J. Microbiol. Biotechnol. (2005), **15**(5), 1146–1151

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

cDNA Cloning of Farnesoic Acid-Induced Genes in *Candida albicans* by Differential Display Analysis

CHUNG, SOON-CHUN[1], JI-YOON LEE[1], AND KI-BONG OH[1,2]*

[1]*School of Agricultural Biotechnology, Seoul National University, Seoul 151-921, Korea*
[2]*Natural Products Research Institute, Seoul National University, Seoul 110-460, Korea*

Received: February 14, 2005
Accepted: March 21, 2005

Abstract The yeast *Candida albicans* has a distinguishing feature, dimorphism, which is the ability to switch between two morphological forms: a budding yeast form and a multicellular invasive filamentous form. This ability has been postulated to contribute to the virulence of this organism. Previously, we reported that the yeast-to-hypha transition in this organism is suppressed by farnesoic acid, a morphogenic autoregulatory substance that accumulates in the medium as the cells proliferate. In this study, using a differential display reverse transcription polymerase chain reaction (DDRT-PCR) technique, we have identified several genes induced in *C. albicans* by farnesoic acid treatment. These observations indicate that farnesoic acid can alter the expressivity of multiple genes, including the DNA replication machinery and cell-cycle-control proteins.

Key words: *Candida albicans*, morphological transition, farnesoic acid, differential display analysis, Northern blot analysis, respondent genes

Candida albicans undergoes reversible morphogenetic transitions during budding, pseudohyphal growth forms mainly in response to environmental conditions, and the switch from a yeastlike form to a filamentous form often correlates with pathogenicity. Morphogenesis of *C. albicans* is triggered by various signals *in vitro*: serum, high temperature, neutral pH, and poor media stimulate yeast cells to sprout true hyphae [4, 22]. Conversely, low temperature, air, acidic pH, and enriched media promote yeast cell growth. In addition, *C. albicans* has been reported to respond to exogenous human hormones such as estradiol and progesterone [15, 34], suggesting that hyphal induction could be modulated by other host factors *in vivo* [25, 26, 33]. Molecular studies of dimorphism have led to the identification of several genes that regulate hyphal morphogenesis [8, 21]. These include the two genes *CPH1* and *EFG1*, whose products play positive roles in promoting the formation of filamentous forms. *C. albicans CPH1* is regulated by a mitogen-activated protein (MAP) kinase cascade. *EFG1* has a complex role in the regulation of filamentous growth in *C. albicans*, having both positive and negative effects [18, 19, 28]. Two other *C. albicans* genes, *TUP1* and *RBF1*, act negatively to repress the ability to grow in filamentous forms [3, 32]. The regulatory networks that control *C. albicans* morphogenesis have been elucidated. However, despite these investigations, the mechanisms of dimorphism remain unclear, possibly because the environmental factors that induce the dimorphic transition have many effects on cells, thus making it difficult to identify which factors are essential for the transition.

It has been reported that yeast-to-hypha transition in *C. albicans* is under the control of at least three morphogenic autoregulatory substances, which accumulate in the medium as the cells proliferate: farnesoic acid [24] and farnesol [12], which inhibit transition, and tyrosol [5], which promotes it. However, the mechanism behind these substances is unclear. Recently, it has been shown that the mRNAs (*HST7* and *CPH1*) in MAP kinase cascades were decreased in farnesol-treated *C. albicans*, but *CST20* (a MAP kinase) was not [29]. These studies on farnesol provide insight into the function of farnesoic acid in *C. albicans*. Based on the our previous works [14, 24], we performed a differential display analysis to identify farnesoic acid-induced genes as the first step to elucidate the cellular control mechanism of farnesoic acid in *C. albicans* morphogenesis. As a result, we have identified several genes induced by farnesoic acid treatment.

C. albicans strain ATCC10231 was cultured in YPD medium (1% yeast extract, 2% peptone, and 2% dextrose) at 28°C for 48 h. Cells (early stationary phase) were collected

*Corresponding author
Phone: 82-2-880-4646; Fax: 82-2-873-3112;
E-mail: ohkibong@snu.ac.kr

by centrifugation, washed with glucose salts (GS) medium [24], and re-inoculated (2×10^6 cells/ml) in either GS or GS plus synthetic farnesoic acid (40 μM) [14]. Cultures were allowed to grow at 37°C. The *C. albicans* cells exposed to farnesoic acid for indicated time and nonexposed cells (control) were collected, and the total RNA was isolated using an RNeasy Midi Kit (Qiagen, Hilden, Germany). Cells were collected by centrifugation and resuspended in 1.5 ml of RLT lysis buffer containing 10 μl/ml β-mercaptoethanol. Then, 0.3 g of acid-washed glass beads (0.5 μm) (Sigma, St. Louis, MO, U.S.A.) was added and the mixture was agitated in a Mini-BeadBeater (Biospec Products Inc., Bartlesville, OK, U.S.A.). After centrifugation at 10,000 ×*g* for 5 min, the supernatant was recovered and the total RNA was isolated by following the manufacturer's suggested protocols. For some experiments, mRNA was subsequently isolated with the Oligotex mRNA Kit (Qiagen) by following the manufacturer's instructions.

A differential display reverse transcription polymerase chain reaction (DDRT-PCR) was performed as described previously [17] with the following modifications. mRNA (0.5 μg) was heated to 65°C for 10 min and then chilled in iced water. To acquire cDNA fragments, we performed reverse transcription using the TaKaRa RNA PCR Kit (version 2.1) (Takara Shuzo), following the manufacturer's instructions. The second-strand cDNA synthesis was carried out by PCR using randomly amplified polymorphic DNA (RAPD) primer. Forty 10-base GC-rich RAPD primers (A-01 to A-20 and I-01 to I-20) were obtained from Operon Technologies (Alameda, CA, U.S.A.). The sequences of these primers are available from the manufacturer. Each PCR mixture contained 2 μl of the products from one of the reverse transcriptase reactions above and 48 μl of a solution containing *Taq*-PCR buffer, four dNTPs (1 mM each), 50 pmol of one of the RAPD primers, and 2.5 units of *Taq* polymerase. After denaturation at 92°C for 5 min, PCR was carried out for 37 cycles of 92°C for 1 min, 35°C for 1 min, and 72°C for 2 min, followed by a 5-min elongation period at 72°C. The products of PCR were resolved by electrophoresis in 1.25% SeaPlaque GTG agarose gels (Takara Shuzo) and visualized by staining with ethidium bromide. Specific fragments from PCR were cut out from the agarose gels. The cDNA was then recovered from the gel and concentrated using Suprec-01 and -02 cartridges (Takara Shuzo, Kyoto, Japan), respectively. Gel-purified cDNAs were directly inserted into the T-A cloning vector pBAD/Thio-TOPO (Invitrogen, Carlsbad, CA, U.S.A.) [20]. DNA inserts were subsequently sequenced using the ABIPrism BigDye Terminator version 3.1 Cycle Sequencing Kit in the ABIPRISM 377 Stretch Laser-induced Fluorescence automatic sequencer (Applied Biosystems, Foster City, CA, U.S.A.). The analysis of the nucleotide sequences confirmed was performed by a comparison with those nucleotide sequences registered in GenBank, using the BLAST Network Service provided by the NCBI (National Center for Biotechnology Information, Bethesda, MD, U.S.A.), and with those registered for the Stanford *Candida* database.

To examine the regulation of obtained genes at the transcriptional level, Northern analyses were performed. Aliquots (10 μg) of total RNA were denatured and subjected to electrophoresis in 1.2% agarose in the presence of 1 M formaldehyde [17]. Size-fractionated RNAs were then transferred onto nylon membrane (Hybond-N$^+$; Amersham Biosciences, Little Chalfont, United Kingdom) and fixed by UV irradiation. cDNAs were labeled with [α-^{32}P]dCTP (Amersham) using the Random Primer DNA Labeling Kit (Ver. 2) (Takara Shuzo). Membranes were hybridized at 65°C for 12 h in phosphate buffer solution (0.5 M sodium phosphate buffer, pH 7.2) containing 7% SDS, 1 mM EDTA, 1% BSA, and heat-denatured probe. Following hybridization, membranes were washed at a high stringency of 65°C for 30 min in a washing buffer (40 mM sodium phosphate buffer, pH 7.2, 5% SDS, 1 mM EDTA). Detection and quantification of radioactivity was performed using the Bio-Imaging Analyzer System (BAS 2500, Fuji, Tokyo, Japan).

In the previous works, we observed that, when *C. albicans* grew in GS medium at 37°C, about 50–70% of the inoculum yeast cells converted into hyphae after 1 h of incubation [14, 24]. Based on these results, we compared the mRNA expression patterns of nontreated cells and those exposed to farnesoic acid (40 μM) at 37°C for 40 min. To avoid bias against isolating differentially displayed cDNAs that are false-positive, only cDNAs whose expression levels

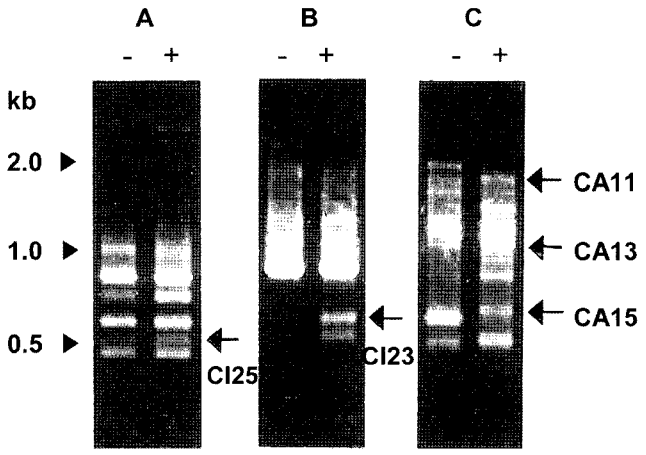

Fig. 1. Representative band patterns on differential display analysis showing induced PCR fragments in farnesoic acid-treated *C. albicans*.

mRNA was extracted from nontreated *C. albicans* cells (-) and those exposed to 40 μM farnesoic acid (+) at 37°C for 40 min, and subjected to differential display analysis. The PCR products were resolved by electrophoresis in 1.25% low-melting-temperature agarose gel and visualized by staining with ethidium bromide. RAPD primers used were as follows: A, 5'-AATGCGGGAG-3'; B, 5'-AAAGTGCGGG-3'; C, 5'-CAGGCCCTTC-3'. Arrows in A-C (CI25, CI23, CA11, CA13, and CA15) indicate signals demonstrating altered expression (see Table 1).

were induced by farnesoic acid in triplicate experiments were selected for further analysis. We have identified a set of differentially expressed genes, among which many were poorly induced. Figure 1 shows representative band patterns of PCR products with three different primer combinations. Indicated in Fig. 1 are PCR products (CI25, CI23, CA11, CA13, and CA15) whose band intensities were reproducibly increased by farnesoic acid treatment. In total, we observed 14 PCR fragments that were differentially displayed between *C. albicans* cultured in the absence and presence of farnesoic acid. However, as described below, Northern blot analysis revealed that three of them were difficult to be characterized, probably due to the sensitivity of the Northern blot analysis. Thus, they were excluded from further analysis. To confirm the gene expression patterns observed in differential display analysis, cDNA fragments were recovered from gels and reamplified by PCR using the corresponding primers. The reamplified fragments were then cloned into plasmid pBAD/Thio-TOPO and used as probes for RNA blots prepared with total RNAs from control and farnesoic acid-treated *C. albicans*. Consequently, we were able to identify 11 genes whose mRNA levels were increased after 40 min of farnesoic acid treatment of *C. albicans*, although changes of mRNA expression of other genes that returned to the basal level within 40 min or were induced after 40 min might have been missed (data not shown; the estimated sizes and the fold changes in mRNA levels of the transcripts are summarized in Table 1).

All of 11 cloned cDNA fragments were completely sequenced, and the resulting sequences were compared for homology with the nucleotide sequences in the GenBank of the NCBI. Homology search against the Current Assembly of *C. albicans* sequence database at Stanford indicated that each sequence has a strong identity with a specific contig. Each clone with a confirmed nucleotide sequence was compared for homology with the nucleotide sequences in the GenBank of the NCBI. The results are summarized in Table 1. The sequence of CA13 was found to be 92% identical with a part of the HOK, a repetitive sequence in the chromosomes of *C. albicans* [6]. The CI23 sequence (642 bp) was identical to a part of CI21 (1175 bp), a transcriptional activator of lysine pathway genes [11]. Although the clones CA11, CA14, CA15, CI21, and CI22 showed similarity with parts of the PHO81 [16], SDS 22 [27], GTT1 [7], LYS14 [9], and YKU80 [2], this similarity was less than 30%, indicating no significant homology in the DNA nucleotide sequence. Therefore, the majority of the farnesoic acid-responsive, differentially expressed cDNAs appear to encode novel proteins. We registered the nucleotide sequences of the five clones (CA11, CA15, CI21, CI23, and CI24) in the GenBank and obtained the accession numbers CO535087, CO535090, CO535087, CO535088, and CO553160, respectively.

As shown in Table 1, the results obtained indicate that farnesoic acid can alter the expressivity of multiple genes, including the DNA replication machinery and cell-cycle-

Table 1. cDNA fragments corresponding to mRNAs, where abundance is altered by farnesoic acid treatment in *C. albicans*.

Clone	cDNA length (bp)	Fold changes in mRNA levels	Nucleotide identity	Protein similarity (Organism)	Description	GenBank accession number
CA11	1,743	2.0	98%, Contig6-2516 (28193-29935)	30%, PHO81 (*S. cerevisiae*)	Cyclin-dependent kinase inhibitor	NP_011749
CA13	1,100	3.2	94%, Contig6-1955 (2397-3545)	92%, HOK (*C. albicans*)	Not likely to encode a functional enzyme	Not registered
CA14	905	0.7	98%, Contig6-2308 (15533-16435)	26%, SDS22 (*S. cerevisiae*)	Protein phosphatase type 1 regulator	NP_012728
CA15	692	1.7	99%, Contig6-2410 (2483-3172)	21%, GTT1 (*S. cerevisiae*)	ER-associated glutathione S-transferase	NP_012304
CA16	530	0.8	98%, Contig6-2501 (89045-89593)	32%, DPB3 (*S. cerevisiae*)	Third-largest subunit of DNA polymerase II	NP_009837
CI21	1,175	1.5	98%, Contig6-2019 (2445-3614)	26%, LYS14 (*S. cerevisiae*)	Transcriptional activator of lysine pathway genes	NP_010327
CI22	1,525	0.8	97%, Contig6-2288 (10190-11704)	24%, YKU80 (*S. cerevisiae*)	Forms heterodimer with Yku70p known as Ku	NP_013824
CI23	642	1.3	98%, Contig6-2019 (2445-3079)	A part of CI21 (*S. cerevisiae*)	Transcriptional activator of lysine pathway genes	NP_010327
CI24	550	1.5	98%, Contig6-2441 (6258-6804)	46%, MSH6 (*S. cerevisiae*)	DNA mismatch repair protein	NP_010382
CI25	545	1.2	99%, Contig6-1951 (2116-2657)	51%, GSH1 (*S. cerevisiae*)	Gamma glutamylcysteine synthetase	NP_012434
CI26	779	0.8	98%, Contig6-1951 (4912-5693)	64%, ADE5, 7 (*S. cerevisiae*)	Bifunctional enzyme of the *de novo* purine nucleotide biosynthetic pathway	NP_011280

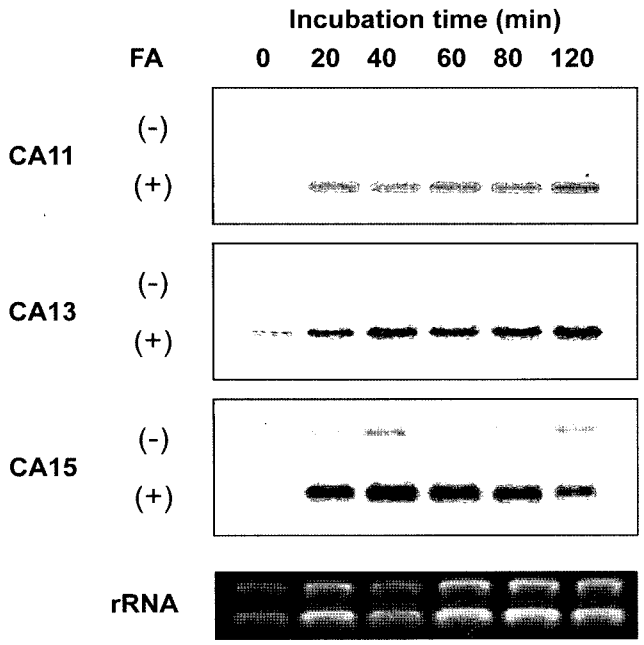

Fig. 2. Effect of farnesoic acid (FA) on CA11, CA13, and CA15 mRNA abundance in cultured *C. albicans* cells.
Total cellular RNA was isolated from nontreated *C. albicans* cells (-) and those exposed to 40 μM farnesoic acid (+) at 37°C for the indicated time and resolved by denaturing agarose gel electrophoresis. Northern blots were hybridized with ^{32}P-labeled cDNA probes prepared from the differential display cDNA fragments. Detection and quantification of radioactivity were performed using the Bio-Imaging Analyzer System. Each lane contains 10 μg of total RNA, and equal loading of RNA was confirmed by ethidium bromide staining of ribosomal RNA.

control proteins. In this study, however, the genes *CPH1*, *EFG1*, *TUP1*, and *RBF1*, which are related to morphogenic regulatory networks in *C. albicans*, were not induced by farnesoic acid. Interestingly, the most highly induced genes identified in farnesoic acid-exposed *C. albicans* were CA11 [30% identical to a part of the PHO81, a cyclin-dependent kinase inhibitor (CKI) in *Saccharomyces cerevisiae*], CA13 (92% identical to a part of the HOK, a repetitive sequence in the chromosomes of *C. albicans*), and CA15 (21% identical to a part of the GTT1, an ER-associated glutathione S-transferase) cDNA fragments. To determine the time courses of mRNA expression of these three genes, total RNA was isolated from *C. albicans* at various times after the addition of 40 μM farnesoic acid, and the mRNA expression levels were analyzed by Northern blot analysis (Fig. 2). These mRNAs were expressed to some extent without stimulation of farnesoic acid. However, a dramatic increase was observed within 40 min of incubation with farnesoic acid. The CA11 and CA13 mRNA expressions continued until at least after 120 min of incubation, whereas CA15 mRNA expression decreased after 40 min of incubation with farnesoic acid.

PHO81 is one of the three CKIs identified in *S. cerevisiae*. It has been reported that PHO81 is a CKI for the kinase PHO80-PHO85 and suggested that the activity of PHO81 is controlled by a posttranslational mechanism in response to extracellular phosphate levels [30]. Deletional analysis has identified both positive and negative regulatory domains located at the carboxy- and amino-terminal regions of the protein, respectively, and it has been suggested that these domains antagonize each other *in vivo* [23]. Between these regions lies a central ankyrin repeat domain of approximately 230 amino acids, containing six ankyrin repeats, which shares significant homology with the $p16^{INK4}$ class of mammalian CKI. This approximately 33-amino-acid motif has been implicated in protein-protein interactions [1, 31]. Although the clone CA11 showed about 30% similarity to that of PHO81 at the protein level, we found that an ankyrin repeat domain of approximately 203 amino acids (eight tandem repeats), which has a strong identity (99%) to that of the PHO81, lies in the central regions in the CA11 clone (GenBank accession no. CO535087). These studies on the PHO81 provide insight into the function of CA11, related to farnesoic acid, in *C. albicans*.

It has been reported that a repetitive sequence, designated as HOK (5,259 bp), is located on all chromosomes in *C. albicans*, except chromosome 3 [6]. These sequences could have some functional significance, since they appear on almost all chromosomes. The conserved region contains the amino acid sequences responsible for binding isocitrate as a substrate as well as the magnesium-ion- and NAD^+-binding sites necessary for enzyme reactions [13]. Nevertheless, this region of HOK is not likely to function as an active enzyme, for several reasons [6]: (i) this ORF is too short to encode a full-size enzyme, (ii) it also contains many stop codons but no ATG initiation codon, and (iii) it is not likely that this kind of gene is located on many chromosomes. It has also been reported that isocitrate dehydrogenase is an RNA-binding protein, although it is not known which region of the enzyme is involved in the actual binding to the RNA [10]. To elucidate the role of HOK in the *C. albicans* morphogenesis, a detailed analysis of the cellular and chromosomal function of CA13 gene is necessary.

In conclusion, this study represents the first investigation into the genes related to the farnesoic acid-mediated morphogenesis in *C. albicans*. Further functional studies on these newly found clones by deletional analysis are in progress and are expected to be published in due course. These results will help elucidate their roles in the *C. albicans* morphogenesis and broaden our understanding of the pathophysiology of candidiasis.

Acknowledgments

This work was supported by Korea Research Foundation Grant (KRF-2001-015-FP0075). S. C. Chung and J. Y. Lee are recipients of a fellowship from the Ministry of Education through the Brain Korea 21 Project.

REFERENCES

1. Bennett, V. 1992. Ankyrins. Adaptors between diverse plasma membrane proteins and the cytoplasm. *J. Biol. Chem.* **267:** 8703–8706.
2. Bertuch, A. A. and V. Lundblad. 2003. The Ku heterodimer performs separable activities at double-strand breaks and chromosome termini. *Mol. Cell Biol.* **23:** 8202–8215.
3. Braun, B. R. and A. D. Johnson. 1997. Control of filament formation in *Candida albicans* by the transcriptional repressor TUP1. *Science* **277:** 105–109.
4. Brown, A. J. P. and N. A. R. Gow. 1999. Regulatory networks controlling *Candida albicans* morphogenesis. *Trends Microbiol.* **7:** 333–338.
5. Chen, H., M. Fujita, Q. Feng, J. Clardy, and G. R. Fink. 2004. Tyrosol is a quorum-sensing molecule in *Candida albicans*. *Proc. Natl. Acad. Sci. USA* **101:** 5048–5052.
6. Chindamporn, A., Y. Nakagawa, I. Mizuguchi, H. Chibana, M. Doi, and K. Tanaka. 1998. Repetitive sequences (RPSs) in the chromosomes of *Candida albicans* are sandwiched between two novel stretches, HOK and RB2, common to each chromosome. *Microbiology* **144:** 849–857.
7. Choi, J. H., W. Lou, and A. Vancura. 1998. A novel membrane-bound glutathione S-transferase functions in the stationary phase of the yeast *Saccharomyces cerevisiae*. *J. Biol. Chem.* **273:** 29915–29922.
8. Dhillon, N. K., S. Sharma, and G. K. Khuller. 2003. Signaling through protein kinases and transcriptional regulators in *Candida albicans*. *Crit. Rev. Microbiol.* **29:** 259–275.
9. El Alami, M., A. Feller, A. Pierard, and E. Dubois. 2002. The proper folding of a long C-terminal segment of the yeast Lys14p regulator is required for activation of LYS genes in response to the metabolic effector. *Mol. Microbiol.* **43:** 1629–1639.
10. Elzinga, S. D., A. L. Bednarz, K. Van Oosterum, P. J. Dekker, and L. A. Grivell. 1993. Yeast mitochondrial NAD(+)-dependent isocitrate dehydrogenase is an RNA-binding protein. *Nucleic Acids Res.* **21:** 5328–5331.
11. Feller A., E. Dubois, F. Ramos, and A. Pierard. 1994. Repression of the genes for lysine biosynthesis in *Saccharomyces cerevisiae* is caused by limitation of Lys14-dependent transcriptional activation. *Mol. Cell. Biol.* **14:** 6411–6418.
12. Hornby, J. M., E. C. Jensen, A. D. Lisec, J. J. Tasto, B. Jahnke, R. Shoemaker, P. Dussault, and K. W. Nickerson. 2001. Quorum sensing in the dimorphic fungus *Candida albicans* is mediated by farnesol. *Appl. Environ. Microbiol.* **67:** 2982–2992.
13. Hurley, J. H., A. M. Dean, Jr. D. E. Koshland, and R. M. Stroud. 1991. Catalytic mechanism of NADP(+)-dependent isocitrate dehydrogenase: Implications from the structures of magnesium-isocitrate and NADP+ complexes. *Biochemistry* **30:** 8671–8678.
14. Kim, S., E. Kim, D. S. Shin, H. Kang, and K. B. Oh. 2002. Evaluation of morphogenic regulatory activity of farnesoic acid and its derivatives against *Candida albicans* dimorphism. *Bioorg. Med. Chem. Lett.* **12:** 895–898.
15. Kinsman, O. S., K. Pitblado, and C. J. Coulson. 1988. Effect of mammalian steroid hormones and luteinizing hormone on the germination of *Candida albicans* and implications for vaginal candidosis. *Mycoses* **31:** 617–626.
16. Knight, J. P., T. M. Daly, and L. W. Bergman. 2004. Regulation by phosphorylation of Pho81p, a cyclin-dependent kinase inhibitor in *Saccharomyces cerevisiae*. *Curr. Genet.* **46:** 10–19.
17. Kokame, K., H. Kato, and T. Miyata.1996. Homocysteine-respondent genes in vascular endothelial cells identified by differential display analysis. *J. Biol. Chem.* **271:** 29659–29665.
18. Leberer, E., D. Harcus, I. D. Broadbent, K. L. Clark, D. Dignard, K. Ziegelbauer, A. Schmit, N. A. R. Gow, A. J. P. Brown, and D. Y. Thomas. 1996. Signal transduction through homologs of the Ste20p and Ste7p protein kinases can trigger hyphal formation in the pathogenic fungus *Candida albicans*. *Proc. Natl. Acad. Sci. USA* **93:** 13217–13222.
19. Leberer, E., D. Harcus, D. Dignard, L. Johnson, S. Ushinsky, D. Y. Thomas, and K. Schroppel. 2001. Ras links cellular morphogenesis to virulence by regulation of the MAP kinase and cAMP signalling pathways in the pathogenic fungus *Candida albicans*. *Mol. Microbiol.* **42:** 673–687.
20. Lee, J.-H., Y.-D. Cho, J. J. Choi, Y.-J. Lee, H.-S. Hoe, H.-K. Kim, and S.-T. Kwon. 2003. High-level expression in *Escherichia coli* of alkaline phosphatase from *Thermus caldophilus* GK24 and purification of the recombinant enzyme. *J. Microbiol. Biotechnol.* **13:** 660–665.
21. Liu, H. 2001. Transcriptional control of dimorphism in *Candida albicans*. *Curr. Opin. Microbiol.* **4:** 728–735.
22. Odds, F. C. 1985. Morphogenesis in *Candida albicans*. *Crit. Rev. Microbiol.* **12:** 45–93.
23. Ogawa, N., K. Noguchi, H. Sawai, Y. Yamashita, C. Yompakdee, and Y. Oshima. 1995. Functional domains of Pho81p, an inhibitor of Pho85p protein kinase, in the transduction pathway of Pi signals in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.* **15:** 997–1004.
24. Oh, K. B., H. Miyazawa, T. Naito, and H. Matsuoka. 2001. Purification and characterization of an autoregulatory substance capable of regulating the morphological transition in *Candida albicans*. *Proc. Natl. Acad. Sci. USA* **98:** 4664–4668.
25. Park, H. S., G. J. Jhon, and W. J. Choi. 1998. Deer antler extract selectively suppresses hyphal growth in dimorphic fungus, *Candida albicans*. *J. Microbiol. Biotechnol.* **8:** 291–294.
26. Park, K.-S., H.-I. Kang, J. W. Lee, and Y.-K. Paik. 2004. Anti-candida activity of YH-1715R, a new triazole derivative. *J. Microbiol. Biotechnol.* **14:** 693–697.
27. Peggie, M. W., S. H. MacKelvie, A. Bloecher, E. V. Knatko, K. Tatchell, and M. J. Stark. 2002. Essential functions of Sds22p in chromosome stability and nuclear localization of PP1. *J. Cell Sci.* **115:** 195–206.
28. Rocha, C. R., K. Schroppel, D. Harcus, A. Marcil, D. Dignard, B. N. Taylor, D. Y. Thomas, M. Whiteway, and E. Leberer. 2001. Signaling through adenylyl cyclase is essential for hyphal growth and virulence in the pathogenic fungus *Candida albicans*. *Mol. Biol. Cell* **12:** 3631–3643.

29. Sato, T., T. Watanabe, T. Mikami, and T. Matsumoto. 2004. Farnesol, a morphogenetic autoregulatory substance in the dimorphic fungus *Candida albicans*, inhibits hyphae growth through suppression of a mitogen-activated protein kinase cascade. *Biol. Pharm. Bull.* **27:** 751–752.
30. Schneider, K. R., R. L. Smith, and E. K. O'Shea. 1994. Phosphate-regulated inactivation of the kinase PHO80-PHO85 by the CDK inhibitor PHO81. *Science* **266:** 122–126.
31. Sedgwick, S. G. and S. J. Smerdon. 1999. The ankyrin repeat: A diversity of interactions on a common structural framework. *Trends Biochem. Sci.* **24:** 311–316.
32. Sharkey, L. L., M. D. McNemar, S. M. Saporito-Irwin, P. S. Sypherd, and W. A. Fonzi. 1999. HWP1 functions in the morphological development of *Candida albicans* downstream of EFG1, TUP1, and RBF1. *J. Bacteriol.* **181:** 5273–5279.
33. Shin, D. H., W. Y. Choi, Y. J. Yoo, M. K. Kim, and W. J. Choi. 2004. Lysophosphatidylcholine suppresses the expression of Phr1p and Pra1p, surface proteins involved in the morphogenesis of *Candida albicans*. *J. Microbiol. Biotechnol.* **14:** 868–871.
34. Zhao, X., P. J. Malloy, C. M. Ardies, and D. Feldman. 1995. Oestrogen-binding protein in *Candida albicans*: Antibody development and cellular localization by electron immunocytochemistry. *Microbiology* **141:** 2685–2692.