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

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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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Introduction

*Candida albicans* commensally colonizes skin and mucosal surfaces; however, this commensalism may 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.\(^5\) 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.\(^6\) 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.\(^9\) 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*.\(^9\) The aspartate metabolic pathway is related to the SA pathway and is another potential antifungal target, as humans lack enzymes involved in this pathway.\(^9\) 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). Betaspartate 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 reintegation

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-Apal/CaHOM6-up-R-Xhol and CaHOM6-dn-F-SacI/CaHOM6-dn-R-SacI, respectively. All the primers used in this study are listed in Supplementary Table S2. These flanking sequences were independently cloned into plasmid pSF52A to generate pSF52A-CaHOM6.\(^13\) For HOM6 reintegration, the 5′-flanking region plus full-length HOM6 were amplified with the primer pair CaHOM6-up-F-Apal/CaHOM6-R2-Xhol. This DNA fragment and the 3′-flanking region of HOM6 were independently cloned into pSF52A to generate pSF52A-CaHOM6-6. The HOM6 deletion and reintegratin cassettes were excised by Apal/SacI digestion from pSF52A-CaHOM6 and pSF52A-CaHOM6-6, respectively, and transformed into the *C. albicans* SC5314 strain as described.\(^13\)

The HOM6-homozygous 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 p-nitrophenylsulfonyl 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.
between each vortex. The protein extract was collected by centrifugation and quantified using a bicinchoninic acid (BCA) protein assay kit (Thermo Scientific Pierce, Rockford, IL, USA).

Homoserine dehydrogenase activity was measured as described. Whole-cell extracts (25 μg) were mixed with a solution [100mM Tris-HCl (pH 9.0), 2mM NADP, and 50mM L-homoserine] and incubated at 30°C for 20 minutes. The production of NADPH was measured by reading the absorbance at 340 nm (OD340). A mixture that lacked L-homoserine was used as a control, and a mixture that lacked protein extract was used as a blank. Homoserine dehydro- genase activity was calculated as: ([OD340 with homoserine − OD340 without homoserine])/6.22 × 1000 (nmol NADP/ mg/min). The value 6.22 represents the absorption coefficient of NADPH.

Amino acid analysis

Cells grown in SM medium were centrifuged, washed, and resuspended in 200 μL water containing 1mM phenylmethylsulfonyl fluoride. The suspension was mixed with glass beads and vortexed 10 times (45 s each), with 30 seconds on ice between each vortex. After centrifugation, the protein extract was assayed using the BCA protein assay kit.

To quantify intracellular amino acids, a Pico-Tag work station and a 2796 bioseparations module (Waters Corporation, Milford, MA, USA) were used. Briefly, protein samples were freeze dried, resuspended in ethanol:water:triethylamine [2:2:1 (v/v)], and then freeze dried again. The samples were then incubated with derivatization reagent [phenylisothiocyanate in ethanol:water:triethylamine, 1:7:1:1 (v/v)], and then freeze dried again. The samples were freeze dried, resuspended in ethanol:water:triethylamine [0.07% Na2HPO4 (pH 7.4) and 5% acetonitrile] were added, and the samples were assayed. Levels of intracellular amino acids were normalized to the number of cells.

Polysome profiling

Cells grown in YPD (OD600 = 1.0–1.5) were transferred to SC or SC–Thr medium for an additional 2 hours. Immediately before harvesting cells, 2 mL of 10 mg/mL cycloheximide and 1/3 volume (~67 mL) of crushed ice were added to the cultures. Cells were collected, washed twice with 5 mL ice-cold lysis buffer [10mM Tris-HCl (pH 7.5), 100mM NaCl, 30mM MgCl₂, 100 μg/mL cycloheximide, 200 μg/mL heparin, and 0.2% diethylpyrocarbonate (DEPC)], and resuspended in 500 μL ice-cold lysis buffer. The cells were mixed with glass beads and lysed by vortexing six times (30 s each), with 30 seconds on ice between each vortex. Cell lysates were collected by centrifugations (6500 g for 5 min at 4°C, followed by centrifugation again at 9200 g for 10 min at 4°C) and stored at –80°C. Sucrose was dissolved in a solution containing 50mM Tris-acetate (pH 7.0), 50mM NH₄Cl, 12mM MgCl₂, 1mM DTT, and 0.1% DEPC, and a 7% to 50% sucrose density gradient was prepared using a Foxy Jr. fraction collector with a Tris pump (Teledyne Isco Inc., Lincoln, NE, USA). Cell extracts (OD260 = 10–20) were loaded onto the sucrose gradient, centrifuged with a Beckman SW-41 rotor (21,000 g for 3.5 h at 4°C), and analyzed at 254 nm using an UA-6 UV/VIS detector (Teledyne Isco, Inc.).

Ribosomes were dissociated from polysomes as described with modifications. Early exponential-phase cells (OD600 = 1.0–1.5) were mixed with crushed ice and centrifuged. The cell pellets were washed twice with 5 mL ribosome-dissociation buffer [1mM Tris-HCl (pH 7.5), 50mM KCl, and 0.1mM ethylenediaminetetraacetic acid], resuspended in 500 μL ribosome-dissociation buffer, and lysed by vortexing (as described above). Whole-cell extracts (OD340 = 10) were loaded onto a 5% to 21% sucrose gradient and centrifuged (35,000 rpm for 5 h at 4°C). The ribosome-dissociation profile was analyzed at 254 nm using a UA-6 UV/VIS detector (Teledyne Isco, Inc.). To quantify the polysome profile, the area of integration for each peak was defined as the area enclosed by the basal lines and the boundary lines using Unicorn 4.00 software (GE Healthcare Life Sciences, Taipei, Taiwan). The boundary line was defined by the two points at which the slope of the trace line exhibited the biggest difference with that of the adjacent point.

Adhesion of C. albicans to polystyrene

To assess cell adhesion to polystyrene, exponential-phase cells were suspended in Roswell Park Memorial Institute (RPMI)-1640 medium at a concentration of 6 × 10⁷ cells/mL. Next, 250 μL of cell suspension was loaded into each well of a 24-well plate and incubated at 37°C for 30 minutes with shaking (100 rpm). Cells were then washed twice with phosphate-buffered saline (PBS) and analyzed for the 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT) reduction. Absorbance at 490 nm was determined using a microplate reader, and data were normalized to the wild-type control.

Statistical analysis

Differences between groups were statistically analyzed using the two-tailed Student t test. Differences were considered significant at p < 0.05.

Results

C. albicans Hom6 is a homoserine dehydrogenase

In the Candida Genome Database (http://candidagenome.org), orf19.2951 is named HOM6, but its function is mostly uncharacterized. In this work, we first aligned/compared its amino acid sequence between C. albicans and S. cerevisiae using ClustalW2 (http://www.ebi.ac.uk/Tools/msa/clustalw2/), which revealed 64% amino acid similarity and 79% identity (Fig. 1). Additionally, searching the C. albicans sequence against the Prosite database (http://prosite.expasy.org/) indicated that residues 199–221 represent the homoserine dehydrogenase signature.

To functionally characterize C. albicans Hom6, we generated HOM6-deleted (HOM6Δ/hom6Δ and hom6Δ/hom6Δ) and HOM6-reintegrated (hom6Δ/hom6Δ::HOM6
and hom6.1::HOM6/hom6.1::HOM6) strains. The successful strain constructions were verified by Southern blot and RT-PCR (Fig. 2A). To determine whether C. albicans Hom6 is a homoserine dehydrogenase, enzymatic activity was measured using L-homoserine as a substrate and NADP as a cofactor. Almost no homoserine dehydrogenase activity was detected for HOM6-homozygous mutants as compared with the wild-type variant (Fig. 2B). When both HOM6 alleles were reintegrated, homoserine dehydrogenase activity was largely restored (Fig. 2B).

C. albicans Hom6p is involved in the response to amino acid starvation

Amino acid starvation-induced Hom6 expression11 raises a possibility that C. albicans Hom6 is involved in cellular response to amino acid starvation. We analyzed cell proliferation in different liquid media. In amino acid–rich YPD medium, there was no significant differences in cell proliferation among the strains tested (data not shown). However, Hom6-homozygous mutants exhibited a growth delay when starved for amino acids (Fig. 3). These results suggested that Hom6 plays a role in cellular responses to amino acid starvation, perhaps by helping to synthesize certain amino acids.

To test whether C. albicans Hom6 is involved in amino acid biosynthesis, a spot assay was performed. HOM6-homozygous mutants exhibited a severe growth defect in amino acid–starved SM medium as compared with that growth observed in amino acid–rich YPD medium (Fig. 4). HOM6 reintegration rescued the growth defect observed in the HOM6-homozygous mutants (Fig. 4). Moreover, supplementing SM agar with methionine did not restore wild-type levels of growth to HOM6-homozygous mutants, whereas...
threonine supplementation partially restored this growth phenotype (Fig. 4). Importantly, HOM6-homozygous mutants grew at wild-type levels when both threonine and methionine were added to SM or homoserine was added alone. These results suggested that C. albicans Hom6 participates in the biosynthesis of methionine and threonine.

To further investigate the role of Hom6 in amino acid biosynthesis, levels of intracellular amino acids were

Figure 3. HOM6-homozygous mutants show a severe growth delay when starved for amino acids. Cells were grown in SM medium, and samples were collected at different times. Results are the mean ± SD of three independent assays. SD = standard deviation; SM = synthetic minimal medium.

Figure 4. Candida albicans Hom6 affects methionine and threonine synthesis. HOM6-homozygous mutants did not grow when starved for amino acids (SM), but growth was recovered by adding threonine plus methionine, or homoserine alone. Cells in the early exponential phase of growth (3 × 10⁷ cells/mL) were serially diluted with PBS and then spotted on agar plates as indicated. PBS = phosphate-buffered saline; SM = synthetic minimal medium.
measured after 1 hour or 2 hours of growth in SM medium. Intracellular threonine was not detected in HOM6-homozygous mutants (Table 1), and levels of isoleucine and glycine, which are synthesized from threonine, were reduced, while levels of methionine were slightly lower in HOM6-homozygous mutants (Table 1). Intracellular levels of other amino acids were also affected in HOM6-homozygous mutants (Supplementary Table S3). Thus, Hom6 affected both amino acid biosynthesis via the aspartate pathway and the size of the intracellular amino acid pool.

### C. albicans Hom6 affects translation

Low levels of intracellular amino acids can downregulate translation. To determine the effects of HOM6 on translation, we assessed cell susceptibility to translation inhibitors. HOM6-homozygous mutants were hypersensitive to hygromycin B and cycloheximide as compared with wild-type, HOM6-heterozygous, and HOM6-reintegrated strains (Fig. 5A).

To further investigate whether C. albicans Hom6 affects translation, we performed polysome profiling.\(^{19}\) Wild-type and HOM6-homozygous mutant strains showed very similar polysome profiles in threonine-rich SC medium (Fig. 5B). In SC medium lacking threonine (SC-Thr), however, polysome levels were lower in HOM6-homozygous mutants as compared with wild-type cells (Fig. 5B). Simultaneously, the levels of 40S and 60S ribosomal subunits increased in HOM6-homozygous mutants as compared with wild-type variants (Fig. 5B). These results suggested that HOM6 deletion affected translation and led to the accumulation of free ribosomes, as indicated by the increases in 40S and 60S subunits. Alternatively, the increase in free ribosomal 40S and 60S subunits may represent an indirect effect of HOM6 deletion in the event that HOM6 affects the synthesis of other ribosomal subunits.

To differentiate between these possibilities, total levels of 40S and 60S subunits were determined by measuring both available free ribosomes and subunits obtained from the dissociation of existing polysomes. Total amounts of 40S and 60S subunits did not differ between HOM6-homozygous mutants and wild-type variants (Fig. 5C), suggesting that increases in free 40S and 60S subunits resulted from a translational defect (i.e., they were released from polysomes).

### C. albicans Hom6 plays a role in C. albicans adhesion

Using a Hom6-green fluorescent protein (GFP) construct, the fusion protein was found in both the cytosolic and cell-wall fractions (Supplementary Fig. 5I). Therefore, we tested whether Hom6 could affect cell adhesion. HOM6-homozygous mutants adhered poorly to polystyrene as compared with other strains (Fig. 6), indicating a role for Hom6 in C. albicans adhesion.

### Discussion

Here, we showed that Hom6 may participate in the biosynthesis of threonine and methionine through the aspartate pathway. Wild-type and HOM6-homozygous mutant strains grew at similar rates in amino acid–rich YPD medium (data not shown); however, in amino acid–depleted SM medium, HOM6-homozygous mutants exhibited slow growth (Fig. 3). Supplementing the SM medium with both methionine and threonine rescued the growth of HOM6-homozygous mutants (Fig. 4). Addition of threonine alone to the SM medium partially rescued the growth phenotype, whereas addition of methionine alone did not (Fig. 4). One explanation is that methionine may be synthesized by the aspartate pathway and alternative routes, such as the SA pathway and cysteine metabolism.\(^{4,20}\) This hypothesis is partially supported by our analysis of intracellular amino acids. After amino acid starvation, similar levels of methionine were detected in wild-type, HOM6-homozygous, and HOM6-reintegrated strains. In contrast, threonine was undetectable in HOM6-homozygous mutants (Table 1). Similarly, isoleucine and glycine can be generated from threonine or through the citraconate pathway (isoleucine)\(^{22}\) and serine metabolism (glycine).\(^{20}\) Moreover, methionine synthesis involves biofilm formation in C. albicans.\(^{23}\) Deletion of ECM17, which encodes a sulfite reductase involved in methionine/cysteine biosynthesis, reduces cell adhesion and biofilm formation in C. albicans.\(^{22,24}\) Our results highlighted complex interactions between different pathways of amino acid biosynthesis and possible links between host amino acid levels and C. albicans infection.

HOM6-homozygous mutants were hypersensitive to hygromycin B and cycloheximide (Fig. 5A). A previous study reported a correlation between hygromycin B sensitivity in S. cerevisiae and defects in protein glycosylation.\(^{25}\)

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**Table 1** Intracellular concentrations of threonine, methionine, isoleucine, and glycine (nmol/10⁹ cells) for cells in amino acid-starvation conditions.

<table>
<thead>
<tr>
<th></th>
<th>1 h in SM medium</th>
<th>2 h in SM medium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wild type</td>
<td>hom6Δ/</td>
</tr>
<tr>
<td>Thr</td>
<td>17.84 ± 0.49 ND</td>
<td>20.13 ± 0.54</td>
</tr>
<tr>
<td>Met</td>
<td>4.65 ± 0.13 4.30 ± 0.46</td>
<td>5.98 ± 0.03</td>
</tr>
<tr>
<td>Ile</td>
<td>9.86 ± 0.07 3.35 ± 0.39</td>
<td>10.14 ± 0.49</td>
</tr>
<tr>
<td>Gly</td>
<td>38.86 ± 1.39 23.86 ± 3.68</td>
<td>45.53 ± 0.40</td>
</tr>
</tbody>
</table>

Gly = glycine; Ile = isoleucine; Met = methionine; ND = not detected; SM = synthetic minimal medium.; Thr = threonine.

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Notably, glycosylation can modulate the structure and function of cell-surface proteins in yeast. Interestingly, \textit{C. albicans} Hom6-GFP fusion protein localized to both the cytosolic and cell-wall fractions. The localization of \textit{C. albicans} Hom6 within the cell-wall fraction was also identified by several proteomic studies.\textsuperscript{26,27} Moreover, \textit{S. cerevisiae} Hom6 localizes to the cytosol and the nucleus.\textsuperscript{28}

Proteins that localize to multiple cellular compartments commonly perform more than one function. For example, \textit{C. albicans} Adh1 protein localizes to the cell surface of yeast-form cells and to the cytosol of hyphal cells.\textsuperscript{29,30} Adh1 is an alcohol dehydrogenase involved in biofilm formation and possible adhesion to the extracellular matrix during infection.\textsuperscript{31,32} Here, \textit{C. albicans} adhesion to polystyrene was studied in amino acid-containing RPMI medium, resulting in reduced adhesion of \textit{HOM6}-deleted strains (Fig. 6). Since wild-type and \textit{HOM6}-deleted strains grew at similar rates in amino acid-rich YPD medium, the reduced adhesion observed in the mutant strain may not be due to a growth defect. Threonine is the key residue for O\textsuperscript{-}linked mannosylation,\textsuperscript{33} and threonine deficiencies likely reduce levels of mannoproteins, which are thought to be important for \textit{C. albicans} adhesion and virulence.\textsuperscript{34} Because Hom6 is involved in threonine and methionine biosynthesis, the possible mechanisms by which Hom6 affects O\textsuperscript{-}linked mannosylation and adhesion require further study.

Figure 5. \textit{HOM6} affects \textit{Candida albicans} translation. (A) \textit{HOM6}-homozygous mutants were hypersensitive to hygromycin B and cycloheximide. Cells in the early exponential phase of growth were serially diluted with PBS as indicated and then spotted onto YPD plates with or without 150 $\mu$g/mL hygromycin B or 2 mg/mL cycloheximide; (B) \textit{HOM6} deletion affected polysome levels. Wild-type or \textit{HOM6}-homozygous mutant cells in the exponential phase of growth were transferred into SC (top panel) or SC–Thr (bottom panel) medium and incubated for 2 hours. The polysome profile shows ribosomal subunits (40S and 60S), monosomes (80S), and polysomes from different fractions of the sucrose gradient. Representative plots from two (upper) or three (lower) independent experiments with similar results are shown. The area under each peak (mean $\pm$ SD) is shown. *$p < 0.05$; and (C) Total levels of 40S and 60S ribosomal subunits (including those derived from dissociated polysomes) were similar between wild-type and mutant cells in SC–Thr medium. A representative plot from three independent experiments with similar results is shown. The area under each peak (mean $\pm$ SD) is indicated. PBS = phosphate-buffered saline; SC = synthetic complete medium; SC–Thr = synthetic complete medium lacking threonine; SD = standard deviation; YPD = yeast-extract peptone dextrose.
with cell-wall components. Although the possible effects of LL-37 interfere with our previous study showed that antimicrobial peptide LL-37 can inhibit the cofactor NAD(P) binding region within the active site. Finally, this compound is effective against Candida albicans, and Cryptococcus neoformans, a promising new target for developing antifungal drugs. 2H-tetrazolium-5-carboxanilide.

The antifungal agent 5-hydroxy-4-oxonorvaline [(S)-2-amino-4-oxo-5-hydroxypentanoic acid; RI-331] inhibits homoserine dehydrogenase by forming an adduct with the cofactor NAD(P) within the active site. This compound is effective against Saccharomyces cerevisiae, Candida albicans, and Cryptococcus neoformans. Moreover, a new class of phenolic inhibitors blocks homoserine dehydrogenase activity by binding to the amino acid-binding region within the active site. Finally, our previous study showed that antimicrobial peptide LL-37 interferes with C. albicans adhesion through its interaction with cell-wall components. Although the possible effects of LL-37 on Hom6 must still be examined, Hom6 represents a promising new target for developing antifungal drugs.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jmii.2016.03.001.