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Roles of dihydrolipoamide dehydrogenase Lpd1 in *Candida albicans* filamentation

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Acetyl coenzyme A, a key intermediate of the mitochondrial carbon metabolism, is formed by the mitochondrial pyruvate dehydrogenase complex (PDC). The dihydrolipoamide dehydrogenase Lpd1 is a catalytic component of PDC. Lpd1 has been recovered during 2D-PAGE screening for the hypha-specific proteins in *Candida albicans*. The Lpd1 protein, as visualized by a GFP-fusion, was localized in the mitochondria during the logarithmic yeast growth and the filamentous growth. The GFP signal was prevalent and relatively uniform toward the tip of the hyphae. The functions of the *LPD1* gene were investigated by construction of *lpd1/lpd1* mutant strain. This homozygous deletion mutant was unable to grow on non-fermentable carbon sources including glycerol, ethanol, acetate, and citrate. In addition, the *lpd1/lpd1* strain exhibited a slow-growth phenotype on glucose-containing media and a marked sensitivity to 0.5 mM of hydrogen peroxide. *LPD1* was shown to be required for filamentous growth under a serum-containing hyphal-inducing condition. These results suggest a possible relationship between mitochondrial respiration and filamentous growth.

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1. Introduction

The mitochondrial pyruvate dehydrogenase complex (PDC) controls the key committed step in carbohydrate utilization, namely the conversion of pyruvate to acetyl-CoA and NADH (Flores et al., 2000; Harris et al., 2002). The *Candida albicans* *LPD1* gene encodes a dihydrolipoamide dehydrogenase which is a component of the PDC (Cheng et al., 2003; Vellucci et al., 2007). Two other catalytic components of the PDC are pyruvate dehydrogenase and dihydrolipoamide acetyltransferase. In *Saccharomyces cerevisiae* and other organisms, the dihydrolipoamide dehydrogenase is also a subunit of the α-ketoglutarate dehydrogenase complex (KGDC) which catalyzes the oxidative decarboxylation of α-ketoglutarate to succinyl-CoA in the citric acid cycle and the glycine decarboxylase multienzyme complex, known as the glycine cleavage system (Roy and Dawes, 1987; Patel and Harris, 1995; Sinclair and Dawes, 1995; Przybyla-Zawislak et al., 1999).

LPD1 was identified in *C. albicans* during antibody-based screening for genes expressed within infected hosts or in a two-dimensional polyacrylamide gel-electrophoresis of hypha-specific proteins (Cheng et al., 2003; Hernandez et al., 2004; Fernandez-Arenas et al., 2007). However, it is not yet elucidated whether *LPD1* is functionally relevant to *C. albicans* pathogenesis, even though its homologous genes in *Streptococcus pneumoniae* and *Mycobacterium turberculosis* are known to encode virulence determinants (Smith et al., 2002; Rajashankar et al., 2005). In humans, a wide range of diseases including metabolic acidosis, autoimmune disease, and neurodegenerative diseases such as Alzheimer's disease have been shown to be correlated with changes in the PDC activities (Gibson et al., 1998; Brown et al., 2004).

The major virulence factor for *C. albicans* pathogenesis is the morphological transition among unicellular yeast, pseudohyphal, and hyphal growth forms (Calderone and Fonzi, 2001; Liu, 2001). Several signaling pathways and transcription factors including the mitogen-activated protein kinase (transcription factor, Cph1) and the cyclic AMP-dependent protein kinase A (transcription factor, Efg1) have been shown to participate in the regulation of these morphological transitions (Liu, 2001; Harcus et al., 2004; Lorenz et al., 2004). However, the involvement of acetyl-CoA metabolism or mitochondrial respiration in filamentous growth has been less well studied in *C. albicans*. A recent study showed that deletion of a structural component of the PDC, Pdx1, resulted in a filamentation defect (Vellucci et al., 2007).

In this study, we investigated the role of the *LPD1* gene in *C. albicans* filamentation. The *lpd1/lpd1* mutant strain showed a slow-growth phenotype, an inability to utilize non-fermentable carbon sources, and a filamentation defect. We also showed the mitochondrial localization of the Lpd1-GFP protein during mitotic yeast growth and filamentation growth.

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2. Materials and methods

2.1. Yeast strains and growth conditions

The *C. albicans* strains used in this study were wild-type CAI4 (*ura3::imm434/ura3::imm434*) and BWP17 (*ura3::imm434/ura3::imm434 his1::hisG/his1::hisG arg4::hisG/arg4::hisG*), and null mutant JKC64 (*lpd1::hph/lpd1::hisG*) derived from CAI4. JKC67 (*LPD1/LPD1::GFP*) was derived from BWP17. *C. albicans* strains were cultured on standard yeast media including YEPD (1% yeast extract, 2% peptone, 2% glucose) and SC-Ura (YNB plus all amino acids except uracil, 0.67% yeast nitrogen base w/o amino acid, 2% glucose, all amino acids required) (Adams et al., 1997). For URA3 pop-outs, cells were streaked on 5-fluoroorotic acid medium (YNB plus 0.2 mM uridine, and 0.1% 5-fluoroorotic acid). The filamentation phenotype of the *C. albicans* cells was tested with serum-containing medium (1% yeast extract, 2% peptone, 10% fetal bovine serum). The solid media contained 2% agar. To induce hyphal growth in the liquid culture, cells were pre-grown in YEPD media at 30 °C overnight, diluted (1×10^6 cells/ml) into the indicated inducing medium, and incubated at 37 °C.

2.2. Plasmid construction

To isolate the *LPD1* gene, a 3.6-kb DNA fragment was PCR-amplified from the genomic DNA of the CAI4 strain using primers LPD1-pcr-F and LPD1-pcr-R (Table 1). The resulting PCR product was digested with *Bam*HI–*Kpn*I and ligated to the *Bam*HI and *Kpn*I sites of *C. albicans* vector pRC18 to generate plasmid pJI315. Disruption plasmid pJI403 (pRS316-*lpd1::hph-CaURA3-hph*) was constructed as follows: two DNA fragments of *LPD1* were generated by PCR-amplification (primers LPD1-cloning-s and LPD1-pcr-R) and subsequent digestion with *Sac*I or digestion with *Kpn*I and *Hin*dIII. These fragments were then ligated into the pRS316 vector, generating pRS316-lpd1-KO1. The *Bam*HI fragment of the *hph-CaURA3-hph* cassette from pQF86 (Feng et al., 1999) was inserted at the *Bam*HI site of pRS316-lpd1-KO1, generating pJI403. To construct a disruption plasmid pJI406 (pRS316-*lpd1::hisG-CaURA3-hisG*), two DNA fragments of *LPD1* were amplified. One was generated by PCR-amplification using primers LPD1-cloning-s and LPD1-KO2-R, after which they were digested with *Sac*I and *Bam*HI. The other fragment was amplified using primers LPD1-KO2-F and LPD1-pcr-R and digested with *Hin*dIII and *Bam*HI. These fragments were ligated into the pRS316 vector, generating pRS316-lpd1-KO2. The *Bam*HI-*Bgl*II fragment of the *hisG-CaURA3-hisG* cassette from pCUB-6 (Fonzi and Irwin, 1993) was inserted at the *Bam*HI site of pRS316-lpd1-KO2.

2.3. lpd1/lpd1 null mutant construction

Disruption plasmid pJI403 was digested with *Nhe*I and *Acc*I. The resulting DNA fragment was transformed into *C. albicans* strain CAI4 to obtain the heterozygote *lpd1::hph-CaURA3-hph/LPD1*. This colony was then grown on 5-FOA media to select for the loss of *URA3*. A second copy of *LPD1* was disrupted by transforming *lpd1::hph/LPD1* strain with the linear DNA fragment from pJI406 digested with *Pst*I, *Sac*I, and *Hin*dIII. The *lpd1::hph/lpd1::hisG* isolates were screened by PCR.

Table 1
Primers used in this study.

Primer	Sequence
LPD1-pcr-F	GGGGGATCCAAACTAGACGCCAACG
LPD1-pcr-R	GGGGGTACCCTTCTGGAACCTCGC
LPD1-cloning-s	AAAACTAGACGCCAACG
LPD1-KO2-F	GGGGGATCCCCCAGAAGTTGCCTGGG
LPD1-KO2-R	GGGGGATCCTTGAGCGGCCTTGATGG
LPD1::GFP-F	AATAAAATAATGATAAACAAAAAAACAACTA CTACTACTATAACCATAACTATAACTACAACTA TAACTATCTAGAAGGACCACCTTTGATTG
LPD1::GFP-R	TGAACAAAAAGTTGAATTCATGTCGCATGATGCT TCCAATACTAGAGGCGCTGAACCTCAAATTG TTTTGGGTGGTGGTTCTAAAGGTGAAGAATTATT

2.4. GFP-tagging at the chromosomal location

The *GFP-HIS1*-encoding region in the plasmid pGFP-HIS1 (Gerami-Nejad et al., 2001) was PCR-amplified using primers LPD1-GFP-F and LPD1-GFP-R. The PCR primers were designed so that the amplified cassette harbors a 70-nucleotide flanking region that is homologous with the C-terminal end of the *LPD1* ORF. The PCR product was utilized for transformation of the *C. albicans* wild-type strain BWP17. The chromosomal integration of the cassette DNA and the subsequent excision of *CaHIS1* resulted in strain JKC67, which is heterozygous for *LPD1::GFP*.

2.5. Sequence analysis of C. albicans LPD1

The complete genomic DNA sequences of *LPD1* were searched in the *C. albicans* genome database (http://www.candidagenome.org/). Sequence analysis was conducted by BLAST searches of the Genbank database (http://www.ncbi.nlm.nih.gov/BLAST/). Sequence comparisons were conducted with the multiple alignment programs designed to apply bioinformatics searches (http://bioinfo.genotoul.fr/). The common motifs in proteins were obtained by searching the PROSITE (http://www.expasy.ch/prosite/) database and the European Bioinformatics Institute's InterProScan database (http://www.ebi.ac.uk/Tools/InterProScan/).

2.6. Growth assays on non-fermentable carbon sources

Alternative carbon utilization assays were carried out on solid SC media containing 2% potassium acetate, ethanol, citrate, or glycerol as the sole carbon source. For spot dilution assays, strains were grown in liquid SC media at 30 °C overnight and washed with H_2O. Tenfold serial dilutions of overnight cultures were spotted onto SC medium containing the indicated carbon sources and plates were incubated for 48 h.

2.7. H_2O_2 resistance test

Strains were cultured in liquid SC media overnight. Culture quantities were determined by measuring the absorbance at 600 nm. Cells were spotted onto solid SC medium in the absence or presence of 1 mM or 0.5 mM H_2O_2 and then incubated at 30 °C for 48 h.

2.8. Cell staining and confocal microscopy

Cells were grown to 2×10^7 cells/ml in the appropriate medium and washed twice with PBS (Sigma Aldrich). To stain the mitochondria, the cells were incubated in the presence of 100 nM MitoTracker Red CMXRos (1 mM, Molecular Probes) at 30 °C for 30 min, and then washed twice with PBS. Observations were made using a Carl Zeiss LSM5 live confocal laser scan microscope by using the 60× objective and the Adobe Photoshop CS3 software (version 10.0).

3. Results and discussion

3.1. Identification of the LPD1 homolog in C. albicans

C. albicans has a close homolog of the *S. cerevisiae LPD1* gene, which encodes a dihydrolipoamide dehydrogenase. The Lpd1 pro-

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