The highly immunogenic enolase and Hsp70p are adventitious *Candida albicans* cell wall proteins

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Screening cDNA libraries with polyclonal antibody preparations against *Candida albicans* yeast or mycelial cell walls resulted in isolation of several positive clones. Some of them encoded enolase; others encoded a protein of the 70 kDa heat-shock protein family (Hsp70p), etc. The presence of these cytosolic proteins in the cell wall of actively growing *C. albicans* was discovered by analytical (SDS-PAGE and Western blot) and cytological (indirect immunofluorescence) experiments. Supplementation of cell cultures with papulacandin B, an antibiotic that inhibits formation of the β -glucan skeleton, resulted in the release of enolase to the supernatant fluids; this release was prevented when 0.6 M KCl was present as an osmotic stabilizer. The cell wall of *C. albicans* incorporated exogenously added proteins (enolase and *Escherichia coli* and *C. albicans* cytosolic proteins). The presence in the *C. albicans* cell wall of enolase, Hsp70p, and probably other intracellular proteins that are highly immunogenic might help the fungal cells to evade the host defences, and consequently could represent a survival mechanism for *C. albicans* 'in vivo'.

Keywords: enolase, Hsp70p, cell wall, *Candida albicans*, antibodies

INTRODUCTION

Candida albicans is an opportunistic dimorphic pathogen that is emerging as a leading cause of deep-rooted infections mainly in immunocompromised patients. It is widely distributed in healthy individuals and initiation of infection is determined by the host conditions, its development depending on breakdown of the equilibrium between resistance and disease. Interaction between cell wall components and the immune system plays a fundamental role in controlling the infection and this is why the cell wall is considered to contain virulence factors (Elorza *et al.*, 1985, 1988; Nelson *et al.*, 1991; Calderone & Fukayama, 1992; Gomez *et al.*, 1993; Hazen & Glee, 1994).

The *C. albicans* cell wall is composed mainly of β -glucans and mannoproteins, chitin being a minor component (Shepherd *et al.*, 1985; Sentandreu *et al.*, 1994). Some wall proteins are loosely associated with the cell walls and are released therefrom by chaotropic agents (SDS, urea, etc.). The remaining mannoproteins are solubilized after digestion with β -glucanases and chitinases, implying that they are covalently bonded to

the β -glucan–chitin skeleton of the wall (Elorza *et al.*, 1985, 1988; Kapteyn *et al.*, 1995). Some wall manno-proteins are in addition linked to 1,6- β -glucose residues (Kapteyn *et al.*, 1994; Sanjuan *et al.*, 1995).

The role of the proteins ranges from a structural function to a receptor-like function that mediates interactions between the fungus and tissues. Among these proteins, receptors for complement factors (Calderone *et al.*, 1988; Gustafson *et al.*, 1991; Alaei *et al.*, 1993), as well as for fibrinogen (Bouali *et al.*, 1987; Martínez *et al.*, 1994), fibronectin (Kalon *et al.*, 1988; Klotz & Smith, 1991; Klotz *et al.*, 1994), laminin (Bouchara *et al.*, 1990; López-Ribot *et al.*, 1994) and other extracellular matrix components (Klotz & Maca, 1988; Klotz, 1990; López-Ribot & Chaffin, 1994), are included.

During our efforts to isolate genes encoding cell wall proteins, we have cloned different known cytosolic proteins such as enolase (Sentandreu *et al.*, 1995) and Hsp70p (Eroles *et al.*, 1995). Both proteins have been reported to be circulating antigens in blood and to elicit an immune response during infection (Franklyn *et al.*, 1990; Walsh *et al.*, 1991). To confirm the finding of the

genes encoding these proteins, the presence of these proteins on the cell surface was investigated and it was concluded that these and probably other cytosolic proteins can be bound to cell walls *in vitro*, as can happen *in vivo*. Their presence in the *C. albicans* cell wall seems to be a consequence of cell lysis and not a result of a secretory process.

METHODS

Strains and growth conditions. The strain of *C. albicans* used in this study was ATCC 26555. Yeast cell cultures were maintained by subculturing every 2–3 weeks on slopes of Sabouraud Dextrose Agar and propagated in minimal medium supplemented with amino acids (Lee *et al.*, 1975). Induction of germ tubes (mycelial cells) was carried out after cell starvation in sterile distilled water as previously described by Casanova *et al.* (1989). Cells were collected from the culture medium by spinning at 3000 g for 10 min at room temperature.

Escherichia coli Y1090 was used in the preparation of phage-adsorbed polyclonal antibodies and to obtain cytosolic proteins. A loopful of *E. coli* was inoculated in 5 ml LB medium (10 g bactopeptone, 5 g yeast extract and 10 g NaCl l⁻¹) supplemented with 0.2% (w/v) maltose and 10 mM magnesium sulphate, and grown overnight (see below).

Western blot techniques. Different subcellular fractions (cytosol, or SDS-, urea- or mercaptoethanol-solubilized cell wall material) and culture media from yeast cells and mycelial cells were obtained as follows. Cell walls were isolated by centrifugation and washing following cell breakage with glass beads as described by Elorza *et al.* (1988); non-covalently bound proteins were extracted with hot SDS or urea. In the first case, the cell walls were extracted with 2% (w/v) SDS in a boiling water-bath and the extracted material was recovered by spinning the suspension at 5000 g for 15 min. Urea treatment was carried out by suspending the isolated cell walls (20 mg) in 12 M urea and incubating at 30 °C overnight. After spinning at 5000 g for 10 min, the supernatant was dialysed against 8, 6, 4 and 2 M urea and distilled water for 42 h each at 4 °C. Treatment with β -mercaptoethanol was carried out by suspending isolated cell walls in 100 ml 1 M ammonium acetate (pH 6.3)/200 ml β -mercaptoethanol/9.5 ml distilled water and incubating at 30 °C overnight. The solubilized material was obtained by centrifugation at 5000 g for 10 min. Cytosolic fractions were prepared after separation of the cell walls by spinning the supernatant at 40000 g for 60 min. The pellet corresponded to the membrane preparation, whereas the supernatant corresponded to the cytosolic preparation. The proteins were fractionated basically according to Laemmli (1970) on SDS-10% polyacrylamide gels at a constant current of 25 mA. The prestained molecular mass standards phosphorylase B (106 kDa), bovine serum albumin (80 kDa), ovalbumin (49.5 kDa), carbonic anhydrase (35.5 kDa), soyabean trypsin inhibitor (27.5 kDa) and lysozyme (18.5 kDa) (Bio-Rad) were run in parallel. After blotting to a nitrocellulose membrane (Bio-Rad Transblot cell), the proteins were detected by the ECL system (Western Blotting Protocols; Amersham) essentially as described in the Bio-Rad Immunoblot (GAR-HRP) assay kit based on the procedure of Burnette (1981) and Towbin *et al.* (1979) using polyclonal antibodies adsorbed against phage 11L (enolase) or 2M (Hsp70p) at a final concentration of 1:500 in 10 mM Tris/HCl (pH 7.4)/3% (w/v) bovine serum albumin as a blocking agent/0.9% NaCl/0.05% Tween 20 (TBST). The blots were subsequently

washed three times with TBST and incubated with a 1:12000 dilution of the peroxidase-labelled goat anti-rabbit antibody (Bio-Rad). Antiserum binding was visualized by use of the ECL fluorescent labelling kit and the chemiluminescent exposure of X-ray film.

Preparation of polyclonal antibodies adsorbed against phage 11L (enolase) or 2M (Hsp70p). Specific antibody preparations against enolase and Hsp70p were affinity-purified from those of polyclonal antibodies by adsorbing against the corresponding positive recombinant plaques of clones 2M and 11L. *E. coli* was grown overnight in 5 ml LB, and 100 μ l of this suspension was infected with different amounts of the corresponding phage in 20 mM Tris/HCl (pH 7.4)/100 mM NaCl/10 mM magnesium sulphate and kept at 37 °C for 30 min. These suspensions were then mixed with 12 ml melted LB medium plus 1% (w/v) agar, kept at 42 °C for 30 min, and were then spread on Petri dishes (25 cm) with LB plus 2% agar and incubated for 3 h at 42 °C for the induction of the lytic phage cycle. The specific phage proteins were blotted onto nitrocellulose (Hybond-C) membranes (Amersham) by covering the plate with this membrane saturated in 10 mM IPTG to induce the expression of the β -galactosidase fusion protein. The blotted membrane was then used to immunoadsorb the polyclonal antibody preparations (5 ml of the original polyclonal preparation) for 30 min and after several washes in 20 ml TBST; the adsorbed antibodies were eluted from the membrane with 4 ml 100 mM glycine (pH 2.8). The eluted material was neutralized with drops of a 3 M Tris/HCl (pH 8.5) solution.

Incorporation of purified enolase by *C. albicans* cell walls. Yeast cell walls (20 mg wet wt) were extracted with 1 ml of boiling 2% SDS solution in glass-distilled water for 10 min at 100 °C, and following three washes with distilled water (3000 g, 10 min) were incubated with 10 mg *Saccharomyces cerevisiae* enolase (Sigma) in a final volume of 1 ml distilled water. After 30 min at 37 or 0 °C, the cell walls were washed again three times as described above, extracted with hot 2% SDS, analysed by SDS-PAGE and after blotting stained with specific adsorbed antibodies.

Treatment of *C. albicans* cells with antibiotics. Starved *C. albicans* cells (1 mg wet wt ml⁻¹) were resuspended in Lee medium (Lee *et al.*, 1975) and after 2 h at 28 or 37 °C supplemented with papulacandin B (2 mg ml⁻¹) or papulacandin B (2 mg ml⁻¹) in 0.6 M KCl and incubated again at either 28 or 37 °C. The spent media were collected by centrifugation at 3000 g for 10 min, dialysed against distilled water (three times) and after freeze-drying were analysed by SDS-PAGE and Western blotting with monospecific polyclonal antibodies.

Indirect immunofluorescence labelling. Cells or cell walls were washed three times with PBS (10 mM sodium phosphate, 150 mM sodium chloride, pH 7.4) and incubated at 37 °C for 1 h with the appropriate dilution in PBS of the specific phage-adsorbed polyclonal antibodies. After three washes with PBS, the cells were incubated with fluorescein-isothiocyanate-conjugated anti-rabbit IgG (Boehringer Mannheim) diluted 1:10 in PBS at 37 °C for 1 h. After three washes with PBS, a few drops of the suspension were placed on slides and examined with an ACAS 470 laser confocal microscope (Meridian Instruments), equipped with a 5 W argon-ion laser, operating at an excitation wavelength of 488 nm and with an emission filter of 510 nm, using a $\times 100$ oil immersion objective lens. The confocal optical section thickness was 0.5 mm, corresponding to the upper surface of the cells, and

the final power of the laser was 0.1 mW. All immunofluorescence localization experiments were performed at least three times and the micrographs depicted representative results. Image acquisition and fluorescence intensity measurements were coordinated by computer software (Meridian Instruments).

Incorporation of *E. coli* and *C. albicans* cytosolic proteins in the *C. albicans* cell wall. *E. coli* was grown in 50 ml LB medium supplemented with radiolabelled protein hydrolysate [$0.2 \mu\text{Ci}$ ($7.4 \times 10^3 \text{ Bq}$) ml^{-1} ; Moravsek Biochemicals] and incubated by shaking at 37°C for 60 min. The cells were then harvested by centrifugation at $10000 g$ for 20 min, washed three times with PBS and the suspension of *E. coli* in PBS (5 ml) was sonicated for 30 s (four times). During the intervals (2 min) the suspension was kept in an ice-bath. The suspension was spun down at $40000 g$ for 30 min and the cytosolic fraction was collected.

C. albicans cells ($0.5 \text{ mg dry wt ml}^{-1}$) were suspended in Lee medium (40 ml) containing radiolabelled protein hydrolysate [$0.2 \mu\text{Ci}$ (7.4 kBq) ml^{-1} ; Moravsek Biochemicals], incubated at 28°C for 3 h and following breakage in a Braun homogenizer (Melsungen) (Casanova *et al.*, 1989) the cytosol was prepared by spinning at $40000 g$ for 5 min.

The pellet of non-radioactive *C. albicans* cell walls (equivalent to 40 mg wet wt) was supplemented with the *E. coli* or *C. albicans* cytosolic fractions. After 30 min at 28°C , the walls were collected by centrifugation at $4000 g$ for 10 min, washed three times with PBS and the radioactivity was measured before and after extraction with hot 2% SDS solutions.

RESULTS

We have previously described the isolation of different cDNA clones by immunoscreening expression libraries with polyclonal antibodies raised against cell wall components (Sentandreu *et al.*, 1995). From 29 positives, 12 clones (11L and others) encoded the enzyme enolase (Sentandreu *et al.*, 1995) and three clones (2M plus two more) encoded a protein belonging to the 70 kDa heat-shock proteins (Eroles *et al.*, 1995). Other clones encoded novel proteins (results to be described elsewhere). These results were surprising as enolase and Hsp70p are located intracellularly (Sundstrom & Aliaga, 1992; Pelham, 1986; Boorstein *et al.*, 1994) and the lambda gt11 libraries had been screened with polyclonal antibody preparations against isolated cell walls.

Location of the proteins encoded by clones 11L and 2M

To find out the actual location of the gene products encoded, phage-adsorbed polyclonal antibodies were used (see Methods). These antibody preparations were employed to probe the presence of the corresponding proteins in cytoplasmic extracts of both yeast and mycelial cells and in the material released from isolated walls treated with either SDS, urea or β -mercaptoethanol, and the material solubilized by Zymolyase after SDS extraction. For the 11L clone (enolase), a protein band of 48 kDa was detected. This band was found in the cytosol, and in the SDS, mercaptoethanol and urea

Fig. 1. Immunoblots of 10% slab gels loaded with the 2% SDS (lane 1), mercaptoethanol (lane 2) and urea (lane 3) extracts of the yeast cell walls, with the Zymolyase digest (lane 4) of the yeast cell walls and the cytosol (lane 5) stained with polyclonal antibodies adsorbed against phage 11L (enolase) (a) or 2M (Hsp70p) (b) as described in Methods. Molecular masses of standard proteins are indicated on the left.

extracts of isolated cell walls (Fig. 1). The 11L antibody detected nothing in the material solubilized with Zymolyase. When the experiment was repeated and the blots were immunoreacted with the phage-adsorbed polyclonal antibody against 2M (Hsp70p), a band of 70 kDa was again detected in the cytosol and the SDS, urea and β -mercaptoethanol extracts. The electrophoretic mobility of both proteins located in the walls was the same as that of the proteins detected in the cytosol (Fig. 1). No protein was detected in the materials released by the Zymolyase (Fig. 1).

Effect of the antibiotic papulacandin B

To find out the effect of the inhibition of the β -glucans on the localization of the enolase and Hsp70p the following experiments were done. Yeast cells growing actively in Lee medium were supplemented with papulacandin B, an inhibitor of β -glucan formation (Baguley *et al.*, 1979; Font de Mora *et al.*, 1993), and after 4 h incubation at 28°C , the spent media were collected by centrifugation, dialysed and freeze-dried. The material collected was analysed by SDS-PAGE, blotted to nitrocellulose and probed with the anti-enolase phage-adsorbed polyclonal antibodies. The antigen was detected only in the cultures where *C. albicans* had been grown in the presence of papulacandin B (Fig. 2). In contrast, the antigen could not be detected in the spent media of the control cells. Interestingly enough, when the cells were grown in papulacandin B but in a hypertonic medium (0.6 M KCl), the enolase could not be detected in the cell supernatant (Fig. 2). These results suggest that the origin of enolase in the walls could be due to its previous release from dead cells.

Fig. 2. Immunoblot of a 10% slab gel loaded with concentrated Lee growth medium (Lee *et al.*, 1975) of yeast cells grown at 28 °C (lane 1) or at 37 °C (lane 4), following supplementation with papulacandin B at 28 °C (lane 2) or at 37 °C (lane 5), or with papulacandin B plus 0.6 M KCl at 28 °C (lane 3) or at 37 °C (lane 6). The molecular mass of enolase is indicated on the right.

Location of enolase and Hsp70p on the cell surface

The results reported above indicate that enolase and Hsp70p proteins are localized cytoplasmically but are also on the cell surface. To demonstrate that this was so, the presence of the two proteins on the surface of *C. albicans* was analysed with the help of a confocal laser microscope. Fig. 3 illustrates the fluorescent pseudo-colour images of the surface of yeast cells and mycelial cells, both of which showed relatively low but significant amounts of enolase and Hsp70p (Fig. 3). This experiment was carried out with living cells and antibody did not get into the cytoplasm under the conditions used. Distribution of the antigens was irregular, resembling mosaics, giving a variegated appearance to the walls. In the mycelial cells, the receptors of the phage-adsorbed antibodies, i.e. the antigens, seemed more abundant in apical and subapical areas. When the isolated walls were treated with hot 2% SDS solutions, neither the enolase nor Hsp70p could be detected on the wall surface (Fig. 3).

Incorporation of purified enolase into the isolated yeast wall

An additional experiment was carried out to understand why the enolase was located in *C. albicans* cell walls. Yeast cell walls were extracted with hot 2% SDS solutions, and after removal of detergent by washing, were supplemented with a solution of 10 µg commercial yeast enolase in 1.0 ml 1.0 mM PMSF in distilled water for 30 min, either at 0 or 30 °C. The walls were then washed several times with 1.0 mM PMSF to eliminate the residual enolase, extracted with hot 2% SDS solutions and blotted to nitrocellulose paper and probed with anti-11L antibodies. In the native walls, the antibodies detected a single band of 49 kDa corresponding to enolase. A similar band was produced by the walls incubated in the presence of commercial yeast

enolase independently of temperature (Fig. 4). *C. albicans* enolase migrates slightly faster than *S. cerevisiae* enolase (Sundstrom & Aliaga, 1994).

Incorporation of *E. coli* and *C. albicans* cytosolic proteins into the *C. albicans* cell wall

The experiments previously described suggest that enolase and Hsp70p present on the surface of *C. albicans* could have a cytoplasmic origin due to their release by cell death or by other unknown means. To obtain additional information, the following experiment was carried out. *E. coli* and *C. albicans* cells were grown independently in ¹⁴C-labelled protein hydrolysate and the radioactive cytosolic preparations (Methods) were added to a suspension of purified *C. albicans* cell walls that had grown in a 'cold' non-radioactive medium. Significant radioactivity was retained in all cases. Practically all proteins retained by the walls were released by hot 2% SDS solutions (Table 1).

DISCUSSION

The ability of *C. albicans* to change from a yeast to a mycelial morphology and to switch between different colonial morphologies is thought to give additional advantages to this pathogenic fungus in its ability to colonize different tissues and to evade the host defence mechanisms simultaneously. These phenomena are probably dependent on many parameters, one of them being the composition of the cell wall (Nelson *et al.*, 1991; Glee *et al.*, 1995; Bromuro *et al.*, 1994). The use of classical genetic approaches to understand the molecular mechanisms implicated in the regulation of the morphological changes in *C. albicans* is very difficult due to the lack of a sexual cycle. Consequently we tried to identify genes present in cDNA expression libraries of yeast cells and mycelia whose products are components of the cell wall. With this aim, we obtained polyclonal antibody preparations raised against yeast and mycelial cell walls. The screened cDNA libraries gave several positive clones, more than 50% of which, as detected with antibodies against yeast cell walls, encode enolase (a glycolytic enzyme). A heat-shock protein of 70 kDa (Hsp70p) (another cytosolic protein) and another glycolytic enzyme, phosphoglycerate mutase, among other proteins, were detected with antibodies against mycelial walls. Similar results have also been obtained by various authors using different antibody preparations (Sundstrom & Aliaga, 1992; La Valle *et al.*, 1995).

Cloning of genes encoding enolase, Hsp70p and other proteins (results to be described elsewhere) was surprising because these proteins are located in the cytoplasm (Sundstrom & Aliaga, 1992; Pelham, 1986). The presence of these proteins in the cell wall was ascertained by analytical (SDS-PAGE and Western blot) and cytological (indirect immunofluorescence) experiments. Although in the blotting experiments the origin of the proteins could be due to the inevitable immersion, at least briefly, of the isolated cell wall in the cytosol, the

Fig. 3. Indirect immunofluorescence of yeast cells (a, b), mycelial cells (c, d) and yeast cell walls treated with 2% SDS (e, f) stained with polyclonal antibodies adsorbed against phage 11L (enolase) (a, c, e) or 2M (Hsp70p) (b, d, f). The observations were carried out with a confocal laser microscope (Methods) and the relative value of the fluorescence emitted is shown to the right.

Fig. 4. Immunoblot of a 10% slab gel loaded with a 2% SDS extract of yeast cell walls (lane 1), with a second SDS extract (lane 2), with walls treated with 2% SDS after incubation with 10 mg enolase at 37 °C for 30 min (lane 3) or at 0 °C for 30 min (lane 4) and 2 mg enolase (lane 5) stained with polyclonal antibodies adsorbed against phage 11L (enolase). The molecular mass of enolase is indicated on the right.

microscopic experiments were carried out *in vivo* and the antibodies do not penetrate into the cytoplasm in the absence of cell permeabilization. Therefore it is possible that proteins that had been cloned were also present in the cell walls. Confirmation of this hypothesis was obtained by three complementary experiments: (i) supplementation of a cell culture with papulacandin B, an antibiotic that blocks formation of the β -glucan skeleton (Baguley *et al.*, 1979; Font de Mora *et al.*, 1993), resulted in the release of enolase to the supernatant fluids; (ii) this release could be avoided if the cells were grown in the presence of an osmotic stabilizer (0.6 M KCl); and (iii) *C. albicans* yeast cell walls could, in addition, incorporate exogenously added proteins (enolase and *E. coli* and *C. albicans* cytosolic proteins). These results suggest that enolase and Hsp70p, and probably other proteins in the *C. albicans* cell wall, have a cytoplasmic origin.

In agreement with this interpretation, Sundstrom & Aliaga (1994) have demonstrated that enolase is found in

Table 1. Incorporation of *C. albicans* and *E. coli* proteins by *C. albicans* isolated cell walls

^{14}C -labelled cytosolic proteins obtained from *C. albicans* and *E. coli* were added to a suspension of isolated non-radioactive SDS-extracted *C. albicans* cell walls. The cell walls were, after 30 min at 28 °C, washed three times with 50 ml 0.010 M phosphate buffer (pH 7.4) plus 0.9% NaCl and their radioactivity was measured before and after extraction with hot SDS solutions. The values are the mean of at least three separate experiments and the SD values were never higher than 10% of the mean.

Cytosolic proteins	Radioactivity				
	Added (c.p.m.)	Retained (c.p.m.)	Percentage retained	SDS-extracted (c.p.m.)	Percentage incorporated by isolated walls
<i>C. albicans</i>	173 346	162 150	93.54	155 580	95.94
<i>E. coli</i>	867 056	158 840	18.32	154 005	97.00

cell supernatants of *C. albicans* and suggested that this is due to either cell lysis or leakage. Even though they failed to detect enolase on the cell surface of formalin-killed hyphal cells, their interpretation of the extra-cellular presence of *C. albicans* is in agreement with our results. These authors also indicated that host defences may contribute to fungal death, and as a consequence, to the circulating enolase in haematogenously disseminated candidiasis. The high abundance of the enolase protein and its antigenic and physical properties lead to its selective detection in cell supernatants over other proteins that are also released following rupture or lysis during infection. Here, we present evidence of the cell surface location of enolase, not only by immunofluorescence of whole cells but also by Western blotting of different subcellular fractions.

Recently, Angiolella *et al.* (1996) reported that enolase could be covalently connected to the cell wall 1,3- β -glucan, as it is released following Zymolyase (a β -glucanase complex) digestion. These authors did not extract cell walls with detergents prior to Zymolyase treatment (to remove non-covalently bound proteins), and this may be the reason why they detected enolase in the cell wall digest. Boiling SDS, as well as other ionic detergents and chaotropic agents, releases different proteins from isolated walls of *S. cerevisiae* and *C. albicans* that are loosely associated with the wall. These proteins are also solubilized by Zymolyase digestion in the absence of such treatments. Contrary to enolase, these proteins show typical features of intrinsic cell wall proteins as their sequences display both signal peptide and glycosylation sites that are characteristic of cell wall and secreted proteins.

Both enolase and Hsp70 proteins have been observed to be major antigens in several infectious diseases (Bianco *et al.*, 1986). From our results, we cannot conclude that the presence of Hsp70p in the cell wall is due to secretion, because its sequence, as in the case of enolase, lacks both signal peptide and glycosylation sites (Eroles *et al.*, 1995; Angiolella *et al.*, 1996).

The results described in this paper point to the *C. albicans* cell wall being a 'sticky' structure and that enolase, Hsp70p and other cytoplasmic proteins could be found in it as a result of cell lysis or any other unknown mechanism. Elimination of these proteins from isolated walls by SDS solutions indicates that they are present in the wall and retained by non-covalent bonds.

C. albicans has been in contact with humans for thousands of years and an equilibrium has been reached; the human host controls the growth of the fungal cells under normal living conditions. When this equilibrium breaks down as a result of a stress situation (iatrogenic treatments, e.g. antibiotics; immunodepressing processes, e.g. cancer, HIV; or immunosuppression interventions, e.g. organ transplantation), the ability of the immune system is reduced and reacts mainly with the most immunogenic antigens. It has been reported that

C. albicans infection induces in the host the production of antibodies against enolase (Franklyn *et al.*, 1990; Postlethwait & Sundstrom, 1995; Sundstrom & Aliaga, 1994), a 90 kDa heat-shock protein (Matthews & Burnie, 1989), an antigen of 96 kDa and a heat-shock protein of 75 kDa (Constantino *et al.*, 1994). These antigens and other intracellular proteins could partially cover the cell surface.

Morphological and colonial switching have been considered important characteristics of *C. albicans* in the processes that mediate colonization. Furthermore, other unknown mechanisms surely play important roles, helping penetration into the host tissues and organs. Several parasites express different epitopes during the process of colonization of the host. *Plasmodium falciparum* escapes the immune defences of the host by producing variations in the intragenic repetitive sequences in the genes that encode surface antigens such as MSA1 (Frontali, 1994) and MSA2 (Felger *et al.*, 1994; Marshall *et al.*, 1994; Anders *et al.*, 1993; McColl *et al.*, 1994) and in an Hsp70 homologue found throughout the asexual blood cycle and in gametocytes (Bianco *et al.*, 1986). The results reported in this paper suggest that evading the host defences by incorporating highly immunogenic cytosolic proteins (enolase, Hsp70, etc.) into the cell walls could represent another virulence factor and a survival mechanism for the fungal cells.

In summary, we have found that the cytoplasm is the probable origin of enolase, Hsp70p and other proteins found in the cell walls of *C. albicans*, and we suggest that their presence in the cell wall *in vivo* may produce a 'smokescreen' that could subvert an effective host immune response to critical surface epitopes on fungal antigens.

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NOTE ADDED IN PROOF

A protein related to the Hsp70p family has been described to be present in the cell wall of *C. albicans* that is extracted with β -mercaptoethanol solutions (López-Ribot *et al.*, 1996).

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