaAct1p with the B42 activating domain (AD). The constructed plasmids, pLexA-CaBud6p and pB42AD-CaAct1p, were used to cotransform the *S. cerevisiae* EGY48 strain containing p8op-lacZ. The resultant transformants were tested for β-galactosidase activity and survival on selective media: SD/Gal/Raf/-His/-Trp/-Ura/BU salt/X-gal and SD/Gal/Raf/-His/-Trp/-Ura/-Leu/BU salt/X-gal.

Results

Identification of the CaBUD6 gene

To find a *BUD6* homologous gene in *C. albicans*, we performed a BLAST-homology search of the *C. albicans* genome database (http://www-sequence.stanford.edu/group/candida) using the *S. cerevisiae BUD6* gene and identified an open reading frame (ORF, Contig6-2452) that specifies a protein consisting of 703 amino acids and shows 37% identity in amino-acid sequence with ScBud6p over their entire sequences (Fig. 1A). We designated it *CaBUD6*. *S. cerevisiae* Bud6p has a coiled-coil region near its C-terminus that may mediate the interaction with actin or the homo-oligomerization of Bud6p (Amberg *et al.*, 1997). Interestingly, an analysis of CaBud6p using ISREC program (http://searchlauncher.bcm.tmc.edu/seq-search/struc-predict) revealed that CaBud6p also contains two predicted coiled-coil regions (Fig. 1B).

CaBUD6 is involved in the polarized growth of C. albicans

Deletion of *BUD6* in a diploid *S. cerevisiae* strain caused severe defects in cell polarity (Amberg *et al.*, 1997; Sheu *et al.*, 2000). To determine whether *CaBUD6* plays a critical role in cell polarity and hyphal development in *C. albicans*, we constructed a *CaBUD6* disruption cassette (Fig. 2A) and deleted *CaBUD6* using the cassette, as described in the *Materials and Methods* section. The mutant strains – the heterozygous *CaBUD6/Cabud6* mutants (CB6_1

A

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CaBud6p    1 ----------MSSNNSRLTPDRTSASSSTNNVSHNLSGKR--RSLNTIESSVTRLLVSTKHLLESLTQWARKEADDKF
ScBud6p    1 ------------------MKMAVDDPTYGTPKIKRTASSSS------SIETTVTKLLMSTKHLLQVLTQWSKGTTSGRL
YlBud6p    1 MSYHGGQHPPPPMAHPPHHQSHNQQAPTGSSRRLSAQTPMARNSTAMNSIESSVTHLLVATKQLLESLTQWARGAASEQD

CaBud6p   67 VSDAYVKLGNDFRAAIRAFSNNGIDISDIGNVPQALRIILEAALSEAPSQENLDRFLPSIRNIIVTLLQTLKTKQAKAKA
ScBud6p   56 VSDAYVQLGNDFKVVSKFFMHAKVDMSDVGDVPMALRRVLEVTLREPPSDETLNKHLPKIREIIVTLLDKLKVKQAILKN
YlBud6p   81 VSDIYVNLGNEFNIACRAFLNAGLEVTDMGDVPQALRTVLEKALSEEASQENLDLYLPSIREIIINLLQGLKKKQAALRA

CaBud6p  147 IS----------QEKSTKEELPTENQRPSSLGVEGQQQQQQQQQQQQQRTGDNDSSVE--------------------
ScBud6p  136 MQQEHRISVKSHHQQNPSFTSNLSLGSEGTREGTPLSSRKSSIVRDQRQSDSVENSYGEKVNSTSTGTPSAQSAEATLTK
YlBud6p  161 AQGAQAQQIPPQQSPKNFRPHPPQHQYGPSRQVSAPTSLPQHKDDRDHVRRSQTIGSAPKQPGLPSPPVARQPPQPPHMA

CaBud6p  195 ------------------------------RSDALSQLQKGDVLQRRASKRFSAYQYAKLTNFN---------NPTTS
ScBud6p  216 PRTNIKQNLKSNNAPN-------------ASDDDDALSQLKKGTNLQRRASKRYSAYHMAKLTNQSTTEAAAAAGLMTTP
YlBud6p  241 PPAHMPPGIPGAMAQAMAPAIPQGSPTRRPGSRGDPLAALQRGEALERRASRRFSAYQYAKLTSG----------APGKA

CaBud6p  234 VPKIKPMESPEVRH-------------------------------SKIMETEEPNVTPNKG--------VESVQEDGNTEE
ScBud6p  283 SPSMLHLEETVRKSKLYGNNNNDDDRNINSAENKGKSIDDVSKASPLAKTPLPIENVRASPRRLSSVVTTSPDKAMNGTC
YlBud6p  311 VPDMPMLPS-----------------------------------------TPLPDESDLGD--------VSTATDVGGPGM

CaBud6p  276 YVLLRINAKTKKAKVSLPVTFASLRLLFVEKFAYSPGAGNFPDIYIQDPRSGVLYELEEHLLPDVKSGTLLCLNETDDQN
ScBud6p  363 PVFLRIGDKTKKCHVQLPTTKNALRLLFIERFAYSPGANSFPDIYIMDPQYGVFYELEELNLLDIKEGFVIELKLEENPN
YlBud6p  343 SLFLRLGNATKKVKADSDVNINALRLLFISTFNYSPGADDFPNIYIRDKKSGVEYELEESHLSDIHDGTMLVLHEEEEKE

CaBud6p  356 KSINELNLRLDDLT-----------------------------------KKIDTMGSTIIAQVKDSVASVEIPVPTVAAS
ScBud6p  443 NTIKEFIDTVKMEIS-----------------------------------NSQNDIIRHLKKMSFGSAISGKQTEVLP
YlBud6p  423 KEKEKEKEKSDDIQGILEHVKAITFALNQQREEIGDLKLMVEKKPPSAAVGAGTQGPSIVKATEPIVKTPVIAPPEVSQA

CaBud6p  401 ASAVTAKSGNAN-----EANKIGRKDITNLERDFRAIKQLHNSSSENFKETISNITEQLRKFQEIG-LEVSQNSNRAYME
ScBud6p  486 QPGLEANKHDLVG----QNKKDDDKTIKDIQYELGKIKQVHNINRSNINETIFNILRKVDNEKSLS-FSAKNSSNRMYME
YlBud6p  503 KLSTIAPAPVPTPIADKDLLKAKAADMHALKHELAVIRQLKDSSFTLWKTSMKDISDTIGKLRDYSSLPAAGDNSRAFME

CaBud6p  475 SCNSKLSDDSDLLITKVDDLQDIMEEMRKDVAQRGVRVSDKQLKHILKDINLAKKSLHDMTLYTSKERPVWKKIWEAELD
ScBud6p  561 KSQTELGDLSDTLLSKVDDLQDVIEIMRKDVAERRSQPAKKKLETVSKDLENAQADVLKLQEFIDTEKPHWKKTWEAELD
YlBud6p  583 MANKKVSTDSDALLTRADDLQDVIEALRKDVAQRGVRAEPGQLEVVTKEMETCNTELTALETYINSQKSVWKKVWERELE

CaBud6p  555 KVCEEQQFLNLQDDLIQDLQDDIKKIEETYNLIEQCSIEQIKGASSKRNKIVANLYIPEPGESLHDLKDAVLNDIVGLTP
ScBud6p  641 KVCEEQQFLTLQEELILDLKEDLGKALETFDLIKLCCEEQEKNPSRSKSNPILP---IMRPGTFNQVREQVMVAVQSLNP
YlBud6p  663 TICEEQQFFKLQEELMADLREDLASAASTLALGEQWSNEQLKSAAKNSHKAVL---LEVETESVQDLKEGVLSSVLALKP

CaBud6p  635 NHESRLEAIERAEKLREKERDMMKLTKFQEELGDFVEDKKLKKSGGIEEIEKQRQLRDIENLKSSFGGI-----------
ScBud6p  718 DHDSRVEAIDKAEKMWEMERKLKASNEFDDELENFVGNSNLKKSGGFEEVERIRKQKDEANLRAYFGPGFT---------
YlBud6p  740 DHESRIEAIERSERLR-KFELESRVDKEEKELGEFVGENKLKPSGGVAEAEKQRLEREQRVWREQFGYGGDEDDIDDLDG

CaBud6p      ----------------------------
ScBud6p      ----------------------------
YlBud6p  819 ELQTASEEETDEEESEAETDEDEEEEEGN
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B

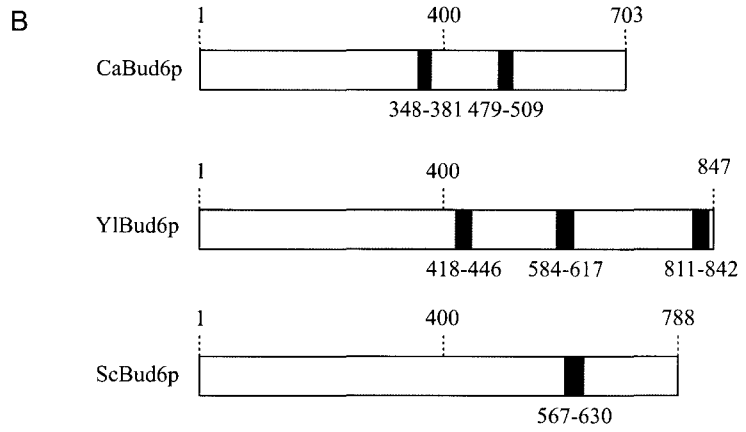

Fig. 1. Comparison of yeast Bud6 sequences. A. The *S. cerevisiae* Bud6p (ScBud6p), *Y. lipolytica* Bud6p (ScBud6p) and *C. albicans* Bud6p (CaBud6p) protein sequences were aligned using ClustalW (Holmes and Bruno, 2001) and visualized using Boxshade. Black shading indicates identical residues and grey shading similar residues. B. Schematic structure of Bud6 proteins. Black boxes represent coiled-coil regions (cc), as predicted by ISREC program.

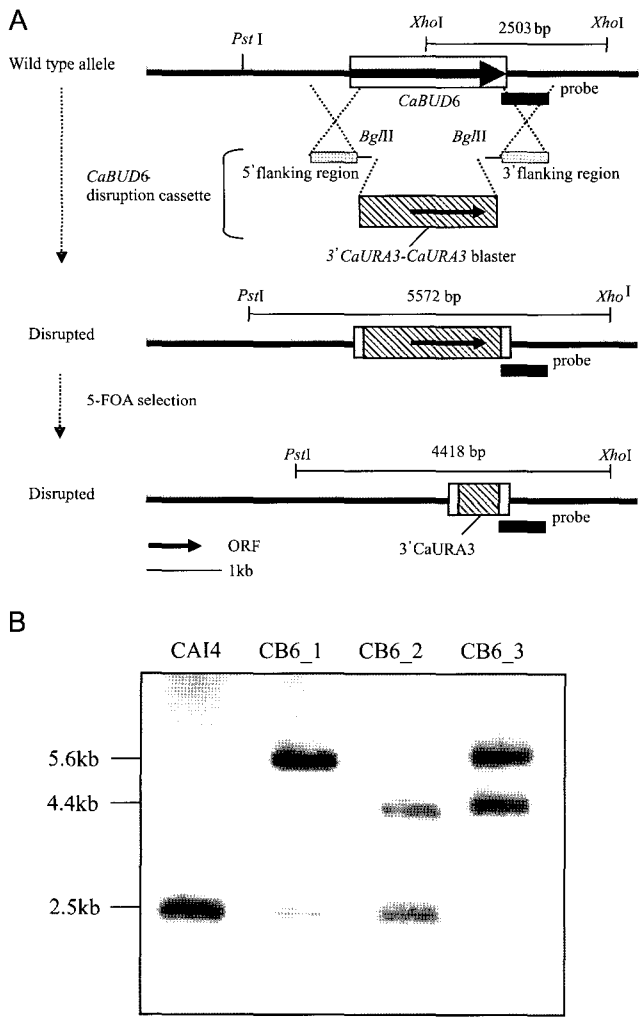

Fig. 2. Disruption of *C. albicans BUD6*. A. Partial restriction map of the *CaBUD6* locus. B. Southern blot analysis. Genotypes of the strains are indicated above each lane. Genomic DNA was isolated from all representative isolates, completely digested with *Pst*I and *Xho*I, and hybridized with a 641 bp fragment amplified by CaBUD6-AF2 and CaBUD6-AB2. The parental strain (CAI4) displayed a single hybridizing band of approximately 2.5 kb. The heterozygous Ura$^+$ *CaBUD6/Cabud6* strain (CB6_1) exhibited one band of 2.5 kb and a second band of approximately 5.6 kb, the predicted size of the replaced allele. One hybridizing band of approximately 4.4 kb, corresponding to the *Cabud6*::3'*CaURA3* allele, and a second band of 5.6 kb were exhibited in the *Cabud6/Cabud6* null mutant strain (CB6_3).

and CB6_2) and the homozygous *Cabud6/Cabud6* mutant (CB6_3)－were verified by Southern blot analysis (Fig. 2B). The generation of homozygous *Cabud6*mutant indicates that *CaBUD6* is not essential for *C. albicans* viability.

We analyzed morphological phenotypes of the wild-type (SC5314), heterozygous *Cabud6* mutant (CB6-1), and homozygous *Cabud6* mutant (CB6-3) strains during the yeast growth stage. In YPD medium, in which *C. albicans* grows in yeast form, the homozygous *Cabud6* mutant cells exhibited several morphological defects, such as round, enlarged cells; widened bud necks; and a random budding pattern (Fig.3A). This result suggests that CaBud6p is important in determining polarity in yeast-form cells.

To investigate the effects of *CaBUD6* deletion on hyphal growth of *C. albicans*, the *Cabud6* mutant strains were grown on several solid hypha-inducing media (Lee's, spider, and serum media) at 37°C. While the wild-type strain formed long, branched filaments that radiated from the colony periphery under all tested conditions, the homozygous *Cabud6* mutant strain grew primarily in its yeast form on Lee's and spider media and formed colonies with short filaments at the colony rims on 10% serum medium (Fig. 3B). The heterozygous *Cabud6* strain showed morphological phenotypes similar to those of the wild-type strain on the serum and spider media, but formed smooth colonies without hyphae on Lee's media (Fig. 3B). We also investigated the ability of the homozygous *Cabud6* mutant to form germ tubes and hyphae in a liquid YPD medium containing 10% serum at 37°C. As shown in Fig. 3C, the homozygous *Cabud6* mutant strain did produce germ tubes. In contrast to the thin and straight germ tubes of the wild-type cells, however, the germ tubes of the mutant cells were thicker and curved. Interestingly, with prolonged incubation, the hyphae of the homozygous *Cabud6* mutant strain were much shorter than those of the wild-type and heterozygous mutant strains. These findings－that the deletion of *CaBUD6* causes severe defects in hyphal growth on various solid hypha-inducing media and prevents maintenance of normal hyphal growth in liquid hypha medium－demonstrate that *CaBUD6* is required for normal hyphal growth of *C. albicans*.

CaBud6p interacts with actin

The actin cytoskeleton is believed to play a major role in the establishment and maintenance of cell polarity (Pruyne and Bretscher, 2000). In *S. cerevisiae*, Bud6p, together with Bni1p, facilitates the assembly of a polarized actin cytoskeleton, perhaps through its ability to interact with actin (Amberg *et al.*, 1997; Evangelista *et al.*, 1997). Because we know that CaBud6p is involved in the polarized growth of *C. albicans*, we expected CaBud6p to form a complex with actin, as occurs in *S. cerevisiae*. On this basis, we performed yeast two-hybrid experiments to obtain evidence of the direct interaction of CaBud6p with actin in *C. albicans*. The strains carrying control plasmids－pLexA-CaBud6p (DNA binding domain-CaBud6p) or pB42AD-CaAct1p (activation domain-CaAct1p)－did not take on a blue color, but the strain

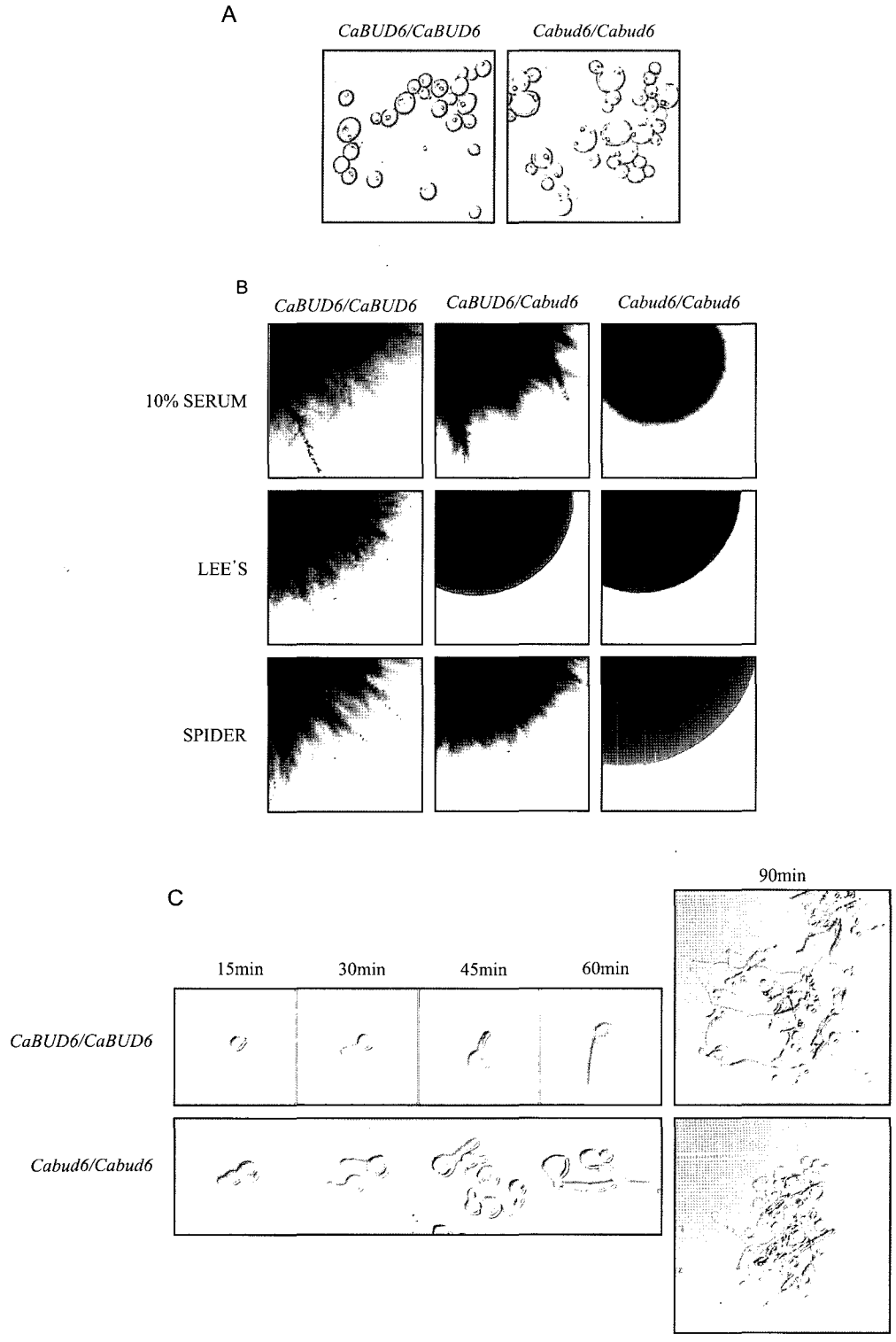

Fig. 3. Effects of *CaBUD6* deletion on colony and cellular morphology. A. The yeast cells of the wild-type strain (SC5314) and the mutant homozygous *Cabud6/Cabud6* mutant (CB6_3) were grown in YPD at 30°C to exponential phase. B. Cells from overnight cultures were washed with sterile water twice and spreaded with 50-100 C.F.U. per plate on the indicated media (Lee's, spider and 10% fetal bovine serum media). All the strains shown are uridine prototrophs; wild-type strain (SC5314), the heterozygous *CaBUD6/Cabud6* mutant (CB6_1) and the homozygous *Cabud6/Cabud6* mutant (CB6_3). Plates were incubated at 37°C for 4 days. C. The overnight cultures were washed with sterile water twice. The same amount of cells (OD_{600}=0.3) were inoculated into liquid YPD media containing 10% fetal bovine serum and grown at 37°C for 1.5 h. Cellular and colony morphologies of each strain were visualized by DIC microscopy with magnification X600 and X40, respectively.

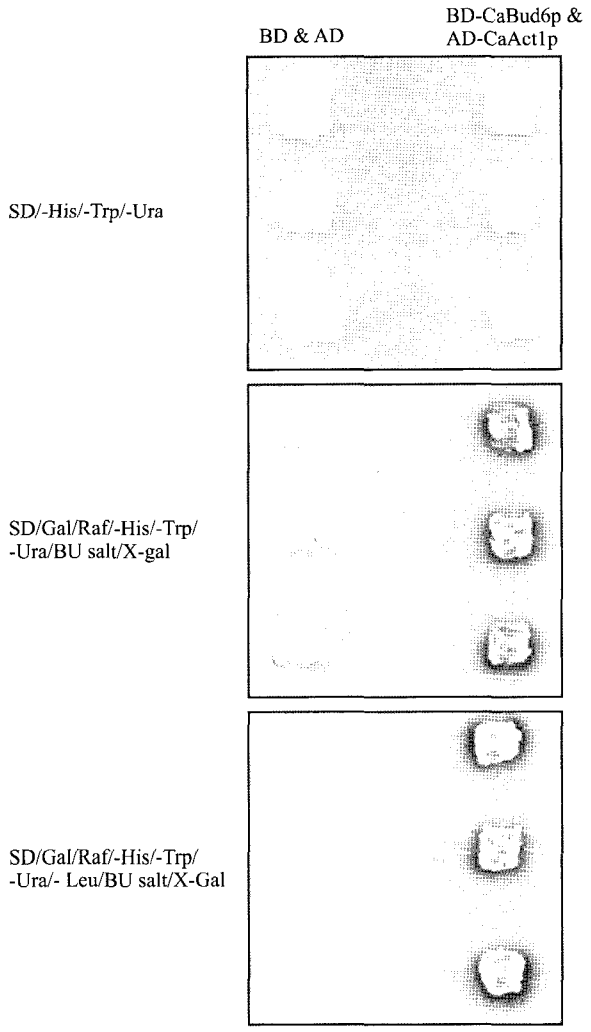

Fig. 4. Interaction between CaBud6p and CaAct1p. Yeast two hybrid analysis was performed with two fusion constructs of BD-CaBud6p (pLexA-CaBud6p) and AD-CaAct1p (pB42AD-CaAct1p). Yeast strain EGY48[p8opLacZ] was cotransformed with pLexA-CaBud6p and pB42AD-CaAct1p. The resulting transformants and control strain (containing pLexA and pB42AD) were tested for β-galactosidase activity and viability on selective media.

carrying both the pLexA-CaBud6p and pB42AD-CaAct1p plasmids highly enhanced *lacZ* expression on a selective medium, SD/Gal/Raf/-His/-Trp/-Ura/BU salt/X-gal. The ability to survive on a selective medium lacking leucine (SD/Gal/Raf/-His/-Trp/-Ura/-Leu/BU salt/X-gal) confirmed the physical interaction of CaBud6p with CaAct1p (Fig. 4).

Discussion

In this study, we demonstrated that the *C. albicans BUD6* plays a role in establishing the cell polarity of *C. albicans*. The homozygous *Cabud6* mutant cells appeared rounder than the wild-type strains during the yeast growth stage, suggesting the loss of cell polarity. The same phenotype has been observed for *S. cerevisiae, Schizosaccharomyces pombe* and *Yarrowia lipolytica bud6* mutants (Amberg *et al.*, 1997; Sheu *et al.*, 2000; Jin and Amberg, 2001; Richard *et al.*, 2001; Song *et al.*, 2003). Therefore, it seems that a common function of the Bud6 protein in various types of yeast is to maintain polarized growth. We also found that the *Cabud6* mutant cells had a random budding pattern. These data, along with the findings in *S. cerevisiae* and *Y. lipolytica bud6* mutants (Amberg *et al.*, 1997; Sheu *et al.*, 2000; Jin and Amberg, 2001; Richard *et al.*, 2001; Song *et al.*, 2003), suggest the importance of CaBud6p in selecting a precise bud site during the yeast growth stage.

We observed an unconditionally severe defect in the ability of homozygous *Cabud6* mutant cells to form hyphae on solid hypha-inducing media. In liquid hypha-inducing serum medium, however, the homozygous *Cabud6* mutant cells formed germ tubes and hyphae, although the hyphae were thicker and much shorter than those seen in the wild-type strain. The *C. albicans* polarisome proteins−Bni1p, Spa2p, and Bud6p−are known to localize predominantly to the sites of polarized growth (Crampin *et al.*, 2005; Li *et al.*,2005; Martin *et al.*, 2005). Based on the observation that *CaBNI1*-deleted mutants successfully formed germ tubes but failed to maintain polarized growth (Li *et al.*, 2005; Martin *et al.*, 2005) and that *CaSPA2*-deleted mutants formed thick hyphae (Zheng *et al.*, 2003), the major roles of the *C. albicans* Bni1 and Spa2 proteins are thought to modulate local polarized growth rather than determine global polarity. The existence of morphological phenotypes for the *CaBUD6*-deleted mutants that are similar to those of *CaBNI1*- and *CaSPA2*-deleted mutants support the idea that the *C. albicans* polarisome proteins maintain cell polarity and polarized growth at sites that are predetermined by factors that regulate global polarity.

The deletion of *CaBUD6* did not completely block hyphal initiation and growth in a liquid medium. In our previous study with *Y. lipolytica* (Song *et al.*, 2003), deletion of *YlBUD6*prevented the formation of hyphae in both solid and liquid hypha-inducing media. It was recently reported that *S. cerevisiae* Bud6p stimulates Bni1p to promote robust cable formation, and Bni1p, in turn, delivers more Bud6p to the bud tip on Bni1p-nucleated actin cables, thereby reinforcing polarized cell growth through a positive feedback loop (Moseley *et al.*, 2004; Moseley and Goode, 2005). It would be interesting to determine whether similar molecular mechanisms are used by the Bud6 and Bni1 proteins of *C. albicans* and *Y. lipolytica* and why differences in the extent of the

defect in hyphal growth arise despite the structural and functional homology of the CaBud6 and YlBud6 proteins.

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