Proteomic Analysis of Hyphae-Specific Proteins That Are Expressed Differentially in *cakem1/cakem1* Mutant Strains of *Candida albicans*

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The yeast-to-hyphal transition is a major virulence factor in the fungal pathogen *Candida albicans*. Mutations in the *CaKEM1* gene, which encodes a 5′-3′ exoribonuclease responsible for mRNA degradation, show a defect in hyphal growth. We applied two-dimensional gel electrophoresis to identify hyphae-specific proteins that have altered expressions in the presence of the *cakem1* mutation. Eight proteins, Eno1, Eps1, Fba1, Imh3, Lpd1, Met6, Pdc11, and Tsa1 were upregulated during hyphal transition in wild-type but not in *cakem1/cakem1* mutant cells. A second group of proteins, Idh1, Idh2, and Ssb1, showed increased levels of expression in *cakem1/cakem1* mutant cells when compared to wild-type cells. Overexpression of Lpd1, a component of the pyruvate dehydrogenase complex, caused slight hyperfilamentation in a wild-type strain and suppressed the filamentation defect of the *cakem1* mutation. The Ssb1 protein, which is a potential heat shock protein, and the Imh3 protein, which is a putative enzyme in GMP biosynthesis also showed the filamentation-associated phenotypes.

Keywords: fungal pathogen, *C. albicans*, filamentation, CaKem1, Lpd1

Candida albicans is one of the leading causes of opportunistic fungal infections in patients with weakened immune systems, including AIDs patients, transplant recipients, and cancer patients (Shepherd *et al.*, 1985; Scherer and Magee, 1990). *C. albicans* also causes mucosal infections in relatively healthy individuals. *C. albicans* is polymorphic and undergoes reversible morphogenetic transitions among unicellular budding yeast as well as pseudohyphal and hyphal growth forms (Calderone and Fonzi, 2001). These transitions are primarily dependent on a variety of environmental signals, such as serum availability, starvation, the presence of specific compounds (such as N-acetylglucosamine), temperature (37°C) and pH. The correlation between hyphal growth and pathogenicity has been of great interest.

Several signaling pathways in conjunction with pathway-specific transcription factors regulate the expressions of hyphae-specific genes, such as *ECE1*, *HWP1*, *HYR1*, *ALS3*, *ALS8*, *RBT1*, and *RBT4* (Liu, 2001; van Burik and Magee, 2001; Biswas *et al.*, 2007). Many hyphae-specific genes have been identified through gene disruption strategies, DNA microarray techniques, and proteomic analyses (Braun and Johnson, 2000; Cheng *et al.*, 2003; Harcus *et al.*, 2004; Lorenz *et al.*, 2004). For example, microarray analysis of the *C. albicans* gene expression during phagocytosis by macrophages or neutrophils revealed that extensive changes in carbon metabolism, translation machinery, or iron transport systems are closely associated with virulence (Lorenz and Fink, 2001; Lorenz *et al.*, 2004; Ramirez and Lorenz, 2007).

The *CaKEM1* gene, which is a *C. albicans* homolog of *Saccharomyces cerevisiae KEM1/XRN1*, encodes a 5′-3′ exori-

bonuclease that is responsible for mRNA degradation (Larimer *et al.*, 1992; An *et al.*, 2004). Kem1/Xrn1 is a major component of cytoplasmic foci known as P-bodies (processing bodies) in which mRNA degradation enzymes colocalize under translationally repressive conditions such as glucose deprivation (Sheth and Parker, 2003; Parker and Sheth, 2007). P-bodies have recently emerged as important players in the posttranscriptional regulation of gene expression. mRNAs within P-bodies can be degraded or stored for return to translation. Mutations in P-body component genes such as *DHH1* and *KEM1* have been reported to affect translational regulation in *S. cerevisiae* (Kim *et al.*, 2004; Coller and Parker, 2005; Park *et al.*, 2006; Ka *et al.*, 2008).

In *C. albicans*, *cakem1/cakem1* mutant shows multiple phenotypes including a slow growth, increased benomyl sensitivity, defects in hyphal growth and biofilm formation (An *et al.*, 2004; Richard *et al.*, 2005). Biofilm formation plays a key role in the life cycles of yeasts and is considered to be a virulent trait. The involvement of *KEM1* in filamentous growth is also conserved in *S. cerevisiae* (Kim and Kim, 2002). Mutations in *KEM1* result in defects in both invasive and pseudohyphal growth.

In a present study, we analyzed the expressions of proteins under hyphal-induction conditions in a wild-type and a *cakem1/cakem1* mutant strain. Two-dimensional gel electrophoresis was applied to identify hyphae-specific proteins that had altered expressions in the presence of the *cakem1* mutation.

Materials and Methods

Strains and plasmids
The *C. albicans* strains used in this study were the wild-type strains,

SC5314 and CAI4 (*ura3::imm434/ura3::imm434*), and the *cakem1/cakem1* mutant strain (*ura3::imm434/ura3::imm434 cakem1::hph/cakem1::hisG*) (An *et al*., 2004). Chromosomal HA-tagging of *LPD1* was conducted as previously described (Lee *et al.*, 2005). The HA-CaURA3-HA cassette was integrated at one of two chromosomal copies of the *LPD1* gene in the CAI4 strain, followed by looping out of *CaURA3* via homologous recombination on 5-FOA (5-fluoroorotic acid) plates.

Plasmids carrying *IMH3* (pJI313), *LPD1* (pJI315), and *SSB1* (pJI317) were constructed as follows. The DNA sequences of target genes were obtained from the *C. albicans* genome data base (http://www.candidagenome.org). The target genes were amplified by PCR (Table 1). The products of target genes were cloned using a TOP blunt cloner (Enzynomics, Korea) by the blunt-end-ligation method. Next, inserts were identified by restriction digestion and subsequent sequencing (Marcrogen, Korea). The inserted DNA sequences were then cloned onto the *C. albicans* vector, pRC18 (Stoldt *et al*., 1997). Specifically, *LPD1* were cloned into the *Ava*I site of pRC18, while *IMH3* and *SSB1* were cloned into the *Hin*dIII site.

Growth conditions and hyphal growth test

The yeast strains were grown at 30°C in YEPD (1% yeast extract, 2% peptone, 2% dextrose) or SC-Ura (synthetic complete without uracil, 0.67% yeast nitrogen base w/o amino acid, 2% dextrose, all amino acids required except uracil) media (Murthy *et al.*, 1975). The filamentation phenotypes of the *C. albicans* strains were tested in the serum-containing media (YEPD with 10% fetal bovine serum) and Spider medium (1% mannitol, 1% nutrient broth, 0.2% K_2HPO_4, pH 7.2) as previously described (Liu *et al.*, 1994). *C. albicans*, cells were plated on hyphal inducing medium (about 120 cells/plate) and then incubated at 37°C for 2-7 days.

Transformation and DNA manipulation technique

C. albicans transformation was performed by the lithium acetate method using 50 μg of salmon sperm carrier DNA (Adams *et al.*, 1997). Standard molecular biological techniques were performed to construct the plasmid (Sambrook and Russell, 2001). Yeast genomic DNA was prepared by the rapid isolation method and then used as a template for PCR amplification (Adams *et al.*, 1997). Restriction enzymes were purchased from Boerhinger Mannheim (BM, Germany), New England Biolab (NEB, USA), and MBI fermentas (USA).

Preparation of cellular extracts

The wild-type and *cakem1/cakem1* mutant strains were grown in YEPD or Spider liquid media for 3 h. Cells were then harvested by centrifugation at 1,500×g for 5 min, after which they were washed twice in extraction buffer (10 mM $CaCl_2$, 10 mM $MgCl_2$, 20 mM NaCl). Equal volumes of glass beads and extraction buffer were added to the pellet and cell disruption was accomplished by vortexing ten times at 4°C for 1 min. Cell debris was pelleted by centrifugation at 13,000×g for 10 min and the supernatant was then centrifuged for an additional 10 min at 13,000×g. Finally, the resulting supernatant was concentrated, desalted by centricon (Millipore, USA) and subjected to two-dimensional gel electrophoresis.

Two-dimensional gel electrophoresis

Proteins were separated by two-dimensional gel electrophoresis according to their isoelectric points and molecular weights. Protein extracts were solubilized in sample buffer (9.8 M urea, 0.2% ampholines, pH 3.0-10.0 or pH 5.0-8.0, 4% CHAPS, and 50 mM dithiothreitol) and then applied to IPG strips (pH 3.0-10 and 5.0-8.0; Bio-Rad) for isoelectric focusing. The strips were then rehydrated at 50 V for 13 h, after which the proteins were focused by subjection to consecutively increasing voltage at 100-9,000 V for 10 h. The strips were equilibrated for 15 min in 10 ml equilibration buffer (6 M urea, 2% SDS, 50 mM Tris-HCl; pH 8.8, 20% glycerol, and 0.002% bromphenol blue) supplemented with 200 mg dithiothreitol. Subsequently, the strips were equilibrated in 10 ml of equilibration buffer containing 250 mg iodoacetamide. Finally, the strips were placed on top of 12% SDS-acrylamide gels and run in SDS electrophoresis buffer

Table 1. Primers used in this study

Primers for gene cloning	Sequence	Source/Reference
IMH3	F : 5′ TTC ACT GTC TTT CGG R : 5′ GTT CCG GCC TAA TAA	This work
LPD1	F : 5′ GGG GGA TCC AAA CTA GAC GCC AAC G R : 5′ GGG GGT ACC CTT CTG GAA CCT CGC	This work
SSB1	F : 5′ TTT CAC AGC CAC CAT GG R : 5′ GGA TCA AAG GCA TAC CCA	This work

Primers for probe	Sequence	Source/Reference
ENO1	F : 5′ GTC TTA CGC CAC TAA AAT CC R : 5′ CTT CAG CAG ATG GAT CTT CA	This work
FBA1	F : 5′ ATG GCT CCT CCA GCA GTT R : 5′ CCA CCG TGG AAA ACC AAG	This work
LPD1	F : 5′ CAA TGG AAA ATT GGC CCA G R : 5′ CGA ATG TGA CAT CCC CAA T	This work
PDC11	F : 5′ TTT CGG TTT ACC AGG GGA R : 5′ TGG CAC AAG CAT CAA CCA	This work
TSA1	F : 5′ ATG GCT CCA GTC GTT CAA CA R : 5′ GCC TCC AAC AAT CTC AAG GA	This work
HWP1	F : 5′ TCA ATT GGG GCC ACT GTC R : 5′ TGG AAT CCA ATC GGT TGG	This work
ACT1	F : 5′ ACC GAA GCT CCA ATG AAT CCA R : 5′ GGA TGG ACC AGA TTC GTC GTA	This work

(25 mM Tris-HCl; pH 8.3, 192 mM glycine, 0.1% SDS) at constant voltage (100 V/gel). After electrophoresis, the protein spots were visualized by silver staining (http://www.expasy.org/ch2d/protocols).

Identification of proteins

Two-dimensional gels were silver stained, and the spots were then detected using the PDQuest program. Differentially expressed protein spots were excised and subjected to in-gel tryptic digestion. Extracted peptides were analyzed by PMF (peptide mass fingerprint). The mass spectrum was analyzed by MALDI-TOF (matrix assisted laser desorption ionization-time of flight) (Amersham, USA). Identification of the open reading frames was conducted by tblastn homology searches using the *C. albicans* genome database (http://www.candidagenome.org).

Northern blot analysis

Total RNA was prepared as previously described (Elder *et al.*, 1983). Briefly, 30 μg of total yeast RNA was fractionated by electrophoresis through 1.0% formaldehyde gel and subsequently transferred onto nylon membranes according to standard protocols (Ingelbrecht *et al.*, 1998). DNA probes were generated by PCR using the primers listed in Table 1. PCR products were purified and subsequently labeled with the Rediprime™ II random primer labeling system from Amersham Biosciences.

End point dilution assay

RAW 264.7 cells were seeded at 1.5×10^5 cells per well in 96-well plates (Costar, USA) and then grown for 24 h to 4.5×10^5 cells/well. Cells were washed three times with DMEM prior to incubation with *C. albicans*. The *C. albicans* cells were grown overnight, washed in PBS and sonicated for 1 min to disrupt any clumped cells. The cells were resuspended in DMEM, after which 1.5×10^5 cells were inoculated into each of the first 8 wells (first column) which contained medium alone or medium with macrophages. Fourfold serial dilution was conducted in subsequent columns, and the plates were incubated at 37°C and 5% CO_2 for 48 h. After incubation, passive lysis buffer (Promega, USA) was added to the wells at a final concentration of 1× to facilitate colony visualization. Dilutions (without macrophages) in which the colonies could be distinctively visualized were counted toward the lower limit and compared to the same dilutions with macrophages.

Results and Discussion

***cakem1/cakem1* mutant strain has a reduced viability in macrophage co-culture**

Previous studies have demonstrated that the *cakem1/cakem1* null mutation causes multiple phenotypes in *C. albicans*, including slow growth in a rich medium and a defect in hyphal development under filamentation-inducing conditions (An *et al*., 2004). To determine if the *cakem1* mutation leads to any defect in a virulence-associated phenotype, we monitored the survival of the wild-type and the *cakem1/cakem1* mutant cells in the presence of macrophage cells using an end point dilution assay (Marcil *et al.*, 2002). After inoculating RAW 264.7 macrophage cells with the wild-type or the *cakem1/cakem1* mutant cells, serial dilutions were conducted prior to incubation. The end point survival ratio was expressed as the number of colonies in the presence of macrophages divided by the number of colonies in the absence of macrophages (Fig. 1). The *cakem1/cakem1* mutant strain showed a reduced survival rate in the macrophage co-culture when compared to the wild-
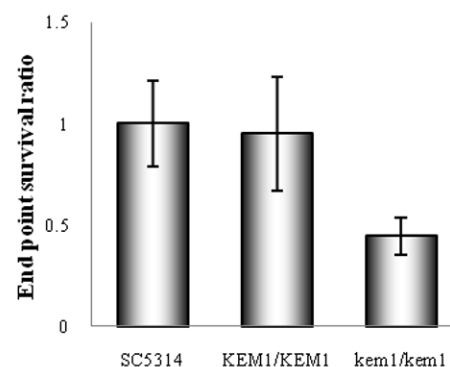

Fig. 1. Virulence test as measured by an end point dilution assay. The wild-type strains, SC5314 and CAI4, and the *cakem1/cakem1* mutant strain derived from CAI4 were used. Survival ratios are expressed as the numbers of colonies in the presence of RAW264.7 macrophages divided by the number of colonies in the absence of macrophages.

type.

Altered protein expression in the *cakem1/cakem1* mutant strain

To determine the effects of the *cakem1* mutation on protein expression, *cakem1/cakem1* mutant cells were grown in rich media YEPD or hyphal-inducing Spider media for 3 h, after which the resulting protein lysates were compared with those from the wild-type cells by 2D-gel electrophoresis (Fig. 2). Among the protein spots with altered expressions in hyphal inducing Spider medium, 11 proteins with differential expression levels in the wild-type and *cakem1/cakem1* mutant were further analyzed (Fig. 3). Peptide mass fingerprint (PMF) and MALDI-TOF analysis were used to identify the protein spots (Table 2).

Eight proteins, Eno1, Eps1, Fba1, Imh3, Lpd1, Met6, Pdc11, and Tsa1 were up-regulated during hyphal transition in wild-type but become constitutive or less induced in *cakem1/cakem1* mutant cells. In the case of Lpd1 and Met6, the *cakem1* mutations showed significantly decreased levels of proteins in both yeast and hyphal conditions. Three proteins, Idh1, Idh2, and Ssb1, showed an increased level of expression in *cakem1/cakem1* mutant cells when compared to wild-type cells; Idh1

Table 2. Descriptions of *C. albicans* genes identified by mass spectrometry

Gene	kDa/pI	Function
ENO1	47.21/5.5	Phosphopyruvate hydratase activity
EPS1	85.54/6.3	Protein disulfide isomerase activity
FBA1	39.37/5.7	Fructose-bisphosphate aldolase activity
IMH3	56.59/6.0	Putative inosine 5-monophosphate dehydrogenase
LPD1	53.07 /6.4	Putative dihydrolipoamide dehydrogenase
MET6	85.80/5.4	Likely cobalamin-independent methionine synthase
PDC11	62.77/5.4	Pyruvate decarboxylase activity
TSA1	21.96/5.0	Thiol peroxidase activity
IDH1	20.72/8.6	Isocitrate dehydrogenase (NAD+) activity
IDH2	39.93/6.7	Isocitrate dehydrogenase (NAD+) activity
SSB1	66.60/5.3	Putative heat shock protein

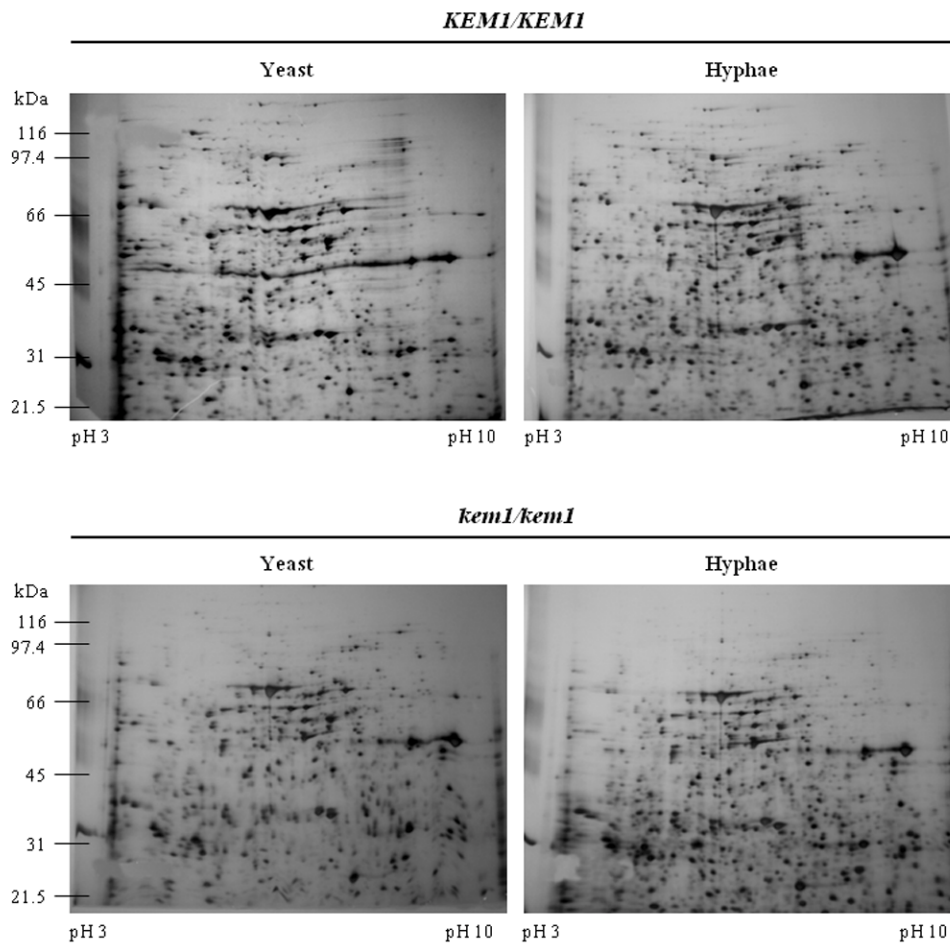**KEM1/KEM1**

kem1/kem1

Fig. 2. Two-dimensional gel electrophoresis of differentially expressed proteins during hyphal transition in wild-type and *cakem1/cakem1* mutant strains of *C. albicans*. Yeast or hyphal cultures were grown in YEPD at 28°C or Spider medium at 37°C. Samples containing 200 μg of protein were suspended in rehydration buffer and subjected to 2D-gel electrophoresis (17-cm pH 3-10 IPG strips; 12% SDS-PAGE). Protein spots were visualized by silver staining. Upper; *CaKEM1/CaKEM1* (CAI4), lower; *cakem1/cakem1*.

and Idh2 showed constitutively higher levels in mutant cells whereas Ssb1 showed higher induction fold. In previous reports, proteins such as Eno1, Fba1, Idh1, Lpd1, Pdc11, Ssb1, and Tsa1 were found to be up-regulated in a wild-type strain when cultured in hyphal-inducing serum media (Pitarch *et al.*, 2002). Lpd1, Ssb1, and Tsa1 were also found to be differentially expressed upon macrophage interaction (Fernandez-Arenas *et al.*, 2007). Our results indicated that several hyphae-specific proteins showed altered levels of protein spots in the *cakem1* strain. Therefore we suggest that the *CaKEM1* gene may regulate the expression of these proteins.

In *S. cereivsiae*, *KEM1/XRN1* has been reported to regulate *KAR4* gene expression at the post-transcriptional level (Kim *et al.*, 2004). The *kem1* mutation caused a significant decrease at the Kar4 protein levels but not at the mRNA levels. To ask the similar question whether the effect of the *cakem1* mutation was mostly at the post-transcriptional level, Northern blot analysis was applied to five genes, *ENO1*, *FBA1*, *LPD1*, *PDC11*, and *TSA1*. We analyzed the transcript level during hyphal transition in wild-type and *cakem1/cakem1* mutant cells. Transcripts of *ENO1*, *FBA1*, and *LPD1* remained at the

similar level during hyphal induction in the wild-type and the *cakem1/cakem1* mutant cells (Fig. 4). It is highly likely that the up-regulation of Eno1, Fba1, and Lpd1 protein levels during filamentation could be at the post-transcriptional level. The *PDC11* transcripts were highly induced during hyphal transition in both wild-type and *cakem1/cakem1* mutant type cells. These results imply that the constitutive level of the Pdc11 protein in the *cakem1* cells was due to the post-transcriptional effect. The transcript levels of *TSA1* were up-regulated during hyphal transition in wild-type but constitutive in *cakem1/cakem1* mutant strain. This regulation pattern is similar to those observed at the protein level. Therefore, it is important to observe their protein expression patterns as well as their transcription profiles.

Filamentation phenotypes of *LPD1*
The Lpd1 protein, which is a component of the pyruvate dehydrogenase complex, was found to be up-regulated during hyphal growth. To confirm our 2D-gel electrophoresis data, one copy of the chromosomal *LPD1* gene was HA-tagged using a 3HA-CaURA3-3HA cassette. Western analysis with
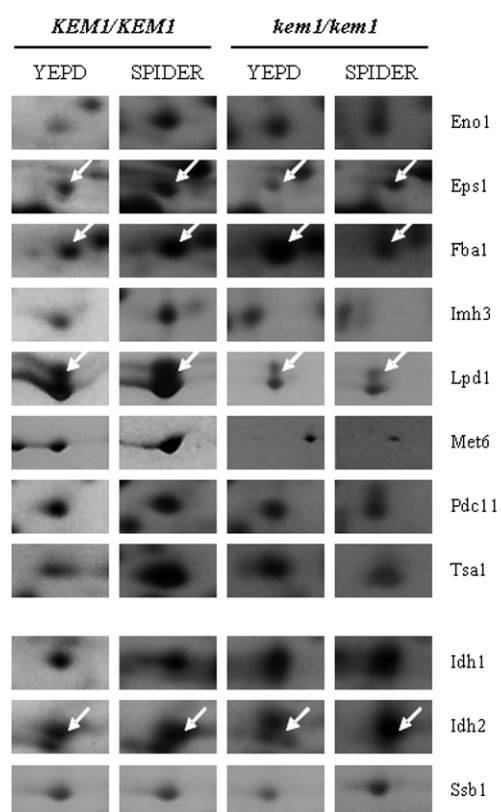
Fig. 3. Protein spots differentially expressed between the wild-type and *cakem1/cakem1* mutant strains during hyphal transition. Spots were detected and visualized using the PDQuest software.

Fig. 4. Northern analysis of *ENO1*, *FBA1*, *LPD1*, *PDC11*, and *TSA1* transcripts in the wild-type and *cakem1/cakem1* mutant strains during hyphal transition. Total RNA was extracted from yeast or hyphal culture after 3 h of induction. rRNA and *ACT1* were used as a loading control. *HWP1* was used as a hypha-specific and *CaKEM1*-dependent control.

anti-HA antibodies revealed moderately increased levels of Lpd1-HA proteins under hyphal-inducing conditions, the Spider media and the serum-containing YEPD media, when compared with the yeast culture (Fig. 5A). In addition, the Pdc11 protein was also tagged with HA and shown to be up-regulated in hyphal culture conditions (data not shown).

To investigate the hyphae-associated phenotypes of the *LPD1* gene, *LPD1* overexpression plasmid was constructed and introduced into the wild-type and *cakem1/cakem1* mutant strains. *LPD1* overexpression caused a slight hyperfilamentation in colony morphology, which was also evident in the *cakem1/cakem1* mutant strain (Fig. 5B). These findings indicate that *LPD1* overexpression suppresses the filamentation defect of the *cakem1* mutation. Interestingly, the slow growth phenotype of *cakem1/cakem1* was not recovered by *LPD1* overexpression (Fig. 5C). These results suggest that the Lpd1 protein, identified in our 2D-gel screening, plays a critical role in *C. albicans* filamentation. Additionally, the *LPD1* functions appeared to be closely related to the cellular functions of *CaKEM1*.

Overexpression of *SSB1* or *IMH3* showed filamentation phenotypes in a wild-type strain

Overexpression analysis demonstrated the filamentation-associated phenotypes of the Ssb1 protein, a potential heat shock protein, and the Imh3 protein, a putative enzyme involved in GMP biosynthesis. *SSB1* overexpression caused the development of a hyperfilamentation in colony morphology
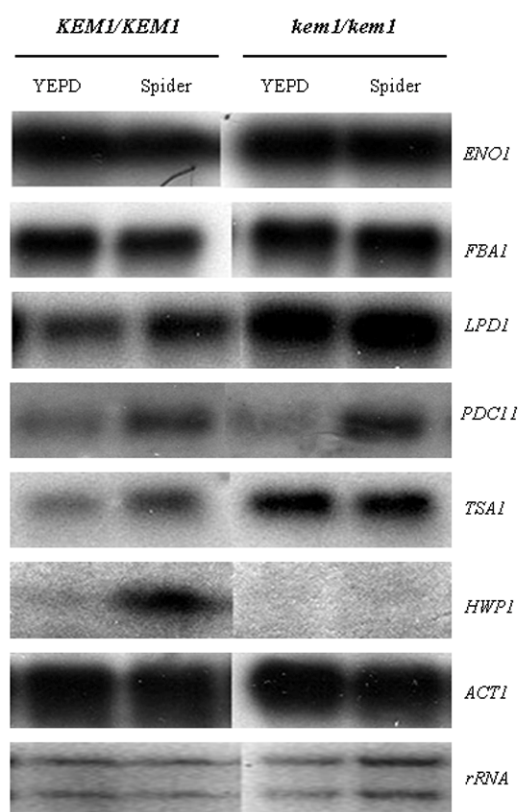
(Fig. 6). This hyperfilamentation phenotype was also evident even in the YEPD medium, which is a rich medium known to induce yeast-form growth. *IMH3* overexpression in a wild-type strain was shown to inhibit hyphal growth in serum-containing media, which is a strong hypha-inducing medium. Therefore, our approach of utilizing the filamentation defective mutations successfully revealed hyphae-specific proteins.

Acknowledgements

References

Adams, A., D.E. Gottschling, C.A. Kaiser, and T. Stearn. 1997. Methods in yeast genetics. Cold Spring Harbor Press, Cold Spring Harbor, New York, N.Y., USA.

An, H.S., K.H. Lee, and J. Kim. 2004. Identification of an exoribonuclease homolog, *cakem1/caxrn1*, in *Candida albicans* and its characterization in filamentous growth. *FEMS Microbiol. Lett.* 235, 297-303.
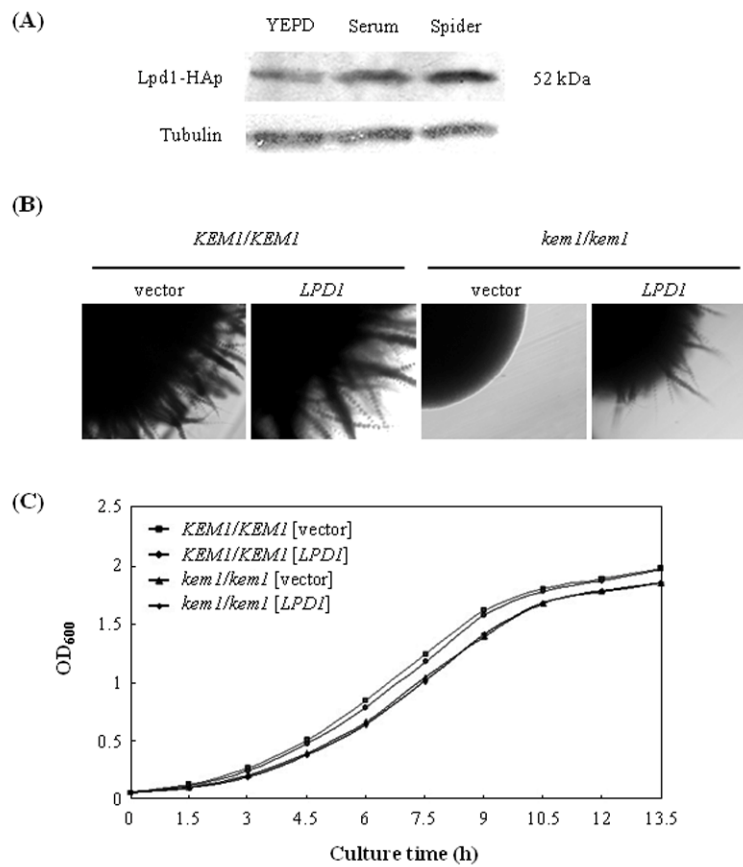
(A)

(B)

(C)

Fig. 5. Filamentation-associated and *cakem1*-suppression phenotypes of *LPD1*. (A) Hyphal-induced protein expression of Lpd1 as analyzed by Western blot. The *LPD1-HA* strain was grown in serum containing YEPD or Spider media to induce the formation of hyphae. Tubulin was used as an internal control. (B) Colony morphology of the wild type or *cakem1/cakem1* strain with *LPD1* overexpression (pJI315). Colonies were incubated on Spider media at 37°C for 4 days. (C) Growth phenotype of the wild type or *cakem1/cakem1* strain with *LPD1* overexpression (pJI315).
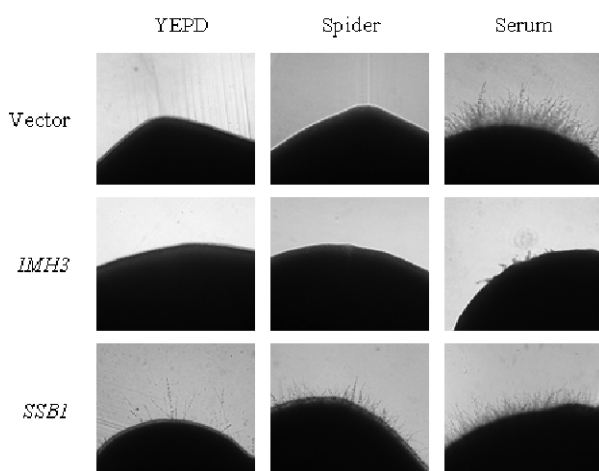

Fig. 6. Filamentation phenotypes of *IMH3* or *SSB1* overexpression. The wild-type strain harboring *IMH3* (pJI313) or *SSB1* (pJI317) on plasmid pRC18 were plated on YEPD, Spider, and serum-containing YEPD media. The plates were incubated at 30°C (YEPD) or 37°C (Spider and Serum) and the colony morphology was photographed after 3-4 days of incubation.

Biswas, S., P. Van Dijck, and A. Datta. 2007. Environmental sensing and signal transduction pathways regulating morphopathogenic determinants of *Candida albicans*. *Microbiol. Mol. Biol. Rev.* 71, 348-376.

Braun, B.R. and A.D. Johnson. 2000. Tup1, Cph1 and Efg1 make independent contributions to filamentation in *Candida albicans*. *Genetics* 155, 57-67.

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

Cheng, S., C.J. Clancy, M.A. Checkley, M. Handfield, J.D. Hillman, A. Progulske-Fox, A.S. Lewin, P.L. Fidel, and M.H. Nguyen. 2003. Identification of *Candida albicans* genes induced during thrush offers insight into pathogenesis. *Mol. Microbiol.* 48, 1275-1288.

Coller, J. and R. Parker. 2005. General translational repression by activators of mRNA decapping. *Cell* 122, 875-886.

Elder, R.T., E.Y. Loh, and R.W. Davis. 1983. RNA from the yeast transposable element Ty1 has both ends in the direct repeats, a structure similar to retrovirus RNA. *Proc. Natl. Acad. Sci. USA* 80, 2432-2436.

Fernandez-Arenas, E., V. Cabezon, C. Bermejo, J. Arroyo, C. Nombela, R. Diez-Orejas, and C. Gil. 2007. Integrated proteomics and genomics strategies bring new insight into *Candida albicans* response upon macrophage interaction. *Mol. Cell Proteomics* 6, 460-478.

Harcus, D., A. Nantel, A. Marcil, T. Rigby, and M. Whiteway. 2004.

Transcription profiling of cyclic AMP signaling in *Candida albicans*. *Mol. Biol. Cell.* 15, 4490-4499.

Ingelbrecht, I.L., C.I. Mandelbaum, and T.E. Mirkov. 1998. Highly sensitive Northern hybridization using a rapid protocol for downward alkaline blotting of RNA. *Biotechniques* 25, 420-423, 425-426.

Ka, M., Y.U. Park, and J. Kim. 2008. The DEAD-box RNA helicase, Dhh1, functions in mating by regulating *STE12* translation in *Saccharomyces cerevisiae*. *Biochem. Biophys. Res. Commun.* 367, 680-686.

Kim, J., S. Jeon, Y.S. Yang, and J. Kim. 2004. Posttranscriptional regulation of the karyogamy gene by Kem1p/Xrn1p exoribonuclease and Rok1p RNA helicase of *Saccharomyces cerevisiae*. *Biochem. Biophys. Res. Commun.* 321, 1032-1039.

Kim, J. and J. Kim. 2002. *KEM1* is involved in filamentous growth of *Saccharomyces cerevisiae*. *FEMS Microbiol. Lett.* 216, 33-38.

Larimer, F.W., C.L. Hsu, M.K. Maupin, and A. Stevens. 1992. Characterization of the *XRN1* gene encoding a 5′-->3′ exoribonuclease: Sequence data and analysis of disparate protein and mRNA levels of gene-disrupted yeast cells. *Gene* 120, 51-57.

Lee, K.H., S. Jun, H.S. Hur, J.J. Ryu, and J. Kim. 2005. *Candida albicans* protein analysis during hyphal differentiation using an integrative HA-tagging method. *Biochem. Biophys. Res. Commun.* 337, 784-790.

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

Liu, H., J. Kohler, and G.R. Fink. 1994. Suppression of hyphal formation in *Candida albicans* by mutation of a *STE12* homolog. *Science* 266, 1723-1726.

Lorenz, M.C., J.A. Bender, and G.R. Fink. 2004. Transcriptional response of *Candida albicans* upon internalization by macrophages. *Eukaryot. Cell* 3, 1076-1087.

Lorenz, M.C. and G.R. Fink. 2001. The glyoxylate cycle is required for fungal virulence. *Nature* 412, 83-86.

Marcil, A., D. Harcus, D.Y. Thomas, and M. Whiteway. 2002. *Candida albicans* killing by RAW264.7 mouse macrophage cells: Effects of *Candida* genotype, infection ratios, and gamma interferon treatment. *Infect. Immun.* 70, 6319-6329.

Murthy, M.S., B.S. Rao, N.M. Reddy, P. Subrahmanyam, and U. Madhvanath. 1975. Non-equivalence of YEPD and synthetic complete media in yeast reversion studies. *Mutat. Res.* 27, 219-223.

Park, Y.U., H. Hur, M. Ka, and J. Kim. 2006. Identification of translational regulation target genes during filamentous growth in *Saccharomyces cerevisiae*: Regulatory role of Caf20 and Dhh1. *Eukaryot. Cell.* 5, 2120-2127.

Parker, R. and U. Sheth. 2007. P-bodies and the control of mRNA translation and degradation. *Mol. Cell.* 25, 635-646.

Pitarch, A., M. Sanchez, C. Nombela, and C. Gil. 2002. Sequential fractionation and two-dimensional gel analysis unravels the complexity of the dimorphic fungus *Candida albicans* cell wall proteome. *Mol. Cell Proteomics* 1, 967-982.

Ramirez, M.A. and M.C. Lorenz. 2007. Mutations in alternative carbon utilization pathways in *Candida albicans* attenuate virulence and confer pleiotropic phenotypes. *Eukaryot. Cell.* 6, 280-290.

Richard, M.L., C.J. Nobile, V.M. Bruno, and A.P. Mitchell. 2005. *Candida albicans* biofilm-defective mutants. *Eukaryot. Cell.* 4, 1493-1502.

Sambrook, J. and D.W. Russell. 2001. Molecular Cloning, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, N.Y., USA.

Scherer, S. and P.T. Magee. 1990. Genetics of *Candida albicans*. *Microbiol. Rev.* 54, 226-241.

Shepherd, M.G., R.T. Poulter, and P.A. Sullivan. 1985. *Candida albicans*: Biology, genetics, and pathogenicity. *Annu. Rev. Microbiol.* 39, 579-614.

Sheth, U. and R. Parker. 2003. Decapping and decay of messenger RNA occur in cytoplasmic processing bodies. *Science* 300, 805-808.

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.

van Burik, J.A. and P.T. Magee. 2001. Aspects of fungal pathogenesis in humans. *Annu. Rev. Microbiol.* 55, 743-772.