Journal of Microbiology, Immunology and Infection (2016) xx, 1–9

Available online at www.sciencedirect.com

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journal homepage: www.e-jmii.com

ORIGINAL ARTICLE

Candida albicans Hom6 is a homoserine dehydrogenase involved in protein synthesis and cell adhesion

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Received 21 October 2015; received in revised form 22 February 2016; accepted 9 March 2016
Available online ■ ■ ■

KEYWORDS
Candida albicans;
cell adhesion;
Hom6;
homoserine dehydrogenase;
protein synthesis

Abstract *Background/Purpose: Candida albicans* is a common fungal pathogen in humans. In healthy individuals, *C. albicans* represents a harmless commensal organism, but infections can be life threatening in immunocompromised patients. The complete genome sequence of *C. albicans* is extremely useful for identifying genes that may be potential drug targets and important for pathogenic virulence. However, there are still many uncharacterized genes in the *Candida* genome database. In this study, we investigated *C. albicans* Hom6, the functions of which remain undetermined experimentally.
Methods: HOM6-deleted and *HOM6*-reintegrated mutant strains were constructed. The mutant strains were compared with wild-type in their growth in various media and enzyme activity. Effects of *HOM6* deletion on translation were further investigated by cell susceptibility to hygromycin B or cycloheximide, as well as by polysome profiling, and cell adhesion to polystyrene was also determined.
Results: C. albicans Hom6 exhibits homoserine dehydrogenase activity and is involved in the biosynthesis of methionine and threonine. *HOM6* deletion caused translational arrest in cells grown under amino acid starvation conditions. Additionally, Hom6 protein was found in both cytosolic and cell-wall fractions of cultured cells. Furthermore, *HOM6* deletion reduced *C. albicans* cell adhesion to polystyrene, which is a common plastic used in many medical devices.
Conclusion: Given that there is no Hom6 homologue in mammalian cells, our results provided an important foundation for future development of new antifungal drugs.

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http://dx.doi.org/10.1016/j.jmii.2016.03.001

Please cite this article in press as: Tsai P-W, et al., *Candida albicans* Hom6 is a homoserine dehydrogenase involved in protein synthesis and cell adhesion, Journal of Microbiology, Immunology and Infection (2016), http://dx.doi.org/10.1016/j.jmii.2016.03.001

Introduction

Candida albicans commensally colonizes skin and mucosal surfaces; however, this commensalism can be disrupted when the immune system is compromised, at which time *C. albicans* can cause superficial, subcutaneous, and even life-threatening invasive infections.[1,2] To date, only a small number of antifungals are available, and these drugs target only a few molecular pathways.[2,3] Moreover, the persistent use of these drugs has led to drug-resistant *C. albicans* in clinical settings.[3] Thus, it is critically important to characterize different aspects of *C. albicans* physiology to identify alternative drug targets that can be used to develop new antifungal therapies.

Metabolic pathways are important targets for developing new antifungals.[4] The sulfur assimilatory (SA) pathways, for example, are significantly different in humans and fungi. The yeast *Saccharomyces cerevisiae* chemically reduces and incorporates inorganic sulfates to synthesize methionine *de novo*, whereas humans are unable to utilize inorganic sulfates.[4] Additionally, the sequential reactions that convert methionine to homocysteine, cystathionine, and cysteine are reversible in most fungi, but in humans, methionine is an essential amino acid that must be obtained from the diet.[4] Moreover, Adenosine-5′-triphosphate (ATP) sulfurylase (Met3), which reduces sulfur for subsequent incorporation into metabolites, is essential for infection by the pathogenic yeast *Cryptococcus neoformans*.[5] The aspartate metabolic pathway is related to the SA pathway and is another potential antifungal target, as humans lack enzymes involved in this pathway.[6] In *S. cerevisiae*, aspartate is initially converted to beta-aspartate semialdehyde. This reaction is sequentially catalyzed by aspartate kinase (Hom3) and aspartate semialdehyde dehydrogenase (Hom2). Beta-aspartate semialdehyde is further converted to homoserine by homoserine dehydrogenase (Hom6). Importantly, homoserine is the intermediate used to synthesize methionine and threonine.[7] In *S. cerevisiae*, homoserine transacetylase (Met2) converts homoserine to *O*-acetylhomoserine, which is then converted to homocysteine and methionine. Homoserine can be also converted to threonine by homoserine kinase (Thr1) and threonine synthase (Thr4),[8] and threonine can be used to synthesize isoleucine. Although several studies have been conducted in *S. cerevisiae*, the aspartate pathway in pathogenic yeast has not been well characterized. *C. albicans THR1*-deleted mutants are serum sensitive, and this phenotype can be suppressed by adding threonine to the growth medium, suggesting that serum sensitivity results from the accumulation of homoserine.[9] Additionally, both *C. albicans THR1* and *C. neoformans MET2* are required for fungal virulence.[9,10] Expression of *C. albicans* Hom6 can be induced by amino acid starvation and macrophage phagocytosis.[11,12] The function of Hom6 in *C. albicans*, however, is mostly unknown.

In this study, we identified functions for *C. albicans* Hom6. Since mammals lack Hom6, our results provide important information for developing new antifungal agents directed toward this enzyme and/or its biosynthetic pathway.

Materials and methods

Strains and growth conditions

C. albicans strains are listed in Supplementary Table S1. Cells were grown in yeast-extract peptone dextrose (YPD) medium (1% yeast extract, 2% peptone, and 2% glucose) or synthetic complete (SC) medium (0.67% yeast nitrogen base with ammonium sulfate, 2% glucose, 0.079% complete supplement mixture of amino acids; MP Biochemicals, Solon, OH, USA). For amino acid-depletion experiments, we used synthetic minimal (SM) medium (0.67% yeast nitrogen base with ammonium sulfate and 2% glucose) or SC−Thr (SM with 0.069% complete supplement mixture lacking threonine; Sunrise Science Products Inc., San Diego, CA, USA). All reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA) unless otherwise indicated.

Gene deletion and reintegration

To delete *C. albicans HOM6*, the *SAT1*-flipper method was used.[13] Briefly, the 5′- and 3′-flanking regions of *HOM6* were amplified from the SC5314 genome with the primer pairs CaHOM6-up-F-*Apa*I/CaHOM6-up-R-*Xho*I and CaHOM6-dn-F-*Sac*II/CaHOM6-dn-R-*Sac*I, respectively. All the primers used in this study are listed in Supplementary Table S2. These flanking sequences were independently cloned into plasmid pSFS2A to generate pSFS2A-CaHOM6.[13] For *HOM6* reintegration, the 5′-flanking region plus full-length *HOM6* were amplified with the primer pair CaHOM6-up-F-*Apa*I/CaHOM6-R2-*Xho*I. This DNA fragment and the 3′-flanking region of *HOM6* were independently cloned into pSFS2A to generate pSFS2A-CaHOM6-6. The *HOM6* deletion and reintegration cassettes were excised by *Apa*I/*Sac*I digestion from pSFS2A-CaHOM6 and pSFS2A-CaHOM6-6, respectively, and transformed into the *C. albicans* SC5314 strain as described.[13] The *HOM6*-heterozygous mutant was used for the second round of cassette integration/excision to generate the *HOM6*-homozygous mutant. To generate the *HOM6* reintegrated strain, the *HOM6*-homozygous mutant was subjected to two rounds of integration/excision of the linear *HOM6* reintegrated cassette.

Southern blot and reverse transcription polymerase chain reaction (RT-PCR)

Genomic DNA and RNA isolation, Southern blot, and RT-PCR were performed as described.[14] For RT-PCR assays, the primer pairs HOM6-forward/HOM6-reverse and ACT1-forward/ACT1-reverse were used for *HOM6* and *ACT1*, respectively.

Assay for homoserine dehydrogenase activity

Cells grown in SM medium were resuspended in 200 μL lysis buffer [100mM Tris-HCl (pH 9.0), 0.6M sorbitol, 1mM phenylmethylsulfonyl fluoride, and 10% glycerol] containing an equal volume of acid-washed glass beads. The mixture was vortexed 10 times (45 s each) and kept on ice for 30 seconds

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