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 CaYst1. It was found in membrane and ribosome fractions but surprisingly, was not found in cell walls. Unlike the human LBP, CaYst1p does not bind laminin. These data indicate that CaYst1p 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 CaYST1-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* (Éroles *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 CaYst1, exhibits over 60% identity with a mammalian LBP, although in our experimental conditions no specific binding to laminin was observed. CaYst1p 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 26555 (serotype A) was employed in this

Abbreviation: LBP, laminin-binding protein.

The EMBL accession number for the nucleotide sequence reported in this paper is Y11434.

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/MAT α ρ^+ *ade2/ade2 can1/can1 his3/his3 ura3/ura3 leu2/leu2 trp1/trp1 YST1/yst1 Δ 1(::URA3) YST2/yst2 Δ 1(::HIS3)*] 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 [1% (w/v) potassium acetate, 0.1% (w/v) yeast extract, 0.05% (w/v) glucose, 2% (w/v) agar] plus the appropriate nutrients in 25% (v/v) of the amounts used in synthetic complete media. *Escherichia coli* DH5 α [F $^-$ ϕ 80d *lacZ* Δ M15 *recA1 endA1 gyrA96 thi-1 hsdR17* ($r_k^- m_k^+$) *supE44 relA deoR* Δ (*lacZYA-argF*)U169] was used routinely for molecular cloning and plasmid amplification. *E. coli* NM522 [*supE thi* Δ (*lac-proAB*) Δ *hsdS* ($r^- m^-$) F' *proAB lacI* q Δ M15] was used as a host strain for the phage λ ExCell (Pharmacia) and amplified by standard procedures (Sambrook *et al.*, 1989). *E. coli* was transformed as described by Hanahan (1983). The transformation of intact yeast cells was performed as described by Ito *et al.* (1983); plasmids were rescued from yeast by transformation of *E. coli* with yeast DNA extracted according to Hoffman & Winston (1987).

cDNA library construction, screening and DNA manipulations. A *C. albicans* cDNA library consisting of 1.16×10^4 clones, with a mean insert of 1.5 kb, was generated from protoplasts regenerating at 37 °C (as mycelia) in phage expression vector λ ExCell. Protoplasts were obtained and regenerated as described by Elorza *et al.* (1983). For mRNA isolation and cDNA synthesis, the mRNA Purification Kit and cDNA Synthesis Kit, both from Pharmacia, were used, respectively, according to the manufacturer's instructions. cDNAs obtained were subsequently linked to *EcoRI*-*NotI* adapters (Pharmacia) prior to their insertion into *EcoRI*-digested λ ExCell. Rabbit antibodies raised against mycelial cell walls from *C. albicans* ATCC 26555 were used to screen the cDNA library, as described previously (Ramón *et al.*, 1996; Sentandreu *et al.*, 1995). Those phage demonstrating enrichment on subsequent screening were isolated and the cDNA inserts rescued by PCA, using universal forward and reverse pUC/M13 primers (Promega). The PCR products were digested with *EcoRI* and then cloned in the plasmid vector pUC18.

Standard DNA manipulation techniques were carried out as described by Sambrook *et al.* (1989). DNA probes for Southern and Northern blot analysis were labelled by random primed incorporation of digoxigenin-labelled deoxyuridine-triphosphate (DIG-labelled DNA) using the DIG DNA Labeling Kit (Boehringer Mannheim) according to 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 3-(4-methoxyphosphoryl-1,2-dioxetane-3,2'-(5'-chloro)tricyclo-[3.3.1.1 3,7] decan-4-yl)phenyl phosphate (CSPD from Boehringer Mannheim) was used as a substrate. RNA concen-

trations were determined by measuring absorbance (A_{260}) in a Gene Quant II RNA/DNA calculator spectrophotometer (Pharmacia).

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; Raué *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 λ ExCell-CaYST1 at a final concentration of 1:100, followed by goat anti-rabbit IgG conjugated to horseradish peroxidase (Bio-Rad). Antiserum binding was visualized by using the ECL (enhanced chemiluminescence) fluorescent labelling kit (Western Blotting Protocols; Amersham) following the manufacturer's instructions.

Preparation of specific antibodies. To obtain specific antibodies against proteins encoded by CaYST1-cDNA, a purified λ ExCell-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.*, 1996). The membranes were then removed and blocked with 3% BSA in TBS [20 mM Tris/HCl (pH 7.5), 150 mM NaCl] for 30 min at 37 °C without shaking and then 30 min at room temperature with gentle shaking. Following blocking, the membranes were incubated overnight at room temperature with rabbit antibodies against *C. albicans* cell walls pre-adsorbed on an *E. coli* Y1090 extract (1 mg ml $^{-1}$ final concentration). The membranes were then washed four times for 10 min at room temperature in TTBS [20 mM Tris/HCl (pH 7.5), 150 mM NaCl plus 0.05% Tween 20], once in TBS and stripped with 0.1 M glycine (pH 2.8) for 20 min at room temperature. The glycine solution was then equilibrated with 3 M Tris/HCl (pH 8.5), collected and stored at -20 °C. This solution contained specific antibodies against epitopes expressed by λ ExCell-CaYST1 (CaYst1p-Abs).

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. YE ϕ CaYST1-ACT and YE ϕ CaYST1-GAL were constructed by cloning the *XbaI*-*PstI* fragment from pCME23 into the *XbaI*-*PstI* sites of YE ϕ lac181ACT (Randez-Gil *et al.*, 1995) and YE ϕ lac112GAL (I. Moukadiri, 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 YE ϕ CaYST1-ACT or YE ϕ CaYST1-GAL. Transformants prototrophic for leucine or tryptophan were sporulated and tetrads dissected by micromanipulation.

1	ATA	GCG	GCC	GCG	CAT	ATA	ATA	GAG	AAT	ATG	TCA	TTA	CCA	GCT	TCA	TTT	GAC	TTA
10	T	P	D	A	K	L	L	L	A	A	N	V	H	L	G	A	K	
55	ACT	CCA	GAA	GAC	GCT	AAA	TTG	TTA	GCT	GCC	AAC	GTC	CAT	TTG	GGT	GCT	AAG	
28	N	V	Q	V	H	N	K	P	Y	V	Y	K	T	R	P	D	G	M
109	AAC	GTT	CAA	GTT	CAC	AAC	AAA	CCA	TAT	GTT	TAC	AAA	ACC	AGA	CCA	GAT	GGT	ATG
46	N	I	I	N	I	G	K	T	W	K	I	V	L	A	A	R	I	
163	AAC	ATC	ATC	AAC	ATT	GGT	AAA	ACT	TGG	GAA	AAA	ATT	GTT	TTG	GCT	GCC	AGA	ATT
64	I	A	A	V	P	N	A	S	D	V	A	V	C	S	S	R	T	F
217	ATT	GCT	GCT	GTT	CCA	AAC	GCT	TCT	GAT	GTT	GCT	GTT	TGT	TCT	TCA	AGA	ACT	TTC
82	G	Q	R	A	V	L	K	F	A	A	H	T	G	A	T	A	I	A
271	GGT	CAA	AGA	GCT	GTT	TTG	AAA	TTT	GCT	GCT	CAC	ACT	GGT	GCT	ACT	GCC	ATT	GCT
100	G	R	F	T	P	G	N	F	T	N	Y	I	T	R	S	F	K	
325	GGT	AGA	TTC	ACT	CCA	GGT	AAC	TTT	ACC	AAT	TAT	ATC	ACT	CGT	TCA	TTC	AAA	GAA
118	P	R	L	V	V	V	T	D	P	R	T	D	A	Q	A	I	K	
379	CCA	AGA	TTA	GTT	GTT	GTT	ACT	GAC	CCA	AGA	ACC	GAT	GCT	CAA	GCC	ATC	AAA	GAA
136	S	S	Y	V	N	I	P	V	I	A	L	T	D	M	Q	S	P	S
433	TCA	TCT	TAT	GTT	AAC	ATT	CCA	GTT	ATT	GCC	TTG	ACT	GAC	ATG	CAG	TCT	CCA	TCT
154	Y	V	D	V	A	I	P	C	N	N	K	G	K	H	S	I	G	
487	GAA	TAC	GTT	GAT	GTT	GCC	ATT	CCA	TGT	AAC	AAC	AAA	GGT	AAA	CAC	TCT	ATT	GGT
172	L	I	W	W	L	L	A	R	V	L	R	L	R	G	I	I	P	
541	TTA	ATC	TGG	TGG	TTG	CTT	GCT	AGA	GAA	GTC	TTG	AGA	TTA	AGA	GGT	ATT	ATC	CCA
190	D	R	T	T	W	S	V	M	P	D	L	Y	F	Y	R	D	P	
595	GAC	AGA	ACT	ACC	GAA	TGG	TCA	GTT	ATG	CCA	GAT	TTG	TAC	TTC	TAC	AGA	GAC	CCA
208	I	I	Q	N	A	V	A	K	T	G	V	G						
649	GAA	GAA	ATT	GAA	CAA	AAT	GCC	GTC	GAA	GAA	GCT	AAA	ACT	GAA	GGA	GTT	GAA	GGA
226	A	P	V	A	A	T	W	T	G	T	D	V	D					
703	GCT	CCA	GTT	GCT	GAA	GCT	GAA	ACC	GAA	TGG	ACT	GGT	GAA	ACT	GAA	GAT	GTT	GAT
244	W	A	D	S	G	A	T	P	S	C	*							
757	TGG	GCT	GAT	TCT	GGT	GCT	ACC	CCC	AGC	TGC	TGA	AGA	TGC	TGC	TGC	TTC	TAA	CTG
811	GTA	AAC	ACT	GAA	ATC	TAC	CAA	TAA	GAA	GTA	GAA	GTA	GAA	GTA	GAA	GAA	GAA	ACA
865	ATA	ACA	ACA	ATA	ACA	ACC	AAA	ATA	AAA	AAA	AGG	TTT	AAT	GAT	GTA	TAT	TAT	CGA
919	TAA	GGA	GAA	AGA	AGA	GAT	TTT	CTT	TTT	TAA	TAA	TGA	GGA	TGC	CAT	TTT	ATA	CAA
973	ATC	CAA	AAT	TGT	AAT	TAA	GAA	AGA	TTA	ATA	AAT	ATA	AAA	TAT	ATA	TAT	ATA	AGT
1027	AAA	AAA	AAA	AAA	AAA	A												

Fig. 1. Nucleotide and predicted amino acid sequences of a cDNA clone encoding CaYst1p. Numbering of bases starts 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 residues are in bold letters. A theoretical transmembrane region is boxed.

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 λ ExCell library obtained from *C. albicans* protoplasts regenerating as mycelium. Approximately 1.2×10^6 recombinant phage were immunoscreened and after three rounds we identified 35 potentially positive clones. cDNA inserts were amplified by PCR using pUC/M13 forward and reverse primers and DIG-labelled. All 35 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

CaYst1	MS LPASFDLTPEDAKLLAANVHLGAKNVQVHKPYVYKTRPDGMNIIN	49
ScYst1	--T-----Q-----T-----R-----QE---FNA---VHV--	49
ScYst2	--T-----Q-----T-----R-----QE---FNA---VHV--	49
PcECMR	-G ILDA QPAA-IEM-IS-C-I-SR-CELRME---F-R-A---VH---	47
p40/37LRP	--GALDVLQMK--VLKF---GT---GT-LDFQMEQ-I---RKS---IY---	50
CaYst1	IGKTWEKIVLAARIIAAVFNASDVAVCSSRTFGQ RAVLKFAAHTG	94
ScYst1	V-----L-----I--PE--VAI-----	94
ScYst2	V-----L-----I--PE--VAI-----	94
PcECMR	L-R---LI-----VTIE-PT-IIAV---SY-- ---H-Y-L---	92
p40/37-LRP	LKR---LL---A-V-IE-PA--S-I---NT--VCGTV-----A--	100
CaYst1	A TAIAGRFTPGNFTNYITRSFKPERLVVVTDPRDQAIKESSYVNIPIV	143
ScYst1	-P-----S-----I-----S-----A-----	143
ScYst2	-P-----S-----I-----L-----A-----	143
PcECMR	-RE--SS-----S-----Y-----II-----S-----A-----	142
p40/37-LRP	-P-----T---Q-QAA-R---L-----A-H-PLT-A---L-T	149
CaYst1	IALTDMQSPSEYVDVAIPCNNGKHSIGLIWLLAREVLRLRGIIPDRIT	193
ScYst1	-----LD---F-----R-----Y-----ALV---Q	193
ScYst2	-----LD---F-----R-----Y-----ALV---Q	193
PcECMR	---C-TD---LQ---T-----M-----TLAN-DV	192
p40/37-LRP	---CNTD--LR---I-----A-V--M--M-----M--T- S-EH	198
CaYst1	EWSVMPDLYFYRDPFEEIEQNAVEEAKTEGVEGA PVAE	230
ScYst1	P--I-----V--QVA---T---EAGEE EAK-	230
ScYst2	P--I-----N--V--V-E-A-AA--GEE- E-K-	230
PcECMR	--GI-V-----T-KDTE-S-Q-AL-ASGN --AQFGSV P	234
p40/37-LRP	P-E-----KEEQAA-EKAVTKKEEFQGEWTA-AP-FTATQP	248
CaYst1	AETEWGTETEDV DWAD SGATPSC	253
ScYst1	EV-- EQA-AT E--E EN-DNVEW	252
ScYst2	EV-- -QA-AT E--E EN-DNVEW	252
PcECMR	VTS-D-EIAAQSTGAGIITNVASNVQSGN-DENFGVETGV--DWAADSIQ	284
p40/37-LRP	EVAD-SEGQV VPSVPIQFPTED-SAQ PATEDWSAAPT	287
CaYst1		253
ScYst1		252
ScYst2		252
PcECMR	QSE--IQHAQNWN	295
p40/37-LRP	QATEWVGATTDWS	300

Fig. 2. Alignments of CaYst1p 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 CaYst1p are denoted by dashes; blank spaces represent gaps introduced to maximize alignment. The boxed region corresponds to peptide G homologues (see text for details).

acids located at position 85–100 (Fig. 1). Two putative myristoylation sites at amino acid positions 105 and 225 may indicate membrane anchoring and/or protein–protein interaction (McIlhinney, 1990).

A comparison of the predicted amino acid sequence with those in the public databases using the BLAST program (Altschul *et al.*, 1990) revealed significant similarity (over 80%) with the ribosomal Yst1 and Yst2 proteins of *S. cerevisiae* (Demianova *et al.*, 1996), the *Pneumocystis carinii* extracellular matrix protein (over 70%) that binds laminin and fibronectin *in vitro* (Narasimhan *et al.*, 1994) and human LBP (over 60%) (Yow *et al.*, 1988) (Fig. 2). The homology included a region similar to peptide G, responsible for laminin binding (Castronovo *et al.*, 1991). Since the highest similarity was found with the Yst proteins from *S. cerevisiae* we have named this protein CaYst1 (Ca for *C. albicans*).

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, con-

Fig. 3. Southern blot of *C. albicans* (lane 1) and *S. cerevisiae* (lane 2) genomic DNA. DNAs from *C. albicans* and *S. cerevisiae* were digested with *EcoRI*, electrophoresed on an agarose gel and transferred to nylon membranes. The blot was hybridized with a DIG-labelled cDNA probe from a pCME22 clone. Molecular mass markers (λ DNA digested with *EcoRI* and *HindIII*) are shown on the left.

firming 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 CaYst1p in *C. albicans*

To determine the cellular localization of CaYst1p, specific antibodies against epitopes expressed by λ ExCell-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

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.

Fig. 5. Western blot of different cell fractions from *C. albicans* mycelial cells. Proteins (5 µg from each fraction) were electrophoresed on SDS-10% polyacrylamide gels, transferred to nitrocellulose membranes and probed with specific antibodies against CaYst1p. (a) C, cytosol; M, particulate membrane fraction; S, SDS-released cell-wall material; Z, Zymolyase-20T-released cell-wall material; ME, β-mercaptoethanol-released cell-wall material. Molecular mass markers are shown on the left. (b) Distribution of CaYst1p in post-ribosomal supernatant (P), membrane (M) and ribosomal (R) fractions. The results obtained with *C. albicans* yeast cells were similar.

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, 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.

Fig. 6. Complementation analysis of the *yst* mutations. (a) *S. cerevisiae* 8-3D, a diploid strain heterozygous for *YST1* and *YST2*, *YST1/yst1-Δ1(::URA3)* and *YST2/yst2-Δ1(::HIS3)*, was transformed with *YEpcAYST1-ACT*, sporulated and tetrads dissected. Individual spores were grown on minimal medium with the appropriate nutrients except leucine. Numbers refer to different tetrads. (b) The four spores of tetrad 1 were analysed for uracil and histidine auxotrophies. Plus and minus designations in front of ura (uracil) and his (histidine) indicate the presence or absence of that nutrient.

Fig. 7. Growth of *S. cerevisiae yst1/yst2* double mutant containing the plasmid *YEpcAYST1-ACT* (1, 3) or *YEpcAYST1-GAL* (2, 4) on galactose (a) or glucose (b).

Fig. 8. Western blot of different cell fractions obtained from *S. cerevisiae yst1/yst2* double mutant transformed with *YEpcAYST1-ACT*. The proteins (5 µg from each fraction) were processed as described in the legend to Fig. 5. P, post-ribosomal supernatant; M, membrane fraction; R, ribosomal fraction. *CaYst1p* is indicated by an arrow.

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

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

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; García-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 homozygous 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 & Worderman, 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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