A Candida albicans 37 kDa polypeptide with homology to the laminin receptor is a component of the translational machinery

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A cDNA encoding a 37 kDa protein was isolated from an expression library using antibodies raised against mycelial cell walls from Candida albicans. The 37 kDa protein has over 60% sequence identity with the 37 kDa laminin-binding protein (LBP) from humans and over 80% identity with the Yst proteins of Saccharomyces cerevisiae. The C. albicans protein was named CaYstl. It was found in membrane and ribosome fractions but surprisingly, was not found in cell walls. Unlike the human LBP, CaYstlp does not bind laminin. These data indicate that CaYstlp is not a cell-surface receptor for laminin as has been proposed for the human LBP. Instead, like the S. cerevisiae Yst proteins, it appears to be a ribosomal protein. This conclusion is supported by the finding that CaYSTl-cDNA complements the lethal phenotype linked to the disruption of both YST genes in S. cerevisiae.

Keywords: Candida albicans, Saccharomyces cerevisiae, yst mutations, laminin receptor, ribosomal protein

INTRODUCTION

Candida albicans is an opportunistic fungus which can cause either septicaemic or mucosal infections in humans (Odds, 1988). The organism is capable of reproducing by budding (yeast cells) or by producing germ tubes (mycelial cells) depending upon environmental factors; this morphological transition has been associated with pathogenicity (Calderone & Braun, 1991; Odds, 1988; Sentandreu et al., 1993). Because the cell wall of C. albicans maintains the characteristic shape for each growth form and is the site of the initial interactions between the organism and its environment, various studies have focused on the biosynthesis and function of this structure (Gozalbo et al., 1994; Sentandreu et al., 1993). The role that glycoproteinaceous components of the cell wall play in morphogenesis has also been studied (Gozalbo et al., 1994; Sentandreu et al., 1993). By immunoscreening of expression libraries from Saccharomyces cerevisiae, C. albicans or Yarrowia lipolytica, genes encoding cell-wall proteins of S. cerevisiae (Moukadiri et al., 1997) and Y. lipolytica (Ramón et al., 1996) have been cloned; however, genes of cytosolic proteins, rather than cell-wall proteins, have been cloned in the case of C. albicans (Eroles et al., 1995, 1997; Sentandreu et al., 1995).

Among the cell-surface proteins described from C. albicans, a 37 kDa laminin-binding protein (LBP) has been reported (López-Ribot et al., 1994, 1996). Additionally, hypothetical LBPs have been described in mammals, Arabidopsis thaliana, Urechis caupo and S. cerevisiae, although each was associated with polysome fractions of cells (Auth & Brawerman, 1992; Demianova et al., 1996; García-Hernández et al., 1994; Rosenthal & Worderman, 1995). In the present study, we report the isolation of a cDNA from C. albicans that encodes a protein closely related (over 80% similarity) to the ribosomal Yst proteins of S. cerevisiae (Demianova et al., 1996). This protein, designated CaYstl, exhibits over 60% identity with a mammalian LBP, although in our experimental conditions no specific binding to laminin was observed. CaYstlp was found in ribosomes, membranes and cytosol fractions of C. albicans and complemented yst mutations in S. cerevisiae.

METHODS

Strains, media, growth conditions and transformations. C. albicans ATCC 26353 (serotype A) was employed in this
study. The organism was propagated as a yeast or mycelium in Lee's medium (Lee et al., 1975), as described previously (Casanova et al., 1989; López-Ribot et al., 1991). S. cerevisiae 8-3D [MATa/MATa Trp1/Trp1 YST1/yst1A::URA3 YST2/ys2A::URA3 H3; his3/ade2 can1/can1 leu2/leu2 2% (w/v) glucose] was used in genetic complementation studies; media used for cultivating yeast were complete YPD medium (Sherman, 1991) and minimal medium [0.7% (w/v) yeast nitrogen base without amino acids, 2% (w/v) glucose] supplemented with the appropriate nutrients in amounts as specified by Sherman (1991). Transformed and untransformed S. cerevisiae 8-3D were sporulated in solid SPO media [10% (v/v)] yeast nitrogen base without amino acids, 2% (w/v) glucose] supplemented with the appropriate nutrients in amounts as specified by Sherman (1991). Transformed and untransformed S. cerevisiae 8-3D were sporulated in solid SPO media as a control. The organism was propagated as a yeast or mycelium in supplemented with the appropriate nutrients in amounts as specified by Sherman (1991). Transformed and untransformed S. cerevisiae 8-3D were sporulated in solid SPO media as a control.

DNA sequencing. DNA sequencing was performed on both strands by the dideoxy chain-termination method (Sanger et al., 1977). Sequencing reactions were carried out with the Auto Read Sequencing Kit (Pharmacia) and cycle sequencing with fmol Cycle Sequencing System (Promega), in both cases according to the specifications of the suppliers. Sequencing was carried out with an ALF DNA Sequencer (Pharmacia Biotech).

SDS-PAGE and Western blot analysis. Different subcellular fractions (cytosol, membranes and ribosomes) and SDS, β-mercaptoethanol or Zymolyase 20T extracts from C. albicans cell walls were obtained as previously described (Casanova et al., 1989, 1992; Elorza et al., 1988; Raue et al., 1991) and run on SDS-10% (w/v) polyacrylamide gels as described by Laemmli (1970). For Western blot analysis, proteins were electrophoretically transferred from SDS-PAGE gels onto nitrocellulose filters according to Towbin et al. (1979). Filters were probed with rabbit antibodies against the epitopes expressed by ßExCell-CaYST1 at a final concentration of 1:100, followed by goat anti-rabbit IgG conjugated to horseradish peroxidase (Bio-Rad). Antiserum binding was visualized using the ECL (enhanced chemiluminescence) fluorescent labelling kit (Western Blotting Protocols; Amer sham) following the manufacturer’s instructions.

Preparation of specific antibodies. To obtain specific antibodies against proteins encoded by CaYST1-cDNA, a purified ßExCell-CaYST1-cDNA clone was plated at a density sufficient to produce confluent plaques in 150 mm plates. After incubation of plates for 4 h at 42°C, they were overlaid with nitrocellulose membranes as previously described (Ramón et al., 1992) and run on SDS-10% (w/v) polyacrylamide gels as described by Laemmli (1970). For Western blot analysis, proteins were electrophoretically transferred from SDS-PAGE gels onto nitrocellulose filters according to Towbin et al. (1979). Filters were probed with rabbit antibodies against the epitopes expressed by ßExCell-CaYST1 at a final concentration of 1:100, followed by goat anti-rabbit IgG conjugated to horseradish peroxidase (Bio-Rad). Antiserum binding was visualized using the ECL (enhanced chemiluminescence) fluorescent labelling kit (Western Blotting Protocols; Amer sham) following the manufacturer’s instructions.

Southern hybridization analysis. Chromosomal DNA from C. albicans and S. cerevisiae X-2180-1A, hybridization conditions and detection of DIG-labelled nucleic acids were performed as described by Ramón et al. (1996).

RNA isolation and Northern hybridization. Total RNA isolation from C. albicans and S. cerevisiae, and Northern hybridization were carried out as previously described (Ramón et al., 1996), except that disodium disuccinate-3,2',5'-chlorotriacyclo[3.3.1.13,7](phenyl)phosphate (CSPD from Boehringer Mannheim) was used as a substrate. RNA concentra-
tions were determined by measuring absorbance (A260) in a Gene Quant II RNA/DNA calculator spectrophotometer (Pharmacia).

Plasmid construction and S. cerevisiae yst complementation. Plasmid pCME22 was made by cloning CaYST1-cDNA into EcoRI-digested pUC18. pCME23 was constructed by cloning a 1.0 kb EcoRI fragment (blunt-ended prior to ligation) from pCME22 into the HincII site of pUC19 and the orientation of inserts checked. YEpCaYST1-ACT and YEpCaYST1-GAL were constructed by cloning the XbaI-PstI fragment from pCME23 into the XbaI-PstI sites of YEpplac181ACT (RandezGil et al., 1995) and YEpplac112GAL (L. Moukadir, unpublished data) so that CaYST1 was under control of the ACT promoter and GAL promoter, respectively.

To examine the effect of CaYST1-cDNA on complementation of S. cerevisiae yst mutation, S. cerevisiae 8-3D was transformed with YEpCaYST1-ACT or YEpCaYST1-GAL. Transfomants prototrophic for leucine or tryptophan were sporulated and tetrads dissected by micromanipulation.
Spores were germinated on minimal medium supplemented with amino acids and analysed by examination for prototrophy for uracil and histidine.

RESULTS

CaYST1-cDNA cloning

With the aim of identifying wall proteins of *C. albicans* mycelial cells, rabbit polyclonal antibodies raised against mycelial cell walls were used for immunoscreening an oligo-dT-primed AExCell library obtained from *C. albicans* protoplasts regenerating as mycelium. Approximately 1.2 × 10^6 recombinant phage were PCR-amplified DNA inserts and DIG-labelled. All PCR-amplified DNA inserts cross-hybridized amongst themselves when analysed by Southern blot analysis. The clone with the largest cDNA fragment (approx. 1.0 kb) was chosen and subcloned in pUC18. The recombinant plasmid was named pCME22. The 1042 bp cDNA sequence (Fig. 1) contained a single ORF of 762 nucleotides beginning with an ATG at position 28-30, followed by an in-frame TGA stop codon at position 787-789. In the 3' region two possible polyadenylation consensus signals (AATAAA) were found at 95 and 209 bp downstream of the stop codon; a poly(A) region was also present at the 3' end. These findings established the orientation of the clone with respect to mRNA transcription and suggested that the entire coding sequence is contained within pCME22. The predicted amino acid sequence from this ORF was 253 residues long with a calculated molecular mass of 27.8 kDa and a theoretical isoelectric point of 4.89.

Further computer analysis revealed the following features (Fig. 1): the protein sequence contained two possible N-glycosylation sites at positions 69 and 106 and four putative serine/threonine phosphorylation sites, three of them for casein kinase II (positions 10, 40 and 151) and one for protein kinase C (position 114). The relatively high abundance and the distribution pattern of glutamic acid residues proved distinctive: 19 residues were evenly distributed throughout the 253 amino acid sequence; 12 residues were present in the last 50 amino acids at the carboxyl terminus of the molecule. Hydropathy analysis (Kyte & Doolittle, 1982) of the deduced amino acid sequence showed a hydrophilic protein containing some weak hydrophobic regions. One of these hydrophobic regions contained a potential transmembrane region (Rao & Argo, 1986) of 16 amino acids and a transmembrane region is boxed.

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![Fig. 1. Nucleotide and predicted amino acid sequences of a cDNA clone encoding CaYst1p. Numbering of bases starts at the first nucleotide and amino acids at the first methionine as potential start site of the ORF. Putative polyadenylation signals are underlined. The stop codon is marked with an asterisk and the putative N-glycosylation sites by a diamond. Squares indicate possible serine/threonine phosphorylation sites and circles putative myristoylation sites. Glutamic acid residues are in bold letters. A theoretical transmembrane region is boxed.](image-url)
Fig. 2. Alignments of CaYstlp amino acid sequence with Yst1 and Yst2 from S. cerevisiae (ScYst), P. carinii extracellular matrix protein laminin receptor (PcECMR) and human p40/37-LRP (laminin receptor protein). Identities with CaYstlp are denoted by dashes; blank spaces represent gaps introduced to maximize alignment. The boxed region corresponds to peptide G, responsible for laminin binding (over 80%) with the ribosomal Ystl and Yst2 proteins of S. cerevisiae (ScYst), human laminin receptor protein (PcECMR) and human p40/37-LRP (over 70%).

To gain an insight into the genomic organization of CaYST1, Southern blot hybridization was performed using chromosomal DNA of C. albicans, digested with EcoRI. The CaYST1-cDNA from DIG-labelled pCME22 hybridized with chromosomal DNA, confirming that this cDNA clone was derived from a nuclear gene. The fact that two signals were detected (Fig. 3) suggests the presence of two copies of CaYST1 since no EcoRI sites were found in the ORF. When chromosomal DNA from S. cerevisiae digested with EcoRI was analysed, two weak signals were also observed in Southern blot using DIG-labelled CaYST1 as a probe (Fig. 3), confirming the similarity between CaYST1 and ScYST genes.

To study the transcription of CaYST1, Northern blots of RNA from mycelial and yeast cells of C. albicans were performed using the DIG-labelled cDNA from pCME22 as a probe. Two transcripts of 1.2 and 1.1 kb from both yeast and mycelium were observed, although both transcripts were more abundant in mycelial forms of C. albicans (Fig. 4). These results are in agreement with those obtained by Southern analysis.

**Cellular distribution of CaYstlp in C. albicans**

To determine the cellular localization of CaYstlp, specific antibodies against epitopes expressed by βExCell-CaYST1 were obtained as described in Methods. Yeast and mycelial cells of C. albicans were broken and the resulting suspension fractionated into cytosol, a particulate membrane preparation and cell walls. The latter were extracted with SDS, β-mercaptoethanol and Zymolyase 20T. SDS-PAGE and Western blots using specific antibodies revealed the presence of a single band of approximately 37 kDa in the
A C. albicans cDNA complementing yst mutations

Fig. 4. Northern blot analysis of total C. albicans RNA isolated from cells growing as yeast (Y) or mycelium (M). Total RNA was electrophoresed through an agarose/formaldehyde gel and transferred to a nylon membrane. The blotted membrane was hybridized with a DIG-labelled cDNA probe from a pCME22 clone. The corresponding ethidium bromide-stained gel is shown on the right and positions of rRNAs (18S and 28S) are indicated by arrowheads. Arrows mark the two transcripts.

cytosol and particulate membrane fractions (Fig. 5a). However, no specific bands were detected in the cell-wall fractions released by the various treatments. In S. cerevisiae, Yst proteins have been shown to be ribosome-associated. To determine whether CaYst1p was also localized to ribosomes, a ribosomal fraction of C. albicans was obtained and analysed by SDS-PAGE, followed by immunoblot with specific antibodies. As shown in Fig. 5(b), CaYst1p is localized in membranes, ribosomes and, to a lesser extent, in the cytosol (postribosomal supernatant).

A 37 kDa cell-surface protein of C. albicans that binds laminin and is recognized by an antibody preparation against the carboxyl-terminal laminin-binding domain of human laminin receptor (pAb 4160) (Castronovo et al., 1991) was identified from P-mercaptoethanol-extractable material from whole cells (López-Ribot et al., 1991, 1994). Because of this observation we then performed assays, as described by López-Ribot et al. (1994), to test whether CaYst1p was able to bind laminin in vitro. Neither the C. albicans native protein nor the fusion protein expressed in E. coli bound laminin under our experimental conditions (data not shown).

CaYST1-cDNA complements yst mutations in S. cerevisiae

To determine the function of CaYst1p, we next investigated whether CaYST1-cDNA could complement the lethal double yst mutation in S. cerevisiae (Demianova et al., 1996). For these experiments, the diploid strain S. cerevisiae 8-3D, heterozygous for YST1 and YST2, was transformed with YEpcAYST1-ACT, as described in Methods. Transformants were sporulated, tetrads dissected and grown in minimal medium supplemented with the appropriate nutrients, except leucine to select only those spores that contained YEpcAYST1-ACT.
Representative results from the growth of individual spores are shown in Fig. 6(a). We assumed that the non-viable spores failed to harbour the plasmid YEpCaYST1-ACT. In tetrad 1, the four spores were analysed for uracil and histidine auxotrophies and the results are summarized in Fig. 6(b). Spore 1D was prototrophic for uracil and histidine, indicating that it was disrupted in YST1 and YST2 genes. This result showed that CaYST1 complemented the yst mutation in S. cerevisiae. Similar results were obtained when CaYST1 was overexpressed under an inducible promoter. The double yst mutant expressing CaYST1 under the GAL promoter could only grow on galactose, failing to grow on glucose (Fig. 7). This fact also demonstrated that CaYST1 complemented the yst mutation.

Because in S. cerevisiae Yst proteins are mainly localized in ribosomes, we next analysed the distribution of CaYst1p in this yeast. Membranes, ribosomes and cytosol were obtained from a double yst mutant transformed with YEPCaYST1-ACT and analysed by Western blot using CaYst1p-Abs. As in C. albicans, the p37 CaYst1p was localized mainly in membranes and ribosomes, and was not present in the cytosol (Fig. 8).

**DISCUSSION**

In the present study, we describe the isolation and characterization of a C. albicans cDNA encoding a protein of an apparent molecular mass of 37 kDa that...
exhibits a high homology (over 80% similarity) to the recently described ribosomal Yst proteins of S. cerevisiae (Demianova et al., 1996). This protein also shares homology with p40/37 LBPs from a number of eukaryotic organisms (Fig. 2). Previous studies have suggested the association of homologous p40/37 LBPs with different cellular structures including the cytoskeleton in Hydra vulgaris (Keppel & Schaller, 1991), ribosomes and also a soluble fraction in Arabidopsis thaliana and mammals (Auth & Brawerman, 1992; Garcia-Hernández et al., 1994). In fact, the Yst proteins are components of the 40S ribosomal subunit in S. cerevisiae (Demianova et al., 1996). Similarly, CaYst1p localizes in the ribosomes of C. albicans, although it was also detected in membranes and in lesser amounts in the cytosol (Fig. 5b). Furthermore, the CaYST1 gene complements a yst mutation in S. cerevisiae when a heterozygous mutant is employed (the homoygous double mutant is inviable), indicating that the encoded protein exerts similar ribosomal functions.

Consistent with the previous observation that genes encoding yeast ribosomal proteins contain two virtually identical genes (Demianova et al., 1996; Rosenthal & Wordeman, 1995; Woolford & Warner, 1991), two genes were detected by Southern analysis using the CaYST1-cDNA as a probe (Fig. 4). Two transcripts, differing by approximately 100–150 bp were also detected by Northern blot, both hybridizing with the CaYST1-cDNA probe, indicating small differences in sequence and length, as occurs in S. cerevisiae (Demianova et al., 1996). Whether these two mRNAs are encoded by two or more genes remains unknown, although Southern blots suggest the presence of two. Interestingly, from enriched ribosomal fractions, no increase in the amount of CaYst1p was observed when compared to a total membranous fraction, where an intense band was detected by CaYst1p-Abs specific antibodies. The protein remained associated with the membrane preparation when these fractions were treated with agents that disorganize ribosomes (data not shown). Such results could suggest a cellular membrane localization, which is in agreement with a potential transmembrane domain found in the primary structure of the predicted amino acid sequence (Fig. 1).

The CaYST1 gene was cloned as result of an immunoscreening using antibodies generated against cell-wall components. While CaYst1p was detected associated with ribosomes, we were unable to detect it at the cell surface using different agents which have been extensively employed to analyse the proteins present in the C. albicans cell wall (Elorza et al., 1988; Marcilla et al., 1991). If CaYst1p kDa is only a cytoplasmic protein, then it must be antigenically related to cell-wall proteins to explain its initial selection from the expression library with antibodies against cell-wall proteins. Interestingly, recent reports have described LBPs from the cell surface of C. albicans germ tubes showing three components of 68, 62 and 60 kDa (Bouchara et al., 1990), and, strikingly, a protein of 37 kDa from C. albicans blastoconidia, which also cross-reacted with antibodies generated against the human laminin receptor (López-Ribot et al., 1994); whether these components are exclusively expressed in response to morphological changes remains unclear. Altogether, these results correlate with those described in mammals where the controversy is still unsolved (Mecham, 1991).

In this study laminin binding in vitro was not detected. CaYst1p expressed either in phage or in E. coli failed to bind laminin in vitro, as also occurred with different C. albicans extracts, even in conditions demonstrated to prevent protein loss (Glee et al., 1996) (data not shown). We might postulate the existence of proteins such as CaYst1p which possess a laminin-binding domain (peptide G) (Castronovo et al., 1991), but which are functionally not related to adhesins. The possible existence of proteins with more than one function should not be dismissed. In this context, several ribosomal proteins have been shown to have a second function apart from a ribosomal one (Wool, 1996). Future analysis should uncover whether this is also the case for CaYst1p.

A significant observation was the localization of CaYst1p when expressed in S. cerevisiae; it was not detected in the cytosol (Fig. 8), in contrast to the situation in C. albicans, suggesting in turn a different processing mechanism, as has also been described in the case of a cell-wall protein from Y. lipolytica (Ramón et al., 1996, 1997).

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