JOURNAL OF BACTERIOLOGY, Oct. 1996, p. 5850–5852 Vol. 178, No. 19
0021-9193/96/$04.00+0

The Mitogen-Activated Protein Kinase Homolog *HOG1* Gene Controls Glycerol Accumulation in the Pathogenic Fungus *Candida albicans*

C. SAN JOSÉ, R. ALONSO MONGE, R. PÉREZ-DÍAZ, J. PLA, AND C. NOMBELA*

Departamento de Microbiología II, Facultad de Farmacia, Universidad Complutense de Madrid, 28040 Madrid, Spain

Received 14 May 1996/Accepted 24 July 1996

The *Candida albicans HOG1* gene (*HOG1*$_{Ca}$) was cloned by functional complementation of the osmosensitive phenotype associated with *Saccharomyces cerevisiae hog1*Δ mutants. *HOG1*$_{Ca}$ codes for a 377-amino-acid protein, 78% identical to *S. cerevisiae* Hog1p. A *C. albicans hog1* null mutant was found to be sensitive to osmotic stress and failed to accumulate glycerol on high-osmolarity media.

Fungi, like other microorganisms, are able to respond to changes in extracellular osmolarity, adjusting their intracellular composition to prevent the dehydration that could impair normal cell growth (4). Recently, different signal transduction pathways involving members of the mitogen-activated protein (MAP) kinase family have been shown to regulate different aspects of cell physiology (2, 7, 10, 18), one of them being responsible for adaptation to high osmolarity in *Saccharomyces cerevisiae* (5). The *HOG1* gene (5) (high-osmolarity glycerol response pathway) encodes a MAP kinase which plays an essential role in osmoadaptation. *Candida albicans* is a commensal dimorphic pathogenic yeast which under situations that diminish the host immune response is able to colonize the human body and seriously compromise human health (20). Interest in this organism as a model pathogen has increased in view of the relevance of the fungal infections that it causes, especially in industrialized countries (12). The identification of signal transduction pathways in pathogenic fungi is essential for understanding fungal mechanisms of adaptation to a complex and changing environment like the human body and also for identifying potential novel targets in antifungal therapy. In this work, we have addressed the existence of a *HOG* pathway in *C. albicans.*

A *C. albicans* gene library (19) was used to screen *S. cerevisiae* JBY10 (*MAT***a** *ura3 leu2 his3 trp1 lys2 ade2 hog1-*Δ*1*::*TRP1*) (obtained from M. Gustin) by electroporation. Of more than 200,000 transformant clones, 121 were initially selected for an osmoresistant phenotype on 1 M sorbitol–minimal SD medium (0.67% yeast nitrogen base without amino acids, 2% glucose) plates. Three of these positive transformants were further characterized. Sequencing of approximately 1.6 kb from the insert present in one of them, pHOG1c24.2 (Fig. 1), allowed us to identify an open reading frame which encoded a putative protein, CaHog1p, of 377 amino acids (42.3 kDa), with an overall 78% identity (86% similarity) to *S. cerevisiae* Hog1p, this similarity not being restricted to the 11 MAP kinase subdomains but also evident within the carboxy-terminal nonkinase domain. CaHog1p was 55.7% identical to *S. cerevisiae* Fus3p, 53.9% identical to *Homo sapiens* Erk1p, 52% identical to *C. albicans* Mkc1p, and 48.8% identical to *S. cerevisiae* Slt2p. A TGY motif, characteristic of hyperosmolarity-activated MAP kinases (6), is found in subdomain VIII in *C. albicans* Hog1p, similar to *Xenopus laevis* Mpk2 (21) and mammalian p38 (14) and CSBP kinases (17), which have been shown to complement *S. cerevisiae hog1*Δ mutants. *HOG1*$_{Ca}$ did not complement *S. cerevisiae slt2* or *fus3* mutants in either centromeric or episomal plasmids (data not shown).

The *C. albicans HOG1* gene was disrupted by a strategy already described (11). A 3-kb *Hin*dIII-*Hin*dIII fragment carrying the *C. albicans HOG1* gene disrupted by introduction of a 4-kb *hisG-URA3-hisG* cassette at the *Hpa*I site (Fig. 1) was used to replace the wild-type *HOG1* gene on strain RM1000 (*ura3*Δ::*imm434*/*ura3*Δ::*imm434 his1*Δ::*hisG*/*his1*Δ::*hisG*), a derivative of strain SC5314 (wild type) (13), yielding the heterozygous *hog1* mutant strain CNC11 (*ura3*Δ::*imm434*/*ura3* Δ::*imm434 his1*Δ::*hisG*/*his1*Δ::*hisG HOG1*/*hog1*::*hisG-URA3-hisG*) and the homozygous *hog1* null strain CNC13 (*ura3* Δ::*imm434*/*ura3*Δ::*imm434 his1*Δ::*hisG*/*his1*Δ::*hisG hog1*::*hisG-URA3-hisG*/*hog1*::*hisG*).

CNC13 mutant cells displayed a clear osmosensitive phenotype when several osmostressing reagents were used, either in minimal SD medium or rich YED medium (1% yeast extract, 2% glucose) plates supplemented with 1.0 M KCl, 1.5 M KCl, 0.9 M NaCl, 1.5 M NaCl, or 1 M sorbitol, with a less dramatic effect with 0.1 M $CaCl_2$ (Fig. 2). CNC13 cells were almost unable to grow on YED–2 M sorbitol plates. On minimal SD liquid medium, both the growth rate (generation time at 37°C is 2 h) and the final A_{600} reached after 24 h of growth under nonselective conditions (A_{600} of 3.7) were similar to those attained by CNC11 and SC5314 strains. However, under selective conditions (0.5 M NaCl), CNC13 was just able to double in mass, reaching a final A_{600} of 0.23 (compared with 2.3 for SC5314) (data not shown). Interestingly, the heterozygous *hog1* (CNC11) strain showed a partial osmosensitive phenotype, being able to resume growth and reach an A_{600} of 1.6 only after a lag period of 10 h. These results indicate that *C. albicans HOG1* is not an essential gene under normal (non-osmotic stress) conditions.

In order to analyze the kinetics of *C. albicans*-compatible intracellular solute formation, cultures from exponentially growing cells in YED medium at an A_{600} of 0.5 were divided into two halves, one receiving NaCl at a 0.5 M final concentration (from a 5 M NaCl stock) and the other receiving a similar volume of YED medium. At fixed intervals of time, a similar amount of cells was collected by low-speed centrifugation and the total intracellular glycerol content was determined

* Corresponding author. Mailing address: Departamento de Microbiología II, Facultad de Farmacia, Universidad Complutense de Madrid, Avda. Ramón y Cajal s/n, 28040 Madrid, Spain. Phone: 34 1 3941743. Fax: 34 1 3941745. Electronic mail address: cnombela@fresno.csic.es.

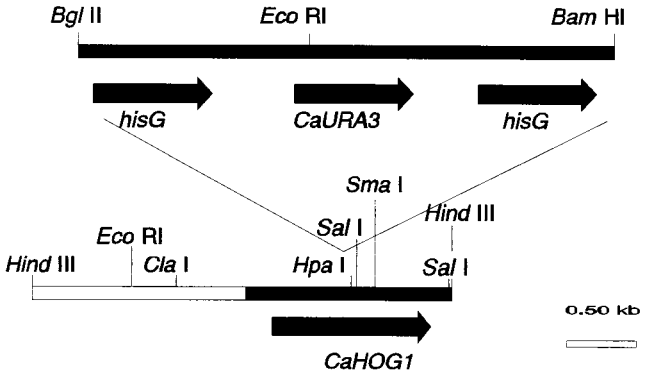

FIG. 1. Physical map of the 3-kb insert comprising the *C. albicans HOG1* locus (*CaHOG1*). Open and solid regions indicate, respectively, nonsequenced and sequenced regions of DNA.

according to the method of Blomberg and Adler (3) by using the Boehringer Mannheim glycerol detection kit. Wild-type cells gave an eightfold increase in glycerol content after 6 h (Fig. 3), while CNC13 cells only doubled it (1.9×). Higher concentrations of external NaCl increased the rate of glycerol accumulation but not the final total intracellular glycerol content (data not shown). We deduce from these results that glycerol is a major osmoprotectant in *C. albicans* and that its synthesis is under the control of the *HOG1* gene. The limited increase in glycerol content in homozygous *hog1* cells when

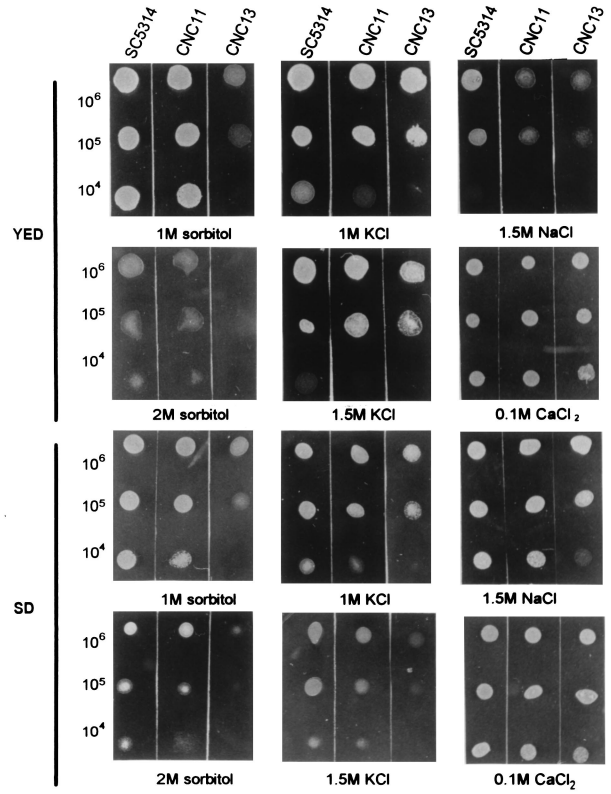

FIG. 2. Osmotic sensitivity of *C. albicans hog1* mutants. 10^6, 10^5, and 10^4 cells of strains SC5314, CNC11, and CNC13 were spotted on solid-agar plates of either rich (YED) or minimal (SD) medium supplemented with different osmostressing reagents at the concentrations indicated. Plates were then further incubated for 3 days at 37°C before microphotographs were taken.

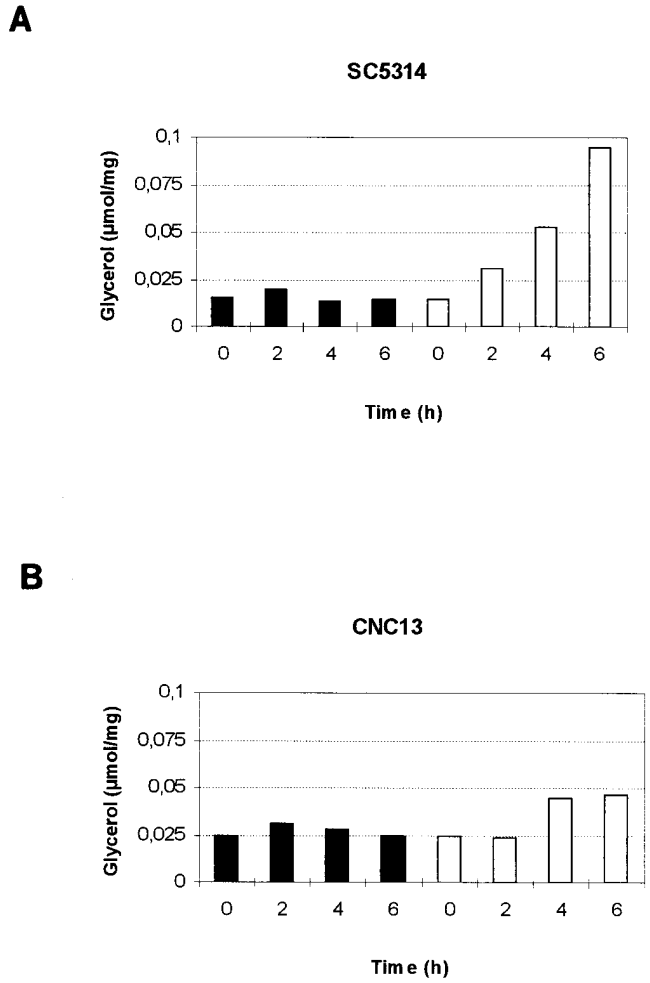

FIG. 3. Failure to accumulate glycerol in *C. albicans hog1* cells subjected to NaCl in YED medium. The intracellular glycerol content is plotted against time after the addition of 0.5 M NaCl (open bars) to exponentially growing cultures of the wild-type strain, SC5314 (A), or the *hog1* mutant strain, CNC13 (B). As a nonosmostressed control, one-half of the culture received just YED medium (solid bars).

osmostressed in NaCl-YED medium suggests the existence of *HOG1*-independent mechanisms in glycerol accumulation. In addition to *GPD1*—regulated by the *HOG* pathway (1)—a second gene involved in glycerol biosynthesis in *S. cerevisiae* has been described (9), and this gene is apparently not subjected to osmoregulation. It is feasible that in *C. albicans* a similar gene could exist, being at least partially inducible on high-osmolarity medium. On the other hand, we cannot rule out the possibility that other *HOG1*-dependent osmolytes different from glycerol, like trehalose or the polyol D-arabinitol, which has been described to be present in substantial amounts in *C. albicans* cells (16) but which has not been assigned a role, could mediate osmoprotection. In addition to having this role in osmoregulation, $HOG1_{Ca}$ could also be involved in a more generalized stress response similar to the situation in *Schizosaccharomyces pombe* (8, 15). This aspect and its putative involvement in pathogenicity are currently being analyzed in our laboratory.

Nucleotide sequence accession number. The sequence of *C. albicans HOG1* has been deposited in the EMBL data bank and assigned the accession number X90586.

We thank M. Gustin for providing us with *S. cerevisiae* JBY10 and F. Navarro-García for critical reading of the manuscript.

R. Alonso Monge is the recipient of a fellowship from the Comunidad Autónoma de Madrid. This study was supported by FIS grant SAF-96-1540.

REFERENCES

1. **Albertyn, J., S. Hohmann, J. M. Thevelein, and B. A. Prior.** 1994. *GPD1*, which encodes glycerol-3-phosphate dehydrogenase, is essential for growth under osmotic stress in *Saccharomyces cerevisiae*, and its expression is regulated by the high-osmolarity glycerol response pathway. Mol. Cell. Biol. **14:**4135–4144.
2. **Ammerer, G.** 1994. Sex, stress and integrity: the importance of MAP kinases in yeast. Curr. Opin. Genet. Dev. **4:**90–95.
3. **Blomberg, A., and L. Adler.** 1989. Roles of glycerol and glycerol-3-phosphate dehydrogenase (NAD^+) in acquired osmotolerance of *Saccharomyces cerevisiae*. J. Bacteriol. **171:**1087–1092.
4. **Blomberg, A., and L. Adler.** 1992. Physiology of osmotolerance in fungi. Adv. Microbiol. Physiol. **33:**145–212.
5. **Brewster, J. L., T. de Valoir, N. D. Dwyer, E. Winter, and M. C. Gustin.** 1993. An osmosensing signal transduction pathway in yeast. Science **259:**1760–1763.
6. **Cano, E., and L. C. Mahadevan.** 1995. Parallel signal processing among mammalian MAPKs. Trends Biochem. Sci. **20:**117–122.
7. **Cid, V. J., A. Duran, F. Delrey, M. P. Snyder, C. Nombela, and M. Sanchez.** 1995. Molecular basis of cell integrity and morphogenesis in *Saccharomyces cerevisiae*. Microbiol. Rev. **59:**345–386.
8. **Degols, G., K. Shiozaki, and P. Russell.** 1996. Activation and regulation of the Spc1 stress-activated protein kinase in *Schizosaccharomyces pombe*. Mol. Cell. Biol. **16:**2870–2877.
9. **Eriksson, P., L. Andre, R. Ansell, A. Blomberg, and L. Adler.** 1995. Cloning and characterization of *GPD2*, a second gene encoding sn-glycerol 3-phosphate dehydrogenase (NAD+) in *Saccharomyces cerevisiae*, and its comparison with *GPD1*. Mol. Microbiol. **17:**95–107.
10. **Errede, B., and D. E. Levin.** 1993. A conserved kinase cascade for MAP kinase activation in yeast. Curr. Opin. Cell Biol. **5:**254–260.
11. **Fonzi, W. A., and M. Y. Irwin.** 1993. Isogenic strain construction and gene mapping in *Candida albicans*. Genetics **134:**717–728.
12. **Fox, J. L.** 1993. Fungal infection rates are increasing. ASM News **10:**515–518.
13. **Gillum, A. M., E. Y. H. Tsay, and D. R. Kirsch.** 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.
14. **Han, J., J.-D. Lee, L. Bibbs, and R. J. Ulevitch.** 1994. A MAP kinase targeted by endotoxin and hyperosmolarity in mammalian cells. Science **265:**808–811.
15. **Kato, T., Jr., K. Okazaki, H. Murakami, S. Stettler, P. A. Fantes, and H. Okayama.** 1996. Stress signal, mediated by a Hog1-like MAP kinase, controls sexual development in fission yeast. FEBS Lett. **378:**207–212.
16. **Kiehn, T. E., E. M. Bernard, J. W. M. Gold, and D. Armstrong.** 1979. Candidiasis: detection by gas-liquid chromatography of D-arabitol, a fungal metabolite, in human serum. Science **206:**577–580.
17. **Kumar, S., M. M. McLaughlin, P. C. McDonnell, J. C. Lee, G. P. Livi, and P. R. Young.** 1995. Human mitogen-activated protein kinase CSBP1, but not CSBP2, complements a *hog1* deletion in yeast. J. Biol. Chem. **270:**29043–29046.
18. **Marshall, C. J.** 1994. MAP kinase kinase kinase, MAP kinase kinase and MAP kinase. Curr. Opin. Genet. Dev. **4:**82–89.
19. **Navarro-García, F., M. Sánchez, J. Pla, and C. Nombela.** 1995. Functional characterization of the *MKC1* gene of *Candida albicans*, which encodes a mitogen-activated protein kinase homolog related to cell integrity. Mol. Cell. Biol. **4:**2197–2206.
20. **Odds, F. C.** 1988. *Candida* and candidosis. Baillière Tindall, London.
21. **Rouse, J., P. Cohen, S. Trigon, M. Morange, A. Alonso-Llamazares, D. Zamanillo, T. Hunt, and A. Nebreda.** 1994. A novel kinase cascade triggered by stress and heat shock that stimulates MAPKAP kinase-2 and phosphorylation of the small heat shock proteins. Cell **78:**1027–1037.