A role for the MAP kinase gene MKC1 in cell wall construction and morphological transitions in Candida albicans

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The Candida albicans MKC1 gene encodes a mitogen-activated protein (MAP) kinase, which has been cloned by complementation of the lytic phenotype associated with Saccharomyces cerevisiae slt2 (mpk1) mutants. In this work, the physiological role of this MAP kinase in the pathogenic fungus C. albicans was characterized and a role for MKC1 in the biogenesis of the cell wall suggested based on the following criteria. First, C. albicans mkc1Δ/mkc1Δ strains displayed alterations in their cell surfaces under specific conditions as evidenced by scanning electron microscopy. Second, an increase in specific cell wall epitopes (O-glycosylated mannoprotein) was shown by confocal microscopy in mkc7Δ/hmkc7Δ mutants. Third, the sensitivity to antifungals which inhibit (1,3)-β-glucan and chitin synthesis was increased in these mutants. In addition, evidence for a role for the MKC1 gene in morphological transitions in C. albicans is presented based on the impairment of pseudohyphal formation of mkc1Δ/mkc1Δ strains on Spider medium and on the effect of its overexpression on Sacch. cerevisiae colony morphology on SLADH medium. Using the two-hybrid system, it was also demonstrated that MKC1 is able to interact specifically with Sacch. cerevisiae Mkk1p and Mkk2p, the MAP-kinase kinases of the PKC1-mediated route of Sacch. cerevisiae, and to activate transcription in Sacch. cerevisiae when bound to a DNA-binding element. These results suggest a role for this MAP kinase in the construction of the cell wall of C. albicans and indicate its potential relevance for the development of novel antifungals.

Keywords: MAP kinase, pseudohyphal growth, antifungal, two-hybrid system, confocal microscopy

INTRODUCTION

In recent years, different signal transduction cascades have been identified in Saccharomyces cerevisiae and found to be based on a common mitogen-activated protein (MAP) kinase module (Herskowitz, 1995). They comprise several pathways which mediate the response to pheromones (Bardwell et al., 1994), diploid and haploid pseudohyphal growth (Roberts & Fink, 1994; Liu et al., 1993), adaptation to high osmolarity media (Brewster et al., 1993), spore cell wall formation (Krisak et al., 1994) and cell integrity (PKC1-mediated pathway) (Levin & Errede, 1995; Errede & Levin, 1993). The cell integrity pathway has attracted much attention in view of its potential implications in fungal physiology and cell wall construction. Different members have been implicated, comprising at least the product of the genes PKC1 (Levin et al., 1990), BCK1 (SLK1) (Costigan et al., 1992; Lee & Levin, 1992), Mkk1p/Mkk2p (Irie et al., 1993) and SLT2 (MPK1) (Lee et al., 1993b; Torres et al., 1991), which transduce by phosphorylation the initial signal. However, and contrary to other routes, this initial triggering event is as yet unknown. SLT2, the MAP kinase of this pathway, was isolated by its ability to complement a lyt2 mutant (Torres et al., 1991; Cabib & Durán, 1975) and shown to encode a member of the MAP kinase family which is activated in response to stimuli such as high temperature and hypo-osmolarity (Davenport et al., 1995; Kamada et al., 1995). Although genetic studies have demonstrated that this gene is not
essential under normal conditions in *Sacch. cerevisiae* (Lee et al., 1993b; Torres et al., 1991), its activity is essential for growth at high temperatures (Martin et al., 1993) unless an osmotic support is provided in the medium, a result which suggests a link with the cell wall machinery.

The study of transduction cascades in pathogenic fungi is especially important in view of their putative implications in pathogenicity as a mechanism of adaptation to a complex environment like the human body. *Candida albicans* is a dimorphic pathogenic yeast which forms part of the commensal flora of humans but which, under certain abnormal conditions (i.e. immune-suppression), is able to colonize different tissues and establish an infection which is frequently life threatening (Odds, 1994, 1988). In *C. albicans*, there is limited knowledge of the role of signal transduction cascades and most of the genes identified to date are close homologues of the kinases found in the pheromone pathway of *Sacch. cerevisiae*. These genes, CST20 (STE20 homologue) (Köhler & Fink, 1996; Leberer et al., 1996), HST7 (STE7 homologue) (Köhler & Fink, 1996; Clark et al., 1995), CEK1 (KSS1 homologue) (Whiteway et al., 1992) and CPH1 (STE12 homologue) (Liu et al., 1994; Malathi et al., 1994) are able to complement certain (but not all) phenotypes of their respective *Sacch. cerevisiae* mutants. We have recently cloned the MKC1 gene (for MAP kinase of *C. albicans*) (Navarro-Garcia et al., 1995) and shown it to be essential for cell growth at high temperatures and thermal shock survival. In this work, we describe how deletion of MKC1 results in cell wall defects in *C. albicans*, a result with potential applications in the search for new antifungal therapies and, in addition, we present evidence for a role in this transduction cascade in some cellular morphogenetic responses.

**METHODS**

**Media, strains, growth conditions and transformation procedures.** Strains used in this work are described in Table 1. *Sacch. cerevisiae* and *C. albicans* strains were grown in YPD medium (2%, w/v, dextrose; 2%, w/v, peptone; 1%, w/v, yeast extract), Lee’s medium (Lee et al., 1975) or SD minimal medium (0-67%, w/v, yeast nitrogen base without amino acids; 2%, w/v, dextrose) with the appropriate auxotrophic requirements. The ability of cells to undergo the yeast-to-hypha transition was tested using Lee’s medium (Lee et al., 1975), SD plus foetal bovine serum (10%, v/v), foetal bovine serum or Spider medium (1%, w/v, mannitol; 1%, w/v, nutrient broth; 0-2%, w/v, K2HPO4; 1-35%, w/v, agar) (Liu et al., 1994). *Sacch. cerevisiae* pseudohyphal growth was tested in SLAHD medium as described by Gimeno et al. (1992). *Escherichia coli* strains were grown at 37°C in Luria-Bertani (LB) broth or terrific broth (TB) supplemented, when necessary, with ampicillin (100 µg ml⁻¹) for plasmid selection.

Other phenotypes were tested as follows. To test ion and drug sensitivities, exponential- (or stationary-) phase cells were tested on YPD plates supplemented with different concentrations of the required agent. Viability was estimated as the percentage of surviving colonies under these conditions compared to plates devoid of the indicated substance. Starvation sensitivity in solid media was assayed using a nitrogen starvation medium (2%, w/v, dextrose; 0-17%, w/v, yeast nitrogen base without amino acids and ammonium sulfate) (Toda et al., 1985): cells grown for 1 week on YPD plates were replica-plated to nitrogen starvation medium and, after 7 d, to YPD plates. Glycogen accumulation was assayed as described by Costigan et al. (1992): strains were grown at 30°C for 1 week on YPD plates and glycogen was visualized by inverting the plates over iodine crystals. Stationary-phase viability assays were performed using stationary-phase *C. albicans* cells grown in liquid YPD at 30°C: cells were then washed and diluted in fresh YPD or water and aliquots were taken and plated on YPD plates over 15 d to assess viability. Carbon source utilization (potassium acetate or glycerol) was checked by addition (2% final concentration) to YPD medium devoid of dextrose. Certain metabolic traits were assayed using the Yeast Identification System (Biomerieux) according to the manufacturer’s specifications.

*Sacch. cerevisiae* was transformed using a modification of the classical lithium acetate protocol (Chen et al., 1992). *C. albicans* protoplast transformation has been described previously (Navarro-Garcia et al., 1995; Herreros et al., 1992). *E. coli* transformation was performed using the procedure of Hanahan (1988) or electroporation (Navarro-Garcia et al., 1995).

**Plasmids and recombinant DNA methods.** YEp352 (a URA3 vector), YEp352H (SLT2 in YEp352) and pSN6 plasmid (MKC1 in YEp352) have been described elsewhere (Navarro-Garcia et al., 1995; Torres et al., 1991; Hill et al., 1986). pCENMKC1, a centromeric plasmid carrying the MKC1 gene and its promoter, was obtained by inserting an EcoRV fragment from pSN6 into the SmaI site of pYEUra3 (centromeric plasmid, Clontech). The MKC1 ORF was obtained by PCR amplification using the primers 5’ GGATCCGGCAATGTGATCAACAAGAAGCACCC 3’ (upper) and 5’ CCCGGGATAACGTGTTGTGTG3’ (lower) (annealing temperature 54°C) and subcloned into pT7Blue (Novagen) to create pT7MKC10. pPGKMKC1, an episomal plasmid carrying the PCR-amplified MKC1 gene under the control of the PGK1 promoter, was obtained by inserting a BamHI-BamHI fragment from pT7MKC10 into the BgIII site of YEpK. YEpK contains the PGK1 promoter as well as the transcriptional termination (3’) region from PGK1 inserted into the HindIII site of YEp352. The CEK1 ORF was PCR-amplified using primers 5’ GGATCCTCGGAATTCTGATG3’ and 5’ GGATCCGGCAATGTGATCAACAAGAAGCACCC 3’ (annealing temperature 49°C) and subcloned into pT7Blue (Novagen) to create pT7CEK10. A pT7CEK10 BamHI–BamHI fragment was inserted into the BgIII site of YEpK to obtain pPGKCEK10. pPGKSLT2 has been previously described as YEpK-WT (Martin et al., 1996).

pGAD424 and pGBT9 used for the ‘two-hybrid’ assay carry the Gal4p activation domain (amino acids 768-881) and the Gal4p binding domain (amino acids 1-147), respectively. pSE1111 bears SNF4 fused to the activation domain of Gal4p in pACT. pGADMKC11 and pGBTMKC17 were constructed by inserting a BamHI-BamHI fragment carrying the MKC1 gene from pT7MKC10 into the BamHI site of pGAD424 and pGBT9, respectively. The functionality of all these MKC constructions was assayed through complementation of the caffeine hypersensitivity of slt2 mutants on 9 mM caffeine plates (Costigan & Snyder, 1994; Costigan et al., 1992).

pGAD424 and pGBT9 plasmids, which comprise specific fragments of SLT2, MKK1 and MKK2 genes, have been
Table 1. Strains used in this work

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
<th>Source</th>
</tr>
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<tbody>
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<td>Sacch. cerevisiae</td>
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<td></td>
</tr>
<tr>
<td>YS26</td>
<td>MATa ade2-101 can¹ gal4-542 gal80-538 his3-200 leu2-3,112 lys2-801 trp1-901 ura3A52 URA3::GAL1-lacZ</td>
<td>Fields &amp; Song (1989)</td>
</tr>
<tr>
<td>YPH98</td>
<td>MATa ura3A52 lys2-801 ade2-101 leu2Δ1 trp1Δ1</td>
<td>Sikorski &amp; Hieter (1989)</td>
</tr>
<tr>
<td>YPNA98</td>
<td>MATa ura3A52 lys2-801 ade2-101 leu2Δ1 trp1Δ1 slt2::TRPI can¹</td>
<td>Navarro-Garcia et al. (1995)</td>
</tr>
<tr>
<td>L3c46c; DL454</td>
<td>MATa ura3A52 lys2-801 ade2-101 leu2Δ1 trp1Δ1 slt2::TRPI can¹</td>
<td>Torres et al. (1991)</td>
</tr>
<tr>
<td>CGX69</td>
<td>MATa/MATa ura3-52/ura3-52</td>
<td>Lee et al. (1993b)</td>
</tr>
<tr>
<td>Y782</td>
<td>MATa ura3-52 lys2-801 ade2-101 trp1Δ1 his3-Δ200 bck1Δ1::TRPI</td>
<td>Costigan et al. (1992)</td>
</tr>
<tr>
<td>C. albicans</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SC314</td>
<td>Wild-type</td>
<td>Gillum et al. (1984)</td>
</tr>
<tr>
<td>CAI4</td>
<td>Δura3::imm434/Δura3::imm434</td>
<td>Fonzi &amp; Irwin (1993)</td>
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<td>CAI-49</td>
<td>MKC1/mkc1Δ::hisG-URA3-hisG</td>
<td>Navarro-García et al. (1995)</td>
</tr>
<tr>
<td>CM-16</td>
<td>MKC1/mkc1Δ::hisG</td>
<td>Navarro-García et al. (1995)</td>
</tr>
<tr>
<td>CM-1613</td>
<td>mkc1Δ::hisG/mkc1Δ::hisG-URA3-hisG</td>
<td>Navarro-García et al. (1995)</td>
</tr>
<tr>
<td>CM-1613C</td>
<td>mkc1Δ::hisG/mkc1Δ::hisG</td>
<td>Navarro-García et al. (1995)</td>
</tr>
<tr>
<td>E. coli</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MC1061</td>
<td>araD139 Δ(ara-leu)7697 Δ(lac)X74 galU galK straA</td>
<td>Meissner et al. (1987)</td>
</tr>
</tbody>
</table>

described previously. MKK1ΔC and MKK2ΔC are engineered alleles of MKK1 and MKK2 whose encoded proteins lack their C-terminal fragment bearing the protein kinase domain (Soler et al., 1995).

Antifungal assays. MICs were determined using the microdilution method in 96-well plates using SD medium without uridine. Briefly, 1/2 dilutions of each substance were done from the first well through to number 10 in the same row. Well 11 was left as an uninoculated (negative) control and well 12 was used as positive control (without the tested substance). One thousand cells were inoculated in each well (except column 11, as indicated). Plates were incubated at 37 °C and read at 24 and 48 h using a 96-well reader iEMS MF (Labsystems). The MIC of amphotericin B was determined as the concentration causing 100% growth inhibition assessed at 630 nm; the MIC to the azoles was determined as the concentration causing 80% growth inhibition assessed at 405 nm. The MIC of other substances was determined as the concentration causing 100% growth inhibition assessed at 405 nm (National Committee for Clinical Laboratory Standards, 1992). MICs were determined three to five times and the same results were obtained on each occasion.

Scanning and transmission electron microscopy. Exponentially growing cells at 30 °C were washed, resuspended at 2 × 10⁶ cells ml⁻¹ in 50 ml fresh YPD, water or sorbitol and maintained with shaking for 6 h at 30 °C or 42 °C. Cells for scanning electron microscopy were prepared as previously described (Tiedt et al., 1987; Williams & Veldkamp, 1974) and visualized using a JEOL JSM-6400 microscope. Transmission electron microscopy samples were obtained as described by Miret et al. (1992) and embedded in EMbed 812 (Electron Microscopy Sciences). Sections (80 nm) were observed through a Zeiss 902 microscope.

Analysis of cell wall composition. The kinetics of cell wall biogenesis was quantified by measuring the incorporation of ¹⁴C-radio labelled material in the cell wall. Strains SC314 and CM-1613 were grown at 28 °C or 42 °C in Lee’s medium containing 0-5% (w/v) [¹⁴C]glucose [0-3 μCi ml⁻¹ (11-1 kBq ml⁻¹)]. Samples were taken at different times and treated with the same volume of 10% (v/v) trichloroacetic acid at 0 °C. Cells were filtered and washed with 5% (v/v) trichloroacetic acid prior to measurement of the incorporated radioactivity.

Cell wall composition was determined from these cells. C. albicans strains grown in [¹⁴C]-labelled media were washed and broken by vigorous vortexing with glass beads. The homogenate was resuspended with 1 mM PMSF, sedimented (2000 g for 10 min) and washed eight times with 1 mM PMSF to obtain cell walls. Isolated cell walls were stored at −20 °C until used. Cell wall carbohydrate composition analyses were carried out as previously described (Elorza et al., 1983).
**Lectin and immunofluorescence analyses.** For lectin binding assays, cells were washed with potassium phosphate buffer (pH 7.0), fixed with 2% formaldehyde in phosphate buffer for 30 min and incubated with concanavalin A–FITC (0.5 mg ml⁻¹) or wheatgerm agglutinin–FITC (1 mg ml⁻¹) (Sigma).

The mAb IB12 (which recognizes an O-glycosylated cell wall mannanprotein; Marcilla et al., 1993) was used throughout this work. For these studies, fixed cells were washed three times with PBS (0.137 M NaCl, 2.7 mM KCl, 4.3 mM NaH₂PO₄, 7.4 mM KH₂PO₄) and incubated for 1 h at 37 °C with 50 μl mAb IB12 at a dilution of 1:50. Samples were washed three times with PBS and incubated for 1 h at 37 °C with 50 μl FITC-conjugated goat anti-mouse antibody (Boehringer Mannheim) diluted 1:10 in PBS (Sundstrom & Kenny, 1984). After three additional washes with PBS, cells were examined by confocal microscopy.

**Confocal microscopy.** All samples (lectins and mAbs) were analysed under a ×100 oil immersion lens in an ACAS470 laser confocal microscope (Meridian Instruments) that uses a 5 W argon ion laser, operating at an excitation wavelength of 488 nm and with an emission filter of 515 nm. The confocal optical section thickness was 0.3 μm and the final power laser obtained was 0.1 mW. Data were acquired as image scans of a field of cells and were performed at least twice. Analysis of fluorescence images to obtain histograms of each population was done by computer software (Meridian Instruments).

**Flow cytometry analyses.** Analyses were carried out using a Becton-Dickinson FACStar PLUS flow cytometer equipped with an argon ion laser. The laser was tuned to 488 nm (300 mW) using propidium iodide (final concentration 0.005% (w/v) (Sigma) to measure cell death (de la Fuente et al., 1992).

**β-Galactosidase assays.** β-Galactosidase was quantified to estimate protein–protein interactions by the two-hybrid method. Preliminary analyses were achieved using the filter replica method (Breeden & Nasmyth, 1985). Quantitative analyses were carried out on cells grown overnight in SD medium lacking the appropriate amino acids. Cells were diluted in fresh SD and grown to exponential phase for 6–8 h to an OD₆₀₀ of 10. Cells were harvested and β-galactosidase was determined using ONPG as substrate (Soler et al., 1995). Analyses were done using at least three independent transformants in three different experiments.

**RESULTS**

The cell wall of *C. albicans* mkc1Δ/mkc1Δ mutants displays an abnormal cell surface under high temperature and hypo-osmotic conditions

To obtain evidence for a role for MKCl in the biogenesis of the cell wall, we first characterized the phenotype of mkc1Δ/mkc1Δ mutants under restrictive conditions. When mutant cells growing at 30 °C were shifted to 42 °C (strain CM1613C), significant morphological defects in the mkc1Δ/mkc1Δ mutant were seen: cells were elongated and remained attached, appearing as long chains which frequently formed big clumps full of dead cells, whilst other cells increased in size and became spherical (Fig. 1). This process started 4 h after the temperature shift but was more evident after 9 h. A similar (although less dramatic) phenotype was found for mutant mkc1Δ/mkc1Δ in a Ura⁺ background (strain CM1613) although no major differences were found in the cell wall of this mutant with respect to wild-type cells using scanning electron microscopy (data not shown). However, when these cells were subjected to a more drastic treatment at high temperature (42 °C) in hypo-osmotic medium, conditions which in *Sacch. cerevisiae* have been shown to activate the cell integrity pathway (Davenport et al., 1995), clear morphological differences were seen in mutant cells with respect to wild-type cells, the former displaying ruffles on their surfaces (Fig. 2). Such structures were detected even after 1 h and could reflect an underlying physical defect in the cell wall of the mutant. In addition, some mkc1Δ/mkc1Δ cells were larger in size than their wild-type counterparts in water at 42 °C. These effects were not observed when mkc1Δ/mkc1Δ cells were maintained in 1 M sorbitol or in wild-type strains under the same conditions (data not shown), which displayed a smooth
Fig. 2. Cell surface of \( m\kappa c1\Delta/m\kappa c1\Delta \) mutants at 42 °C in water. Wild-type (SC5314) cells (a) and \( m\kappa c1\Delta/m\kappa c1\Delta \) (CM1613) cells (b) were grown exponentially at 30 °C in YPD, washed and transferred to water at 42 °C with shaking. Samples were taken after 6 h and processed for scanning or transmission electron microscopy (see Methods). Bars, 1 μm.

Table 2. Antifungal sensitivity of \( C.\ albicans \) \( m\kappa c1/m\kappa c1 \) mutants to different compounds at 37 °C

The strains used were SC5314 (MKCl/MKCl) and CM-1613 (\( m\kappa c1A/m\kappa c1A \)). All MICs are given in μg ml\(^{-1}\), except for caffeine, where it is given in mM. MICs were determined three to five times and the same results were obtained on each occasion.

<table>
<thead>
<tr>
<th>MIC</th>
<th>MKCl/MKCl</th>
<th>m\kappa c1A/m\kappa c1A</th>
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<tbody>
<tr>
<td>Amphotericin B</td>
<td>1·95</td>
<td>1·95</td>
</tr>
<tr>
<td>Caffeine</td>
<td>25</td>
<td>12·5</td>
</tr>
<tr>
<td>Calcofluor white</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Canavanine</td>
<td>&gt;320</td>
<td>&gt;320</td>
</tr>
<tr>
<td>Cilofungin</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Echinocandin</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td>Fluconazole</td>
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<td>0·24</td>
</tr>
<tr>
<td>Fluorocytosine</td>
<td>0·03</td>
<td>0·03</td>
</tr>
<tr>
<td>Miconazole</td>
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<td>0·97</td>
</tr>
<tr>
<td>Nikkomycin Z</td>
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<td>0·39</td>
</tr>
<tr>
<td>Staurosporine</td>
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<td>10</td>
</tr>
<tr>
<td>Trichodermin</td>
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</table>

C. \( \textit{albicans} \) \( m\kappa c1\Delta/m\kappa c1\Delta \) mutants are more sensitive to antifungals that interfere with (1,3)-β-glucan and chitin synthesis

To characterize the role of MKCl in cell wall construction, we tested the sensitivity of \( C.\ albicans \) \( m\kappa c1\Delta/m\kappa c1\Delta \) cells to a wide array of different antifungal substances (Table 2). We assayed inhibitors of protein synthesis (trichodermin), membrane functionality (amphotericin B, fluconazole, miconazole), chitin synthesis (nikkomycin Z), nucleic acid synthesis (5-fluorocytosine), (1,3)-β-glucan biosynthesis (cilofungin, echinocandin), cell wall interfering agents (calcofluor white), as well as other toxic compounds (canavanine, caffeine). In addition to caffeine, only (1,3)-β-glucan and chitin biosynthesis inhibitors showed a different activity against \( m\kappa c1\Delta/m\kappa c1\Delta \) mutants. When assayed at 30 °C, \( m\kappa c1\Delta/m\kappa c1\Delta \) cells were highly sensitive to nikkomycin Z (MIC 1·56 μg ml\(^{-1}\)) in contrast to wild-type cells (MIC >800 μg ml\(^{-1}\)). As shown in Table 2, at 37 °C this difference was reduced to 6·25 μg ml\(^{-1}\) (wild-type) and 0·39 μg ml\(^{-1}\) (\( m\kappa c1\Delta/m\kappa c1\Delta \)). The differences in the MICs between the homozygous mutant and wild-type cells to cilofungin and echinocandin (twofold) was, however, maintained at all temperatures tested and no differences were observed for the MICs of the heterozygous mutant CAI-49 compared to wild-type strain SC5314 (data not shown). These results suggest that \( m\kappa c1\Delta/m\kappa c1\Delta \) mutants may have differences in the overall cell wall organization which render them more susceptible to some cell wall synthesis inhibitors.

Composition and structure of the cell wall in \( C.\ albicans \) \( m\kappa c1\Delta/m\kappa c1\Delta \) mutants

We performed a detailed analysis of cell wall composition in \( m\kappa c1\Delta/m\kappa c1\Delta \) mutants, analysing both the overall composition as well as the kinetics of incorporation of precursors using radiolabelled \( [\text{14C}]\)glucose. No major differences were observed in the kinetics of incorporation of precursor into the cell wall material among wild-type, heterozygous \( m\kappa c1\Delta \) and homozygous \( m\kappa c1\Delta \) cells, either at 28 °C or 42 °C (data not shown). In addition, the major polymer composition of isolated cell walls from wild-type (SC5314), MKCl/MKCl
Table 3. Polymer composition of wild-type, MKC1/mkc1Δ and mkc1Δ/mkc1Δ cell walls

The strains used were SC5314 (MKC1/MKC1), CAI-49 (MKC1/mkc1Δ) and CM-1613 (mkc1Δ/mkc1Δ). Data are percentages of total cell wall material. Results are means of three determinations from a single whole-cell wall preparation.

<table>
<thead>
<tr>
<th></th>
<th>28 °C</th>
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<th>42 °C</th>
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<tr>
<td></td>
<td>Mannan</td>
<td>Glucan</td>
<td>Chitin</td>
<td>Mannan</td>
<td>Glucan</td>
<td>Chitin</td>
</tr>
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<td>Wild-type</td>
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<td>9</td>
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<td>69</td>
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<tr>
<td>MKC1/mkc1Δ</td>
<td>32</td>
<td>58</td>
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<td>25</td>
<td>69</td>
<td>6</td>
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<tr>
<td>mkc1Δ/mkc1Δ</td>
<td>30</td>
<td>60</td>
<td>10</td>
<td>19</td>
<td>76</td>
<td>5</td>
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</table>

Fig. 3. Detection of 1B12 epitope in C. albicans cells using confocal microscopy. Strains used were SC5314 (wild-type), CAI-49 (MKC1/mkc1Δ) and CM-1613 (mkc1Δ/mkc1Δ). Experiments were done at 28 °C as indicated in Methods. Fluorescence is given in arbitrary units and is related to the relative cell size of each strain. Populations of cells were divided into different phases: phase 1, cells without buds; phase 2, cells with small buds; phase 3, cells with intermediate buds; phase 4, mother cells and buds having the same size. m, mother cell; d, daughter cell.

Since the overall composition of the mkc1Δ/mkc1Δ cell wall was scarcely altered, we looked for changes in the pattern of distribution of these polymers in the cell wall. Wild-type and mkc1Δ/mkc1Δ cells were examined using confocal microscopy with lectins and specific antibodies. The use of wheatgerm agglutinin-FITC (which detects chitin) and concanavalin A-FITC (which detects mannoproteins) did not demonstrate differences in the distribution of chitin and mannoproteins between wild-type and mutant cells at 28 °C or 42 °C (data not shown). However, the signal observed when using mAb 1B12 (which recognizes an O-glycosylated mannoprotein; Marcilla et al., 1993) was found to be consistently higher (almost twofold) in mkc1Δ/mkc1Δ cells with respect to wild-type cells at 28 °C (Fig. 3), as well as at 42 °C (data not shown) and independent of the cell size. We concluded that the cell wall of the mkc1Δ/mkc1Δ mutants is altered in such a way that allows a more efficient detection of this specific O-glycosylated mannoprotein epitope.

Sensitivity of C. albicans mkc1Δ/mkc1Δ mutants to different ions and drugs

This was assayed in YPD medium. mkc1Δ/mkc1Δ mutant cells did not grow at 0.05% (w/v) SDS, whilst exponentially growing wild-type cells (strain SC5314) could grow at this concentration and stationary-phase cells were able to grow even at 1% SDS. At these concentrations, wild-type cells appeared bigger and rounder than cells growing in medium without SDS, whilst mkc1Δ/mkc1Δ cells appeared as ghosts (data not shown). Differential sensitivity to SDS was used to isolate hypo-osmotic-sensitive Sacch. cerevisiae mutants, one of them, hpo2, resulting in a pkc1 mutation (Shimizu et al., 1994).

Recently, it was found that crv3 (slt2) mutants of Sacch. cerevisiae [as well as crv2 (bck1)] show a particular sensitivity to vanadate and Mg2+, Na+, Li+ or Mn2+ ions (Nakamura et al., 1996). None of these phenotypes were observed in C. albicans mkc1Δ/mkc1Δ mutants, in which the MICs of these compounds were similar to those in the wild-type strain (data not shown), and very different from those in slt2 mutants. The sensitivity of mkc1Δ mutants to cyclosporin A (or its derivative
FK506), which has been related in Sacch. cerevisiae to defects in the PKCl pathway (Garrett-Engele et al., 1995), could not be tested due to the intrinsic resistance of C. albicans to this compound. Sensitivity to oxidative stress when cells were subjected to 100 mM H2O2 was found to be similar for mck1Δ/mck1Δ cells and wild-type cells. In both mutant and wild-type strains, exponentially growing cells showed a higher susceptibility to H2O2 (mean survival time 4 min) than stationary-phase cells (30 min) (data not shown). These results suggest that that MKCl does not mediate an oxidative stress response.

**mck1Δ/mck1Δ cells are defective in acquired thermotolerance**

Previous work has shown how exponentially growing C. albicans mck1Δ/mck1Δ mutants are more sensitive than wild-type or MKCl/mkclA cells to thermal shock at 55°C (Navarro-García et al., 1995). In addition, recent data on Sacch. cerevisiae slt2Δ showed that these cells are no more sensitive than wild-type cells to killing by this treatment but are defective in the acquisition of thermotolerance induced by a pretreatment at 37°C (Kamada et al., 1995). When these experiments were carried out with wild-type, MKCl/mkclA or mck1Δ/mkclA strains (Fig. 4) mutant cells died after 50 min at 55°C whilst wild-type and MKCl/mkclA cells survived. The efficiency of thermotolerance acquisition was higher in C. albicans at 42°C than at 37°C, with mck1Δ/mck1Δ cells being able to survive a 50 min period (Fig. 4). Nevertheless, mck1Δ/mkclA cells died after 60 min while their isogenic strains harbouring at least one copy of MKCl were able to survive this period of time (data not shown). Thus, we concluded that mck1Δ/mck1Δ mutants are defective in acquired thermotolerance as are Sacch. cerevisiae slt2 mutants.

**Nutritional phenotypes of C. albicans mck1Δ/mck1Δ mutants**

Sacch. cerevisiae bck1Δ and slt2Δ mutants show some nutritional phenotypes similar to those reported for mutants of the RAS-PKA route in Sacch. cerevisiae (Costigan & Snyder, 1994; H. Martin, M. Molina & C. Nombela, unpublished data). mck1Δ/mck1Δ Ura+ mutants were not able to survive nitrogen starvation at 37°C, in a similar way to slt2 mutants, while MKCl or MKCl/mkclA did so. mck1Δ/mck1Δ Ura+ mutants, however, survived this starvation period even at 42°C, thus suggesting the effect was due to a poorer genetic background. Another phenotype related to the PKA pathway in Sacch. cerevisiae is the loss of viability in stationary phase and the inability to survive on nutrient-depleted medium at 30°C. Even after 15 d, C. albicans mck1Δ/mkclA cells, either Ura+ or Ura-, did not lose viability when maintained in pure water, whilst bck1Δ cells died after 4 d. Glycogen accumulation was not affected either in mck1Δ/mkclA mutants, in contrast to bck1Δ cells (Costigan & Snyder, 1994). Finally, no alteration in the carbon source assimilation pattern was found for the mck1Δ homozygous strain, with cells being able to grow on YP-glycerol (in contrast to slt2Δ or bck1Δ strains) or YP-potassium acetate plates at different temperatures. The Yeast Identification System (Biomerieux), an auxanogram, showed no differences for parental or mutant strains at the different temperatures tested. Thus, we concluded that deletion of MKCl in C. albicans does not produce the nutritional phenotypes reported for Sacch. cerevisiae slt2 or bck1 mutants.

**Role of MKCl in the dimorphic transition of C. albicans on Spider medium**

Recent data suggest the involvement in morphological transitions of some components of a MAP kinase pathway, such as CPH1 and CPH2 (Liu et al., 1994), HST7 (Clark et al., 1995) and CST20 (Köhler & Fink, 1996; Leberer et al., 1996). To test if MKCl could be involved in this important process in C. albicans, cells of wild-type, MKCl/mkclA and mck1Δ/mkclA strains were spread on Spider plates. As shown in Fig. 5(a), mck1Δ/mkclA cells were unable to grow invasively into the agar to the same extent as MKCl/mKCl1 or MKCl/mkclA cells. Nevertheless, mck1Δ homozygous mutants produced true hyphae in other inducing media such as foetal bovine serum or Lee’s medium. Calciofluor-white staining revealed that these hyphae were apparently normal with respect to chitin localization (in the cell wall and bud scars) while chromomycin staining failed to show any defect in nuclei positioning (data not shown). Hyphae were, however, shorter than in wild-type cells, as has also been observed in vivo in experimental infections in mice (Diez-Orejas et al., 1997). We concluded that MKCl does play a role in...
**Fig. 5.** Effect of MKC1 on morphological transitions. (a) Colony phenotype of *C. albicans* mkc1Δ/mkc1Δ mutants on Spider medium. Stationary-phase cells from wild-type (SC5314) and *mkc1Δ/mkc1Δ* (CM-1613) strains were collected, washed, plated on Spider medium and incubated for 5 d at 37 °C as described by Liu et al. (1994). Several colonies (upper row) and magnifications of the colony borders (lower row) were photographed. (b) Colony phenotype of different transformants of *Sacch. cerevisiae* CGX69 on SLAHD medium. Different CGX69 transformants were plated on SLAHD medium and grown for 2 d at 30 °C. Transformants bear the plasmids: YEp352 (2μ), pCENMKC1 (MKC1 in centromeric vector pYEura3), pPGKMK1, pPGKSL2, pPGKCEK1 (MKC1, SLT2 or CEK1 under the control of the PGK1 promoter in YEpk, see Methods), pSN6 (2μ MKC1) and YEpk352H (2μ SLT2). Colony pictures were taken as previously indicated by Gimeno et al. (1992).
morphological transitions of \textit{C. albicans} on certain specific media. Furthermore, overexpression of \textit{MKC1} in \textit{Sacch. cerevisiae} diploid strain CGX69 (E1278b genetic background) promoted pseudohyphal formation on SLAHDM medium (Fig. 5b). Microscopical analyses of the colony border showed enlarged cells budding in a polar way (data not shown). Similar results were observed for \textit{Sacch. cerevisiae SLT2} (Whiteway \textit{et al.}, 1992), and to a lesser extent for \textit{C. albicans CEK1} (Fig. 5b). In contrast, cells transformed with the vector alone did not develop pseudohyphae after 2 d of growth.

**MKC1 is able to act specifically in the PKC1-mediated pathway of \textit{Sacch. cerevisiae}**

The data presented above indicate how deletion of the MKC1 gene results in novel phenotypes in \textit{C. albicans} which have not been described in homologous \textit{Sacch. cerevisiae} mutants. For this reason, we assessed the specificity of MKC1 within the PKC1 pathway to discard a non-specific general suppressor effect on MAP kinase defects. First, MKC1 was found to be able to complement the caffeine or thermosensitive phenotype of \textit{Sacch. cerevisiae slt2} mutants (YPNA98, DL454 or L3c46c) (Fig. 6 and data not shown) in centromeric plasmids under the control of its own \textit{C. albicans} promoter (pCENMKC10). Second, MKC1 complemented the caffeine sensitivity (6 mM) of another mutant in the pathway, bck1Δ, but only when overexpressed, whilst other \textit{C. albicans} MAP kinases like Cek1p could not complement it (Fig. 6), a result consistent with the epistatic relations of these kinases within the pathway. Third, MKC1 was not able to complement the inability to grow in high osmolarity media of \textit{Sacch. cerevisiae hog1Δ} or the mating defect of fus3Δ mutants, even when overexpressed (data not shown). These results indicate that the \textit{C. albicans} MKC1 gene is able to act specifically in the cell integrity-cell growth pathway in \textit{Sacch. cerevisiae}.

**Mkc1p interacts with Mkk1p and Mkk2p in \textit{Sacch. cerevisiae}**

To define more precisely the role of \textit{C. albicans} MKC1 gene within the PKC1 pathway of \textit{Sacch. cerevisiae}, we checked its interaction \textit{in vivo} with other elements of this pathway in \textit{Sacch. cerevisiae} using the ‘two-hybrid’ assay (Fields \& Song, 1989). When Mkc1p was fused to the Gal4p binding domain (plasmid pGKT9), we were able to detect β-galactosidase activity, showing that, as well as Slt2p (Soler \textit{et al.}, 1995), Mkc1p was able to activate the transcription on the lacZ gene reporter even more strongly than \textit{Sacch. cerevisiae} Slt2 (137 versus 50 β-galactosidase units) (data not shown). This transcriptional activity was explained in Slt2p by the presence of a glutamine-rich region and a polyglutamine tract (Soler \textit{et al.}, 1995). The \textit{C. albicans} Mkc1p final tract, which is also enriched in glutamine residues, is, however, increased in favour of glutamic acid residues. The pl of this region in Slt2p (amino acids 368–391) is 6.89 with a net charge at pH 7 of -0.01, whereas the pl of the equivalent fragment in Mkc1p (amino acids 391–414) is 5.1 with a net charge at pH 7 of -1.91. This higher negative charge could explain, at least in part, the increased transcriptional activity of Mkc1p in \textit{Sacch. cerevisiae} when bound to a DNA-binding element. Another \textit{C. albicans} protein, ACPR (CPH1), shows a C-terminal fragment bearing two stretches of glutamine residues which are related to transcriptional activation after binding to pheromone response elements (PREs) of the \textit{Sacch. cerevisiae} mating-activated genes through its N-terminal fragment (Malathi \textit{et al.}, 1994).

Fusions of Mkc1p to the Gal4p activation domain (plasmid pGAD424) either alone or in combination with the empty vector carrying the binding domain of Gal4p, failed to produce significant β-galactosidase activity. This plasmid was used to measure the interaction between Mkc1p and Mkk1p (Mkc1p–Mkk1p) and
Table 4. Two-hybrid interaction between Mkc1p and Mkk1p/Mkk2p

Table 4 shows the results of two-hybrid interactions between different Gal4p activating fusions and different Gal4p binding fusions. Data are means of at least three independent transformants in three different experiments and the standard deviation was less than 30% in each case.

<table>
<thead>
<tr>
<th>Gal4b fusions</th>
<th>Sltp</th>
<th>Mkc1p</th>
<th>Snf4p</th>
<th>None*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mkk1p</td>
<td>8.8</td>
<td>0.26</td>
<td>–</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Mkk2p</td>
<td>3.3</td>
<td>&lt;0.05</td>
<td>–</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Mkk1ACp†</td>
<td>8.2</td>
<td>&lt;0.05</td>
<td>–</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Mkk2ACp†</td>
<td>122</td>
<td>6.2</td>
<td>3.6</td>
<td>1.9</td>
</tr>
<tr>
<td>None</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>–</td>
</tr>
</tbody>
</table>

* ‘None’ indicates the presence of the empty fusion vector.
† Mkk1ACp and Mkk2ACp are the N-terminal portions of Mkk1p and Mkk2p, respectively, lacking the protein kinase moiety (Soler et al., 1995).

Mkk2p (Mkc1p–Mkk2p). The Mkc1p–Mkk1p interaction was found to be 35-fold weaker (0.26 versus 8.8 β-galactosidase units) than the Sltp–Mkk1p interaction in a quantitative assay (Table 4). Despite the fact that Sltp2 interacts more efficiently with Mkk1p than with Mkk2p (Soler et al., 1995), deletion of the kinase domain of Mkk2p (Mkk2AC) significantly increases this interaction (Soler et al., 1995). We therefore checked the behaviour of Mkc1p with this construction. The Mkc1p–Mkk2AC interaction was found to be 20-fold weaker than the Sltp2–Mkk2AC interaction (6.2 versus 122 β-galactosidase units) but, in any case, significantly greater than control constructions. However, we failed to find evidence for any interaction between Mkc1p and the wild-type Mkk2p (Table 4).

These results show that Mkc1p interacts with MAP kinase kinases of the Sacch. cerevisiae PKC1-mediated pathway, presumably through their N-terminal domains, as we have already shown for Mkk2p.

DISCUSSION

Role of MKC1 in morphological transitions

In this work we have characterized the phenotype of C. albicans mck1Δ/mck1Δ mutants to define the role of MKC1 in C. albicans. We show here how deletion of MKC1 impairs hyphal growth on Spider medium, similar to what has been found for other C. albicans kinases homologous to elements of the Sacch. cerevisiae mating pathway (Köhler & Fink, 1996; Leberer et al., 1996; Liu et al., 1994). In addition, Mkc1p promotes pseudohyphal growth in Sacch. cerevisiae when over-expressed, as well as the aforementioned C. albicans kinases (Köhler & Fink, 1996; Clark et al., 1995; Singh et al., 1994; Liu et al., 1994). Our results, then, suggest a relationship between the MKC1-mediated route with morphological transitions in pathogenic fungi. However, mck1Δ/mck1Δ mutants – as well as csl2Δ, hst7Δ and cph1 mutations – are still able to switch to mycelial growth on other inducing media such as serum or Lee’s medium, thus supporting the existence of additional pathways in C. albicans controlling hyphal formation. These routes could act in C. albicans either independently or through some crosstalk between them as has been found in Sacch. cerevisiae for the mating and HOG pathways (Hall et al., 1996) or the mating and the cell integrity pathways (Errede et al., 1995). Furthermore, diploid Sacch. cerevisiae cells deleted in specific protein phosphatases related to the PKC1 pathway (ppz1Δ ppz2Δ) lyse at 37°C with an elongated appearance which resembles pseudohyphae (Lee et al., 1993a). The role of the PKC1 pathway in polarized growth (Mazzoni et al., 1993) could explain in part our results. It is feasible, then, that pseudohyphal growth promoted by SLT2 or MKC1 overexpression could be explained assuming certain crosstalk between the putative cell integrity pathway and other, as yet undefined, additional elements of the pseudohyphal pathway in Sacch. cerevisiae, which may be indicative of a similar mechanism in C. albicans. These different routes could be important for a pathogen to better adapt to changing microenvironments in the human body and its ability to colonize or invade tissues.

A cell integrity pathway in C. albicans

Although homologues of Sacch. cerevisiae PKC1 and SLT2 have been cloned before, the existence of a cell integrity pathway in C. albicans has been inferred only from the phenotypes of their respective mutants (Paravicini et al., 1996; Navarro-García et al., 1995). As we show here, Mkc1p is able to substitute for the activity of Sltp2 in Sacch. cerevisiae and to interact with MAP kinase kinases (MAPKKs) in the cell integrity pathway of Sacch. cerevisiae. Thus, Mkc1p may be playing a similar role in C. albicans.

Assuming that this cell integrity pathway exists in C. albicans and regulates the construction of the cell wall, differences between phenotypes of Sacch. cerevisiae slt2 mutants and C. albicans mck1Δ/mck1Δ mutants could be explained by the existence of functional differences in the construction of the cell wall or the cellular integrity as in the case of Schizosaccharomyces pombe mutants in SLT2 homologues pmk1+ (pmk1Δ) (Zaitsevskaya-Carter & Cooper, 1997; Toda et al., 1996) or BCK1 homologue mkb1+ (Sengar et al., 1997). Although Schiz. pombe Δpmk1 (Δspm1) (SLT2 homologue) (Zaitsevskaya-Carter & Cooper, 1997; Toda et al., 1996) or mkb1Δ (BCK1 homologue) (Sengar et al., 1997) mutants are altered in cell wall integrity, some of the Δpmk1 defects are not similar to Sacch. cerevisiae slt2Δ but to C. albicans mck1Δ/mck1Δ mutants (e.g. growth in the presence of a high concentration of sodium but defective growth on media with high calcium concentrations). In Sacch. cerevisiae, ion homeostasis is related to the calcineurin-mediated pathway (Mendoza et al., 1994), which has been recently related to the cell integrity pathway...
pathway (Nakamura et al., 1996). Both pathways could be possibly interrelated in Sacch. cerevisiae through the phosphatases Ppz1p and Ppz2p (Posas et al., 1993, 1995; Lee et al., 1993a). However, neither the increased sensitivity to high concentrations of Na⁺, Mg²⁺ or Mn²⁺ (Nakamura et al., 1996), nor the augmented susceptibility to cyclosporin A or FKS06 (Nakamura et al., 1996; Garrett-Engele et al., 1995) that Sacch. cerevisiae slt2Δ mutants display are shared by the C. albicans mkc1Δ/mkc1Δ mutants. Thus, our data suggest that a different regulation or relationship between these pathways to control ion homeostasis could exist in C. albicans.

Another difference can be found in the heat shock response. Although slt2Δ and mkc1Δ/mkc1Δ mutants are defective in acquired thermotolerance in the same way, we showed that thermal stress affects C. albicans mkc1Δ/mkc1Δ earlier than wild-type cells (Navarro-García et al., 1995), in contrast to slt2Δ mutants (Kamada et al., 1995). As the expression of several genes regulated by heat shock has the same pattern in Sacch. cerevisiae slt2Δ mutants as in wild-type cells (Kamada et al., 1995), it was inferred that the establishment of a heat-shock response is unrelated to the PKC1 pathway in Sacch. cerevisiae. In contrast, our results could indicate that such a relationship could exist in C. albicans, with MKC1 promoting the acquisition of thermotolerance maybe through the activation of small heat-shock proteins in a similar way to Sacch. cerevisiae HOG1, which is able to induce HSPl2 expression under osmotic stress (Varela et al., 1995).

Role of MKC1 in cell wall construction

Our results demonstrate that MKC1 in C. albicans does play a role in the construction of the cell wall as inferred from other observations: first, mkc1Δ/mkc1Δ mutant cells show alterations in cellular morphology under restrictive conditions; second, they display an enhanced sensitivity to (1,3)-β-glucan and chitin biosynthesis inhibitors; third, they show an altered cellular shape under hypo-osmotic and high temperature stress; fourth, they are more sensitive to SDS (Shimizu et al., 1994); and fifth, they show a different deposition of some epitopes on the cell wall. Recent analyses carried out on mutants of Sacch. cerevisiae such as rho1 (Drškova et al., 1996; Qadota et al., 1996) or pck1 (Roemer et al., 1994; Shimizu et al., 1994) indicated that a direct relationship exists between these proteins and cell wall biogenesis, either as part of the (1,3)-β-glucan synthesis complex or as activators in alternative processes through other elements of the PKC1 cascade. rho1 mutants show a hypersensitivity to (1,3)-β-glucan synthesis-blocking antifungals in both Sacch. cerevisiae (Qadota et al., 1996) and Sch. pombe (Arellano et al., 1996) and Rho1p interacts with Pck1p (Kamada et al., 1996; Nonaka et al., 1995). pck1 mutants are viable when growing in osmotically supported media and the composition of their cell wall shows a 30% reduction in glucan content (Roemer et al., 1994; Shimizu et al., 1994). Although the mkc1Δ/mkc1Δ cell wall does not seem to have a significant reduction in glucan or chitin content, mutant cells need osmotic support at high temperature growth and, similarly, are more sensitive to (1,3)-β-glucan and chitin synthesis inhibitors. In fact, glucan and chitin have been shown to play compensatory roles in cell wall architecture, leading to particular sensitivity to chitin inhibitors (Popolo et al., 1997). In contrast, our data suggest that the cell wall construction in C. albicans could be somehow altered in mkc1Δ/mkc1Δ mutants not through a clearly unbalanced composition of polysaccharides but through another process not as yet unravelled. Deposition of components in the cell wall starts with several interconnected synthetic processes, which end with the extrusion of 'pure bricks' (glucan, mannan, chitin) through the cellular membrane. The development of linkages between the polysaccharides then defines the mature cell wall (Kapteyn et al., 1995; Sanjuan et al., 1995; Kollár et al., 1995; Elorza et al., 1989). Whilst in Sacch. cerevisiae part of the biosynthetic process could be controlled by an alternative branch of the PKC1 route (Roemer et al., 1994), the SLT2 route has been related to some type of transcriptional control of cell wall biosynthetic proteins (Madden et al., 1997; Iugul et al., 1996) or to some type of control of the actin cytoskeleton and the polarized secretion in cell growth (Zarzov et al., 1996; Mazzoni et al., 1993). Since we did not observe significant alterations in cell wall structure when transmission electron microscopy was used to examine mkc1Δ/mkc1Δ mutants (data not shown), the differential detection of the 1B12 epitope, an O-mannosylated protein, in the cell wall of C. albicans mkc1Δ/mkc1Δ mutants could be explained by an altered secretion, an altered deposition or an altered linkage process, which could be caused by some deregulated processes controlled by the MKC1 route in C. albicans. Since the amount of mannosprotein is not significantly augmented in mkc1Δ/mkc1Δ mutants, the simplest explanation could be that the epitope reached by antibody 1B12 is more accessible due to an altered cell wall. This increased 'porosity' of the cell wall could also explain why mkc1Δ/mkc1Δ mutants show an enhanced sensitivity to degradation by cell-wall-digesting enzymes (Navarro-García et al., 1995). The absence of significant modifications in the total amount of glucan, mannan or chitin in C. albicans mkc1Δ/mkc1Δ mutants, in contrast to Sacch. cerevisiae pck1 or slt2 mutants (Roemer et al., 1994; Shimizu et al., 1994; J. Ariño & R. Sentandreu, unpublished results), points to some alteration in the deposition or the maturation process during cell wall construction. Thus, the external shape of mkc1Δ/mkc1Δ mutant cells in hypotonic media at 42 °C could be the consequence of physical discontinuities between the different polymers that constitute the cell wall. This altered structure of the cell wall could explain the diminished virulence of this strain in a mouse model (Diez-Orejas et al., 1997) as a higher susceptibility to immunological defences, since invasive processes in epithelial cells seem not to be affected in mkc1Δ/mkc1Δ mutant cells (F. Navarro-García, F. García del Portillo, J. Pla, & C. Nombela, unpublished data). Susceptibility
to (1,3)-β-glucan and chitin biosynthesis inhibitors in mck1Δ/mck1Δ mutants could be explained as a double blocking process in the synthesis and secretion-maturation of cell wall components. It is clear that C. albicans mck1Δ/mck1Δ mutants have great potential in screens for the detection of new antifungal drugs affecting cellular integrity (Frost et al., 1995). Unraveling these cell integrity processes in pathogenic fungi must, therefore, be important in the development of new strategies—and novel drugs—to fight against fungal infections.

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glucosylated mannoproteins in cell walls of yeast forms of fungus kinase C. 


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