Investigating the Function of Ddr48p in Candida albicans

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Candidiasis now represents the fourth most frequent nosocomial infection both in the United States and worldwide. Candida albicans is an increasingly common threat to human health as a consequence of AIDS, steroid therapy, organ and tissue transplantation, cancer therapy, broad-spectrum antibiotics, and other immune defects. The pathogenic potential of C. albicans is intimately related to certain key processes, including biofilm formation and filamentation. Ddr48p is a damage response protein that is significantly upregulated during both biofilm formation and filamentation, but its actual function is unknown. Previous studies have indicated that this protein may be essential in C. albicans but not Saccharomyces cerevisiae. Here we examined the function of Ddr48p and investigated the role of this protein in biofilm formation and filamentation. We demonstrated that this protein is not essential in C. albicans and appears to be dispensable for filamentation. However, DDR48 is required for the flocculation response stimulated by 3-amino-triazole-induced amino acid starvation. Furthermore, we examined the response of this deletion strain to a wide variety of environmental stressors and antifungal compounds. We observed several mild sensitivity or resistance phenotypes and also found that Ddr48p contributes to the DNA damage response of C. albicans. The results of this study reveal that the role of this highly expressed protein goes beyond a general stress response and impinges on a key facet of pathogenesis, namely, the ability to sense and respond to changes in the host environment.

Candida albicans is a versatile commensal organism that possesses a number of attributes that enhance its ability to survive in diverse environments and enable it to transition from a harmless commensal to an invasive pathogen. C. albicans remains the main causative agent of invasive fungal infection in an expanding population of immunocompromised patients with associated high morbidity and mortality rates (2, 5, 51, 52). C. albicans pathogenesis is a complex phenomenon that results from a delicate balance between its intrinsic virulence attributes and host immune responses (8–11). This gives rise to the highly complex and dynamic nature of the host-fungus interaction that ultimately determines the outcome of an infection. The host responses during systemic candidiasis range from nonspecific innate mechanisms to sophisticated adaptive responses (23, 40, 41, 44). Morphogenetic conversion, the ability to switch reversibly between the yeast cell and filamentous forms, constitutes one of the most important virulence attributes of this organism (7, 14, 26, 42).

Ddr48p is a damage response protein that is significantly upregulated during both biofilm formation and filamentation. It is a low-complexity protein that is thought to be associated with DNA damage but whose actual function is unknown. In Saccharomyces cerevisiae, DDR48 is a member of a set of genes that show increased expression when exposed to heat shock (27), cellular stresses such as cadmium exposure and osmotic stress (28, 29), and treatments that produce DNA lesions (49). However, the antimutator phenotype reported by Treger and McIntee (49) has since been challenged, as Roche et al. could not replicate it (37). Previous studies have indicated that this protein may be essential in C. albicans (15) but not S. cerevisiae. These studies also implicated Ddr48p in filamentation, stress response, and drug resistance (15). This implication was based solely on the inability to generate a homozygous null mutant of C. albicans. In this study, we report the generation of homozygous null mutants in different C. albicans backgrounds and describe several stress response- and environmental-sensing-related phenotypes.

MATERIALS AND METHODS

Strains, plasmids, and media. The C. albicans strains and plasmids used in this study are described in Tables 1 and 2. Yeast strains were routinely maintained at −80°C glycerol freezer stocks and retrieved on yeast extract-peptone-dextrose (YPD) medium as required. Amino acid starvation was imposed using yeast nitrogen base (YNB) medium containing 3-amino-triazole (3-AT) at a final concentration of 9 mM. For filamentation assays in liquid medium, strains were grown overnight in YPD medium at 28°C, diluted 1:20 into fresh YPD medium or fetal bovine serum (FBS; Lonza), and incubated with shaking at 37°C. Samples were taken from these cultures at various time points, and their morphology was evaluated microscopically using a 40× objective lens and photographed using a digital camera. For filamentation assays on solid Spider medium (24) or Lee medium (22) (pH 7), strains were grown in YPD medium overnight at 28°C, washed in sterile phosphate-buffered saline, counted using a hemocytometer, and resuspended to a final concentration of 5 × 10^7/ml. Aliquots of 2 μl (1 × 10^4 cells) were spotted onto agar and incubated at 37°C for 4 days (Lee medium, pH 7) or 7 days (Spider medium). Colonies were examined and photographed using a GL9-280 Stereo Zoom microscope (Jenco) equipped with a digital camera. Biofilms were formed as described previously (35) using the 96-well microtiter plate method, and biofilm growth was assayed by 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT) reduction. All plasmid manipulations were performed with Escherichia coli strain DH5α with selection on Luria-Bertani plates containing 100 μg/ml ampicillin when necessary.

Strain construction. Both copies of DDR48 were sequentially deleted from parental strains SC5314 and BWP17 using the SAT1 flipper method (36). First, two regions flanking the DDR48 coding sequence were PCR amplified by using primers DDR48_LHF_UPS and DDR48_LHF_DS and

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primers DDR48_RHF_UPS and DDR48_RHF_DS. These amplification products were ligated into the Smal site of plasmid pMT3000 (34). The flanking regions were liberated from the plasmid by restriction digestion with Apal and Xhol or SacI and SacI at the sites engineered into the primers and ligated sequentially between the Apal and Xhol or SacI and SacI sites in the proximal and distal cloning regions of plasmid pSF52 (36) to form plasmid pDS3. The entire deletion cassette was then liberated from this plasmid as a KpnI-SacI fragment and transformed into the appropriate species of *C. albicans* (SC5314 or BPW17) using a modified electroporation transformation method (21). Nourseothricin-resistant transformants were selected as described above. Correct insertion at the native locus was confirmed by PCR.

Complemented strains containing a copy of *DDR48* returned to its native locus were constructed as follows. The *DDR48* coding and upstream sequences were amplified from genomic DNA using primers DDR48_LHF_UPS and DDR48_DS_XhoI, and the product was ligated between the XhoI and EcoRI sites in plasmid CIpSATSA (12) (replacing the previous LHF fragment). The inserted DNA was liberated by digestion with Apal and Xhol at the sites engineered into the amplification primers and then ligated between the Apal and Xhol sites in plasmid DS3 (12) (replacing the previous LHF fragment) to produce plasmid pDSLcoding. The entire deletion cassette was then liberated from this plasmid as a KpnI-SacI fragment and transformed into the *ddr48Δ* null strain, and nourseothricin-resistant transformants were selected as described above. Correct insertion at the *DDR48* locus was confirmed by PCR.

**Quantitative PCR.** RNA was isolated from *C. albicans* cells using the MasterPure yeast RNA extraction kit (Epitome Biotechnologies). RNA was treated with amplification grade DNase I (Invitrogen) and used for cDNA synthesis with the MasterScript kit (Promega). The primer sets (Table 3) were used in conjunction with SYBR green PCR master mix (Applied Biosystems) and twin.tec real-time 96-well PCR plates (Eppendorf) in a ABI 7300 real-time PCR system (Applied Biosystems). Dissociation curves were analyzed for all reactions to verify single peaks/products. Expression levels were analyzed using ABI 7300 System SDS software (Applied Biosystems). Results were normalized to *ACT1*.

**Sensitivity assays.** A microdilution method was used to test the sensitivity of the deletion strain to a variety of stressor compounds. A dilution series of each compound was mixed with molten YPD agar and added to a 96-well microtiter plate. After the medium had solidified, an aliquot of 10 µl (200 cells) from a logarithmically growing culture was added to each well. Plates were incubated overnight at 30 or 37°C.

**Results and discussion.**

*DDR48* is a known stress-related protein in both *S. cerevisiae* and *C. albicans* whose expression is elevated in association with several key *C. albicans* processes, i.e., in response to antifungal drugs (3, 6, 16, 20, 25, 38, 46), during filamentation (30–32), and during biofilm formation (45). To investigate its function, we used the *SAT1* flipper methodology to sequentially delete both copies of *DDR48* from several strains of *C. albicans*, including wild-type strain SC5314 and a tri-axotrophic strain BPW17. A study published concurrently with our own work creating a *ddr48Δ* null strain suggested that *DDR48* might be an essential gene in *C. albicans* based on the inability to delete both copies of the gene (15). However, we were able to delete both copies of *DDR48* (Fig. 1A) multiple times, demonstrating that *DDR48* is not an essential gene. Quantitative real-time PCR analysis of transcription confirmed that there was no *DDR48* mRNA in our

**Table 1** Strains used in this study

| Strain | Parent | Genotype | Reference |
|--------|--------|----------|-----------|
| SC5314 | SC5314 | DDR48/ddr48::SAT1-FLIP | 17 |
| DS1    | DS1    | DDR48/ddr48::FRT | This study |
| DS1-3  | DS1-3  | ddr48::SAT1-FLIP/ddr48::FRT | This study |
| ddr48Δ | ddr48Δ | ddr48::FRT/ddr48::FRT | This study |

**Table 2** Plasmids used in this study

| Plasmid       | Reference |
|---------------|-----------|
| ClpSATSA      | 12        |
| ClpSATSAADD48 | This study |
| pSF52         | 36        |
| pDL52         | This study |
| pDS3          | This study |
| pMT3000       | 34        |
| pDL23         | This study |
| pDRHF2        | This study |
| pDDR48-31     | This study |
| pDDR48LHFcoding | This study |
| pDSLcoding    | This study |

**Table 3** Oligonucleotides used in this study

| Oligonucleotide | Sequence | Reference |
|-----------------|----------|-----------|
| DDR48 UPS       | CTGGAGCCATTTATCATAAATTAAAGTTGG | This study |
| DDR48 DSE       | GAATTCGCTTCTTAATTTAAGTTGG | This study |
| DDR48 LHF UPS   | GGGGCCCATTTGGCCCTTCTCC | This study |
| DDR48 LHF DS    | CTGGAGCCATTTGTTATCATAAATTAAAG | This study |
| DDR48 RHF UPS   | CCGGGCTTATCTGTGGTTTGGACCT | This study |
| DDR48 RHF DS    | GAACCTACGTGTGGTAAATGACCTTT | This study |
| DDR48 Xhol      | CTGGAGCCATTTGTTATCATAAATTAAAG | This study |
| DDR48qFOR      | CGAGCAAGAAGAAGAAAGG | This study |
| DDR48qREV      | TCTGTGGAGAGAAGGCGT | This study |
| ACT1-S         | ATGTGACAAGGCCGGTTTTGCGG | 48 |
| ACT1-A         | CCATACTGGCCAGGGTATG | 48 |

Underlined sequences indicate restriction enzyme recognition sequences engineered into the oligonucleotides.
deletion strain (Fig. 1B). Complementation of the deletion with a single copy of DDR48 under the control of the constitutive ACT1 promoter or of the endogenous DDR48 promoter at its native locus restored transcription to levels slightly lower than those observed in the heterozygote strain.

Since elevated DDR48 expression is associated with hyphal induction (25–27), we next tested the growth of our null strain under a variety of hypha-inducing conditions using solid and liquid media (Fig. 2). We observed that the null strain was not deficient in hypha formation on solid Spider or Lee (pH 7) medium and that it formed hyphae equally well in liquid YPD medium and FBS when grown at 37°C (Fig. 2). Unlike the phenotypes reported by Dib et al. in their heterozygote strain (15), we observed no filamentation defect in our heterozygote or deletion strains. We therefore conclude that while DDR48 expression is upregulated during filamentous growth, it is not required for hypha formation.

FIG 1 Southern blotting and expression analysis confirming strains. (A) Southern blot analysis. Lane 1 contains the lambda HindIII marker. Lane 2 is the untransformed parent strain (SC5314). Lane 3 is the heterozygote strain where one copy of DDR48 has been deleted. Lanes 4 shows correct insertion into the second copy of DDR48. Genomic DNA was digested with BglII and probed with DNA flanking DDR48. The expected sizes are as follows: endogenous, 3.2 kb; insert, 6.5 kb; flip, 2.6 kb; WT, wild type. (B) Quantitative real-time PCR analysis. RNA was isolated from cells grown in YPD medium at 37°C. 1, SC5314 (wild type); 2, DDR48 heterozygote; 3, ddr48Δ homozygote; 4, complemented-strain endogenous promoter expression; 5, complemented-strain constitutive expression. Levels were normalized by ACT1 and are expressed relative to that of wild-type control strain SC5314 (which is therefore 1.0). No DDR48 transcript was detected in the deletion strain.

FIG 2 Filamentation assays. Filamentation was induced by growth at 37°C on solid Spider medium, on solid Lee medium (pH 7), or in liquid YPD medium or FBS. Neither the heterozygote nor the null strain displayed a defect in hypha formation in these media.
DDR48 is induced in *S. cerevisiae* and *C. albicans* in response to cellular stresses including oxygen stress (19, 43), exposure to cell wall-perturbing agents (28, 29), and, in the case of *C. albicans*, antifungal drugs (2, 4, 13, 15, 20, 31). We therefore tested the response of our deletion strain to various stress-inducing compounds using a microdilution assay. *DDR48* is normally upregulated by growth at 37°C, so we tested sensitivities at 30°C and 37°C. Interestingly, the deletion strain was more sensitive to 4-nitroquinoline 1-oxide (Fig. 3), which induces oxygen radical formation and DNA lesions (1), at both 30°C and 37°C. For each stressor, the maximum concentration in the dilution series on which growth was observed is graphed. Error bars represent the standard deviation of biological triplicate samples, which is zero in most cases.

**FIG 3** Sensitivity assays. A microdilution method was used to test the impact of *DDR48* deletion on sensitivities to various cellular stresses. Deletion of one or both copies of *DDR48* resulted in increased resistance to SDS, amphotericin B, and rapamycin, which perturb the cell wall, the cell membrane, and the Tor kinase pathway, respectively, at 37°C but not at 30°C. Deletion of *DDR48* increased sensitivity to DNA damage and oxidative stress induced by 4-nitroquinoline 1-oxide at both 30°C and 37°C. For each stressor, the maximum concentration in the dilution series on which growth was observed is graphed. Error bars represent the standard deviation of biological triplicate samples, which is zero in most cases.

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The antifungal compound rapamycin affects signaling through the Tor kinase pathway. The Tor kinase pathway is important for the regulation of cellular growth and proliferation in response to nutrient availability (reviewed in reference 39). One of the multiple downstream targets of Tor signaling is the GCN response, which upregulates amino acid biosynthesis pathways in response to uncharged tRNA accumulation (45). Given our rapamycin resistance result, we examined the impact of nutrient starvation on the growth of our mutant strain. Histidine starvation can be im-
posed on yeast by growth in the presence of 3-AT, a competitive inhibitor of His3p. This impairs the growth of *S. cerevisiae* and activates the GCN amino acid starvation response. In *C. albicans*, 3-AT also induces the pseudohyphal morphology (50). In liquid culture in the presence of 3-AT wild-type cells formed dense clumps consisting of yeast and pseudohyphal cells (Fig. 4A). The heterozygote strain formed smaller yet still dense clumps of cells. The cells of the deletion strain formed only small, loosely aggregated clumps which appeared to be more like a monolayer of clearly visible individual yeast cells with infrequent pseudohyphae (Fig. 4A, bottom row). This is strikingly similar to the results reported by Bastidas et al., who observed that rapamycin treatment induced elevated DDR48 expression and the formation of clumps of cells in a wild-type strain, while under the same conditions, a rapamycin-resistant strain had only slightly elevated DDR48 levels and was not flocculent (4).

Strains with defects in cell-cell adhesion, such as those lacking Als3p, show reduced biofilm formation (13, 33, 53). We therefore tested the capacity of our mutant strain to form biofilms in the presence of 3-AT using the 96-well microtiter plate model (35). In YNB or RPMI 1640 medium, we did not observe a marked difference in biofilm formation in the mutant strain (data not shown), but when 3-AT was added to these media, there was a small but consistent decrease in biofilm formation by the *ddr48/H9004* mutant as determined by the XTT reduction assay (Fig. 4B).

We have demonstrated that DDR48, a gene upregulated under a variety of stress conditions, is not essential in *C. albicans*, as had been previously suggested, nor is it required for filamentation. We have also been able to identify a role in DNA damage repair in *C. albicans*, as it is required for wild-type resistance to DNA lesions caused by 4-nitroquinoline 1-oxide. Interestingly, our results have also revealed a role for DDR48 in sensing or responding to environmental nutritional conditions. Growth in the presence of 3-AT imposes amino acid starvation, while rapamycin affects the func-
tion of the Tor kinase pathway, which is also involved in the stimulation of the amino acid starvation response (45). The reduced-flocculation phenotype of the ddr48Δ strain treated with 3-AT mimics that of a rapamycin-resistant strain exposed to rapamycin, and our mutant strain is also more resistant to rapamycin. The Tor kinase pathway is repressed by rapamycin, and Tor downregulation appears to be necessary for the regulation of factors related to adhesion and biofilm formation. Ddr48 is present in the cell wall and upregulated in C. albicans biofilms (47), and here we have demonstrated that DDR48 is required for the flocculation response stimulated by 3-AT-induced amino acid starvation. The results of this study reveal that the role of this highly expressed protein goes beyond a general stress response and impinges on a key facet of pathogenesis, namely, the ability to sense and respond to changes in the host environment.

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REFERENCES

1. Arima Y, et al. 2006. 4-Nitroquinoline 1-oxide forms 8-hydroxydeoxyguanosine in human fibroblasts through reactive oxygen species. Toxicol. Sci. 91:382–392.
2. Banerjee SN, et al. 1991. Secular trends in nosocomial primary bloodstream infections in the United States, 1980-1989. National Nosocomial Infections Surveillance System. Am. J. Med. 91:865–898.
3. Barker KS, et al. 2004. Genome-wide expression profiling reveals genes associated with amphotericin B and fluconazole resistance in experimentally induced antifungal resistant isolates of Candida albicans. J. Antimicrob. Chemother. 54:376–385.
4. Bastidas RJ, Heitman J, Cardenas ME. 2009. The protein kinase Tor1 regulates adhesion gene expression in Candida albicans. PLoS Pathog. 5:e1000294. doi:10.1371/journal.ppat.1000294.
5. Beck-Sague C, Jarvis WR. 1993. Secular trends in the epidemiology of nosocomial fungal infections in the United States, 1980-1990. National Nosocomial Infections Surveillance System. J. Infect. Dis. 167:1247–1251.
6. Brown VM, et al. 2006. Control of the C. albicans cell wall damage response by transcriptional regulator Cas5. PLoS Pathog. 2:e21. doi:10.1371/journal.ppat.0020021.
7. Calderone RA, Fonzi WA. 2001. Virulence factors of Candida albicans. Trends Microbiol. 9:327–335.
8. Casadella A, Pirofski LA. 1999. Host-pathogen interactions: defining the basic concepts of virulence and pathogenicity. Infect. Immun. 67:3703–3713.
9. Casadella A, Pirofski LA. 2002. What is a pathogen? Ann. Med. 34:2–4.
10. Casadella A, Pirofski LA. 2003. “Anti-virulence” genes—further mud- ding the lexicon? Response from Arturo Casadevall and Liis-anne Pirofski. Trends Microbiol. 11:413–414.
11. Cleary IA, et al. 2010. Pseudohyphal regulation by the transcription factor Rfg1p in Candida albicans. Eukaryot. Cell 9:1363–1373.
12. Cleary IA, et al. 2011. Candida albicans adhesin Als3p is dispensable for virulence in the mouse model of disseminated candidiasis. Microbiology 157:1806–1815.
13. Cutler JE. 1991. Putative virulence factors of Candida albicans. Annu. Rev. Microbiol. 45:187–218.
14. Dib L, Hayek P, Sadek H, Beyrouthy B, Khalaf RA. 2008. The Candida albicans Ddr48 protein is essential for filamentation, stress response, and confers partial antifungal drug resistance. Med. Sci. Monit. 14:BR113–121.
15. Dunkel N, et al. 2008. A gain-of-function mutation in the transcription factor Upc2p causes upregulation of ergosterol biosynthesis genes and increased fluconazole resistance in a clinical Candida albicans isolate. Eukaryot. Cell 7:1180–1190.
16. Gillum AM, Tsay EY, Kirsch DR. 1984. Isolation of the Candida albicans gene for orotidine-5′-phosphate decarboxylase by complementation of S. cerevisiae ura3 and E. coli pyrF mutations. Mol. Gen. Genet. 198:179–182.
17. Hao B, et al. 2009. Candida albicans RFX2 encodes a DNA binding protein involved in DNA damage responses, morphogenesis, and virulence. Eukaryot. Cell 8:627–639.
18. Hromatka BS, Noble SM, Johnson AD. 2005. Transcriptional response of Candida albicans to nitric oxide and the role of the YHB1 gene in nitrosative stress and virulence. Mol. Biol. Cell 16:4814–4826.
19. Karababa M, Coste AT, Rognon B, Bille J, Sanglard D. 2004. Comparison of gene expression profiles of Candida albicans azole-resistant clinical isolates and laboratory strains exposed to drugs inducing multidrug transporters. Antimicrob. Agents Chemother. 48:3064–3079.
20. Köhler GA, White TC, Agabian N. 1997. Overexpression of a cloned IMP dehydrogenase gene of Candida albicans confers resistance to the specific inhibitor mycophenolic acid. J. Bacteriol. 179:2331–2338.
21. Lee KL, Buckley HR, Campbell CC. 1975. An amino acid liquid synthetic medium for the development of mycelial and yeast forms of Candida albicans. Sabouraudia 13:148–153.
22. Levitz SM. 1992. Overview of host defenses in fungal infections. Clin. Infect. Dis. 14(Suppl. 1):S37–S42.
23. Liu H, Kohler J, Fink GR. 1994. Suppression of hyphal formation in Candida albicans by mutation of a STE12 homolog. Science 266:1723–1726.
24. Liu TT, et al. 2005. Genome-wide expression profiling of the response to azole, polycyclic, eichonadin, and pyrimidine antifungal agents in Candida albicans. Antimicrob. Agents Chemother. 49:2226–2236.
25. Lo HJ, et al. 1997. Nonfilamentous C. albicans mutants are avirulent. Cell 90:939–949.
26. McClanahan T, McIntee K. 1986. DNA damage and heat shock dually regulate genes in Saccharomyces cerevisiae. Mol. Cell. Biol. 6:90–96.
27. Miralles VI, Serrano R. 1995. A genomic locus in Saccharomyces cerevisiae with four genes up-regulated by osmotic stress. Mol. Microbiol. 17:653–662.
28. Momose Y, Iwashashi H. 2001. Bioassay of cadmium using a DNA microarray: genome-wide expression patterns of Saccharomyces cerevisiae response to cadmium. Environ. Toxicol. Chem. 20:2353–2360.
29. Murad AM, et al. 2001. Transcript profiling in Candida albicans reveals new cellular functions for the transcriptional repressors CaTup1, CaMig1 and CaNigl. Mol. Microbiol. 42:981–993.
30. Murad AM, et al. 2001. NRGI represses yeast-hypha morphogenesis and hypha-specific gene expression in Candida albicans. EMBO J. 20:4742–4752.
31. Nantel A, et al. 2002. Transcript profiling of Candida albicans cells undergoing the yeast-to-hyphal transition. Mol. Biol. Cell 13:3452–3465.
32. Nobile CJ, et al. 2006. Critical role of Bcr1-dependent adhesins in C. albicans biofilm formation in vitro and in vivo. PLoS Pathog. 2:e63. doi:10.1371/journal.ppat.0020063.
33. Paget MS, Hintermann G, Smith CP. 1994. Construction and application of streptomycete promoter probe vectors which employ the Streptomyces glucosecin tyrosinase-encoding gene as reporter. Gene 146:105–110.
34. Pierce CG, et al. 2008. A simple and reproducible 96-well plate-based method for the formation of fungal biofilms and its application to antifungal susceptibility testing. Nat. Protoc. 3:1494–1500.
35. Reus O, Vík A, Kolter R, Morschhauser J. 2004. The SAT1 flipper, an optimized tool for gene disruption in Candida albicans. Gene 341:119–127.
36. Roche H, Rachmandran K, Kunz BA. 1995. Failure to detect an antimutator phenotype following disruption of the Saccharomyces cerevisiae DDR48 gene. Curr. Genet. 27:496–500.
37. Rogers PD, Barker KS. 2003. Genome-wide expression profile analysis reveals coordinately regulated genes associated with stepwise acquisition of azole resistance in Candida albicans clinical isolates. Antimicrob. Agents Chemother. 47:1220–1227.
38. Rohde JR, Bastidas R, Puria R, Cardenas ME. 2008. Nutritional control via Tor signaling in Saccharomyces cerevisiae. Curr. Opin. Microbiol. 11:153–160.
39. Romani L. 2000. Innate and adaptive immunity in Candida albicans infections and saprophytism. J. Leukoc. Biol. 68:175–179.
41. Romani L. 2004. Immunity to fungal infections. Nat. Rev. Immunol. 4:1–23.
42. Rooney PJ, Klein BS. 2002. Linking fungal morphogenesis with virulence. Cell. Microbiol. 4:127–137.
43. Setiadi ER, Doedt T, Cottier F, Noffz C, Ernst JF. 2006. Transcriptional response of Candida albicans to hypoxia: linkage of oxygen sensing and Efg1p-regulatory networks. J. Mol. Biol. 361:399–411.
44. Shoham S, Levitz SM. 2005. The immune response to fungal infections. Br. J. Haematol. 129:569–582.
45. Staschke KA, et al. 2010. Integration of general amino acid control and target of rapamycin (TOR) regulatory pathways in nitrogen assimilation in yeast. J. Biol. Chem. 285:16993–16991.
46. Synnott JM, Guida A, Mulhern-Haughey S, Higgins DG, Butler G. 2010. Regulation of the hypoxic response in Candida albicans. Eukaryot. Cell 9:1734–1746.
47. Thomas DP, Pitarach A, Montediva L, Gil C, Lopez-Ribot JL. 2006. Proteomics to study Candida albicans biology and pathogenicity. Infect. Disord. Drug Targets 6:335–341.
48. Toyoda M, Cho T, Kaminishi H, Sudoh M, Chibana H. 2004. Transcriptional profiling of the early stages of germination in Candida albicans by real-time RT-PCR. FEMS Yeast Res. 5:287–296.
49. Treger JM, McEntee K. 1990. Structure of the DNA damage-inducible gene DDR48 and evidence for its role in mutagenesis in Saccharomyces cerevisiae. Mol. Cell. Biol. 10:3174–3184.
50. Tripathi G, et al. 2002. Gcn4 co-ordinates morphogenetic and metabolic responses to amino acid starvation in Candida albicans. EMBO J. 21:5448–5456.
51. Viudes A, et al. 2002. Candidemia at a tertiary-care hospital: epidemiology, treatment, clinical outcome and risk factors for death. Eur. J. Clin. Microbiol. Infect. Dis. 21:767–774.
52. Wey SB, Mori M, Pfaller MA, Woolson RF, Wenzel RP. 1988. Hospital-acquired candidemia. The attributable mortality and excess length of stay. Arch. Intern. Med. 148:2642–2645.
53. Zhao X, et al. 2004. ALS3 and ALS8 represent a single locus that encodes a Candida albicans adhesin; functional comparisons between Als3p and Als1p. Microbiology 150:2415–2428.