Cloning and sequencing of the *Candida albicans* homologue of *SRB1/IPSA1/VIG9*, the essential gene encoding GDP-mannose pyrophosphorylase in *Saccharomyces cerevisiae*

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Two genomic fragments have been isolated from *Candida albicans* which strongly hybridize to *SRB1/IPSA1/VIG9*, an essential gene which encodes GDP-mannose pyrophosphorylase in *Saccharomyces cerevisiae*. A common 2.5 kb XbaI–PstI fragment has been identified, which Southern analysis suggests is most likely unique in the *C. albicans* genome. The fragment contains an ORF, which is 82% identical and 90% homologous to the Srblp/PsalpNiggp from *S. cerevisiae*, contains one additional amino acid at position 254 and is able to functionally complement the major phenotypic characteristics of *S. cerevisiae* srbl null and conditional mutations. The authors therefore conclude that they have cloned and sequenced from *C. albicans* the *bona fide* homologue of *SRB1/IPSA1/VIG9*, named hereafter *CaSRB1*. Northern analysis data indicate that the gene is expressed in *C. albicans* under conditions of growth in the yeast and hyphal form and suggest that its expression might be regulated.

**Keywords**: SRB1/IPSA1/VIG9, glycosylation, GDP-mannose, *Candida albicans*, cell wall

INTRODUCTION

Yeast cells, unlike mammalian cells, are surrounded by a thick cell wall, which is a particularly attractive target for antifungal drugs (Debono & Gordee, 1994). The yeast cell wall consists of three major components—glucans, mannans and chitin—and the structural features and interactions of these components are relatively well understood (Klis, 1994). In recent years two MAP kinase signalling pathways (PKCl and HOGI) and the Ras/cAMP dependent pathway have been shown to regulate cell wall integrity in *Saccharomyces cerevisiae* (Cid et al., 1995; G. Tomlin, S. G. Oliver & L. I. Stateva, unpublished results).

The cell wall performs a number of functions, the most important of which are cell shape determination, regulation of permeability, protection against damaging extracellular agents and, in pathogenic species like *Candida albicans*, expression of virulence properties. The cell wall is a highly dynamic organelle which can undergo modifications to its composition and structural features in response to both developmental and environmental signals. Significant variations have been reported for mannoproteins in particular. In *S. cerevisiae*, differences in mannoprotein synthesis during development of the bud and that of mature cells result in differences in permeability (de Nobel et al., 1991). Mannoprotein composition and structure are also very different at the schmoo projection, which develops in response to pheromones (Lipke et al., 1976; Herrero et al., 1985). In *C. albicans*, cell wall mannoproteins specific only for the hyphal form have been described (Elorza et al., 1985; Casanova et al., 1989). For these reasons the mannoproteins, their synthesis and regulation, are a good target for the development of selective antifungal drugs. Mannans are synthesized by an elaborate mechanism of post-translational modifications carried out during passage through the secretory pathway (Lehle & Tanner, 1995; Herscovics & Orlean, 1993) and all of these (N-, O-linked glycosylation and
GPI-anchor synthesis) require a common substrate—GDP-mannose. Its synthesis, is of key importance in the biogenesis of a functional cell envelope in yeast.

The gene which encodes the GDP-mannose pyrophosphorylase in *S. cerevisiae* is an essential, cell-cycle-regulated gene. It has been independently isolated as a monocopy suppressor of both *alg1-PSA1* (Albright & Robbins, 1990; Benton *et al.*, 1996) and *dpm1* (Orlean *et al.*, 1988; J. Schultz & G. Sprague, unpublished data in the SGD database), as the *bona fide* SRB1 (G. Tomlin, S. G. Oliver & L. I. Stateva, unpublished results) and VIG9 (Hashimoto *et al.*, 1997). The existing mutant alleles of the *SRB1* gene produce highly pleiotropic cell wall integrity defects: dependence for growth upon the presence of osmotic stabilizers in the medium, lysis upon osmotic shock (Venkov *et al.*, 1974) and high competence for DNA transformation, including that with large YACs (Heale *et al.*, 1994). Repression of *SRB1/PSA1* expression reveals additional phenotypes suggesting that the gene is also required for bud-site selection and cytokinesis (Zhang, 1997). A number of structural and compositional differences in the major cell wall components glucan and mannan have also been reported (Maerkisch *et al.*, 1983; Blagoeva *et al.*, 1991). Mutations in *SRB1* (*srbl-1*) are synthetically lethal with *pck1*, but cannot be rescued by overexpression of either *PKC1*, or any of the downstream components of the *PKC1*-regulated MAP kinase signal transduction pathway (Cid *et al.*, 1995). However, overexpression of *PDE2* (the gene encoding the high-affinity cAMP phosphodiesterase; Sass *et al.*, 1986) restores the wild-type phenotype of *srbl-1* mutants (G. Tomlin, S. G. Oliver & L. I. Stateva, unpublished results).

In the present paper the cloning and molecular characterization of the *SRB1/PSA1* homologue from *C. albicans* is reported. Southern analysis shows that it is unique in the *C. albicans* genome. Sequence determination indicates that it encodes a highly conserved protein, which is 82% identical and 90% homologous to its *S. cerevisiae* counterpart. The potential role of this gene in the dimorphic switch of *C. albicans* is discussed on the basis of the results of the Northern analysis of its expression under conditions of hyphal induction by serum or changes in pH.

**METHODS**

**Strains and media.** Strains of *Saccharomyces cerevisiae*, *Candida albicans* and *Escherichia coli* used in this study are listed in Table 1. Media to support the growth of *S. cerevisiae* and *C. albicans* (rich YEPD and minimal SD, containing YNB, glucose and appropriate supplements) were prepared according to Sherman *et al.* (1986). All types of media for the propagation of *srbl-1* fragile mutants were supplemented with 10% (w/v) sorbitol. Minimal medium for pH hyphal induction in *C. albicans* was prepared essentially as described by Lee *et al.* (1975). Bacterial strains were grown in LB and LB with ampicillin, as described by Sambrook *et al.* (1989).

**Yeast library and plasmids.** The *C. albicans* genomic library was kindly provided by Dr S. Scherer, University of Minnesota, USA. It was constructed using the fosmid pFOS1 (Kim *et al.*, 1992) and fragments of *SaulAI* partially digested genomic DNA from *C. albicans* 1161. The 1920 clones obtained were transferred to Hybond-N* membranes (Amersham Pharmacia Biotech). The membranes were used for hybridization with a labelled *S. cerevisiae* SRB1/PSA1 0.8 kb EcoRI-BglII DNA fragment, isolated from plasmid SRB1-9b (kindly provided by G. Tomlin). All plasmids used in this study are listed in Table 1.

**DNA preparation and manipulations.** Yeast genomic DNA was isolated from spheroplasts (Davis *et al.*, 1980). Bacterial plasmid DNA, on both a small (1 ml) and a large (50–100 ml) scale, was isolated using the alkaline-lysis method of Birnboim & Doly (1979), as described by Sambrook *et al.* (1989). DNA was purified either by CsCl gradient centrifugation (Sambrook *et al.*, 1989) or by a Qiagen QUA filter plasmid Midi kit. All DNA fragments used for subcloning were gel-purified by the method of Heery *et al.* (1990). Enzymic reactions with restriction enzymes (Boehringer), T4 ligase (Boehringer) and Taq DNA polymerase (Amersham Pharmacia Biotech) were all performed according to the recommendations of the manufacturers. For Southern analysis, the procedure recommended by Amersham Pharmacia Biotech was followed. All DNA probes used for hybridization were labelled with the Rediprime kit and Redivue [a-32P]dCTP (10 mCi ml–1, 1 Ci = 3.7 × 1010 Bq) from Amersham Pharmacia Biotech.

**Hyphal induction, RNA extraction and Northern blotting.** Hyphal induction by serum and pH was achieved essentially by the methods of Bailey *et al.* (1996) and Sundstrom *et al.* (1990), respectively. The control alternative experiments, where the strains were growing only in the yeast form, were performed in both rich (YEPD) and minimal (Lee’s medium, pH 4.5) medium at 23 °C after an initial 2 h starvation in water for synchronization of growth. The cell cycle phase of the cultures was determined by counting the budded and unbudded cells in the course of the experiments. Samples were withdrawn at various times (0.5, 1, 2, 3, 4 and 6 h) after hyphal induction (either method) and during the control experiments, and used for RNA extraction. Total RNA, isolated as described by Brown (1994), was denatured in formaldehyde/formamide, and after electrophoresis under denaturing conditions, transferred to Hybond-N membranes (Amersham Pharmacia Biotech). The membranes were treated and hybridized to labelled DNA probes, as recommended by the manufacturers. After hybridization, they were exposed to a Bio-Rad imaging screen, developed with the Bio-Rad phosphorimager (GS-363) and analysed with Molecular Analysers software. As an alternative probe for loading and transfer of the total RNA, the gene TEF3 (Swoboda *et al.*, 1994) was used. However, CaSRB1 mRNA levels were quantified relative to the rRNAs, loading approximately equal amounts and measuring the ethidium bromide staining intensity in each lane. The levels of expression at given time points were determined relative to that at 30 min after the start of the growth under all conditions.

**DNA sequence analysis.** The method employed for sequencing was essentially as described by James *et al.* (1995). Nucleotide sequence analyses and homology searches were performed using both the *BLAST* search program (http://www.ncbi.nlm.nih.gov/cgi-bin/blast/nph-blast) and the CLUSTAL W (1.60) Multiple Sequence Alignment program (http://www.genome.ad.jp/SIT/SIT.html).

**Genetic methods.** The concomitant loss of plasmid markers and the mitotic stability of transformed strains were tested by growing the cultures to the stationary phase in rich (YEPD)
Table 1. Strains and plasmids used in this study

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant genotype or details</th>
<th>Source/reference</th>
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<tbody>
<tr>
<td><strong>S. cerevisiae</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7SLU</td>
<td>MATa srb1-1 leu2 ura3</td>
<td>Stateva et al. (1991)</td>
</tr>
<tr>
<td>AH22</td>
<td>MATa leu2-3, leu2-112 his4-519 can1</td>
<td>UMIST collection</td>
</tr>
<tr>
<td>20hPS1-2d MET3-PSAl</td>
<td>MATa leu2 ura3 trp1 ade1 his2 psa1::TRP1 pURA3/MET3-PSAl</td>
<td>Zhang (1997)</td>
</tr>
<tr>
<td><strong>C. albicans</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SC5314</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>
</tr>
<tr>
<td><strong>E. coli</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>XL-1 Blue</td>
<td>recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac [F'proABlacZAM15 Tnl O(TetR)]</td>
<td>Bullock et al. (1987)</td>
</tr>
<tr>
<td><strong>Plasmids</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pUC19</td>
<td>E. coli vector</td>
<td>Yanisch-Perron et al. (1985)</td>
</tr>
<tr>
<td>SRB1-4</td>
<td>YCp50 based, SRB1-2 with the ClaI fragment removed</td>
<td>G. Tomlin, Dept of Biomolecular Sciences, UMIST</td>
</tr>
<tr>
<td>SRB1-9b</td>
<td>ClaI–BamHI fragment of SRB1 in pRS316</td>
<td>G. Tomlin, Dept of Biomolecular Sciences, UMIST</td>
</tr>
<tr>
<td>pA301</td>
<td>EcoRI–BamHI fragment of S. cerevisiae ACT1</td>
<td>J. Rosamond, University of Manchester, UK</td>
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</table>

medium containing 10% sorbitol at 30 °C and plating out aliquots for single colonies on plates of the same medium. After incubation at 30 °C the colonies were replica-plated to selective media. Sorbitol dependence of the S. cerevisiae strain 7SLU (Table 1) and transformants, containing C. albicans SRB1/PSA1 putative homologues, was tested by comparing the growth of single colonies on minimal media with and without 10% sorbitol, in the presence of the appropriate nutritional supplements.

Cultures of strains containing the pMET3-regulated SRB1/PSA1 cassette were grown in selective SD medium at 30 °C with vigorous shaking until the early exponential phase (OD600 0.05), at which point they were split into two halves. To one half of each culture, methionine was added to a final concentration of 2 mM; to the other half, an equivalent volume of distilled water was added. The two cultures were then incubated further under the same conditions as before, until stationary phase.

**Lysis test.** The standard procedure, described by Stateva et al. (1991), was followed. All tests were performed after growth of the strains in selective minimal media to ensure maximum stability of the transforming plasmids.

**Transformation.** E. coli XL-1 Blue was transformed either by the calcium chloride method or by the DMSO method, as described by Sambrook et al. (1989). Yeast strains were transformed either by a modification of the lithium procedure of Ito et al. (1983), as described by Stateva et al. (1991) or by the DMSO enhanced procedure developed by Hill et al. (1991).

**PCR.** The SRB1 forward (5’-AGCTCAGAGGTCACCTATACG-3’) and reverse (5’-CGGATCCAATACAGACAT-TGATAGGCC-3’) primers were used to amplify the S. cerevisiae SRB1/PSA1. In 50 µl of PCR reaction, 5 ng DNA template (SRB1-4) was mixed with 200 ng of each primer, 1.5 mM MgCl2, 200 µM dNTPs, 0.5 unit Taq polymerase (Boeringer) and PCR buffer. The PCR amplification was run for 30 cycles (94 °C for 1 min, 55 °C for 1 min and 72 °C for 2 min 15 s).

**RESULTS**

The SRB1/PSA1/VIG9 putative homologue from C. albicans is unique

In *S. cerevisiae* the gene SRB1/PSA1 is essential for growth. Speculating that SRB1/PSA1 might be a highly conserved gene among yeast species, it was decided to attempt the isolation of a *C. albicans* homologue by DNA sequence hybridization with an ordered fosmid library, using the internal 0.8 kb EcoRI–BglII fragment from the *S. cerevisiae* SRB1/PSA1 gene as a probe. After two consecutive rounds of screening, two strongly hybridizing clones (designated A and B) were selected for further analysis. They were shown to have inserts of around 45 kb (data not shown) with a common 6 kb XbaI fragment. At this initial stage of characterization two polymorphisms were observed in the restriction patterns of the two recombinant clones. Clone A contained an EcoRI site, absent from clone B, whilst clone B had a PstI site, not found in clone A. Subcloning experiments defined a 2.5 kb XbaI–PstI fragment as the smallest uniquely hybridizing region of the *C. albicans*
DNA inserts and this was used in subsequent experiments.

Southern analysis was used to check whether the putative SRB1/PSA1 homologue from clones A and B defined unique sequences in the Candida genome. Total genomic DNA was isolated from two C. albicans strains, CAI4 and SC5314, and digested with two enzymes without cleavage sites in the SRB1/PSA1 gene of S. cerevisiae (XbaI and HindIII) and one enzyme with an internal cleavage site (PstI). Genomic DNA from S. cerevisiae AH22 was similarly treated. The 2.5 kb XbaI–PstI fragments from C. albicans clones A and B, and a PCR-amplified fragment of the S. cerevisiae SRB1 gene, were used as probes. The results of the Southern analysis with the three different hybridization probes, together with the restriction map of the XbaI–PstI fragment from C. albicans generated on the basis of the preliminary cloning experiments, are presented in Fig. 1. In all three cases, the signals from the homologous probing experiments were stronger [with S. cerevisiae DNA in Fig. 1(b)], and with C. albicans DNAs in Fig. 1(bII and bIII), than those from the heterologous probing. The hybridization pattern of the S. cerevisiae DNA, seen best in Fig. 1(bI), confirmed the presence of all the expected fragments. The patterns of the two strains of C. albicans, which were identical to each other, were significantly different from that of the S. cerevisiae strain: note the different PstI/XbaI and HindIII patterns. However, they verified the restriction map (Fig. 1a) of the cloned C. albicans fragment, which has unique sites for XbaI and PstI, and two sites for HindIII. There are one or two very faint additional bands which must contain regions of limited similarity; however, given the intensity of the main bands, it was concluded that, like the SRB1/PSA1 gene in S. cerevisiae, its putative homologue from C. albicans is most likely also unique.

**Fig. 1.** Southern analysis. (a) Restriction map of the C. albicans probes. (b) Total genomic DNAs of S. cerevisiae AH22 (lane 1) and C. albicans SC5314 and CAI4 (lanes 2 and 3, respectively) were digested with XbaI, XbaI/PstI and HindIII and hybridized with S. cerevisiae SRB1 probe (I), and with the 2.5 kb XbaI–PstI fragments from clones A and B (II and III, respectively).

**Functional complementation of srb1-1-associated phenotypes with the C. albicans SRB1/PSA1 homologue**

Mutations of SRB1 are highly pleiotropic in their phenotypic effects, with the most characteristic features of srb1-1 being dependence on osmotic stabilizers for
growth and lysis upon osmotic shock. These phenotypes were chosen for the functional complementation test. The S. cerevisiae srbl-1 mutant strain (7SLU) was transformed with pRS415 only (Sikorski & Hieter, 1989), and with recombinant plasmids carrying either the clone A or the clone B 2.5 kb XbaI–PstI fragment. One hundred transformants for each recombinant plasmid were tested and, unlike the homologues of S. cerevisiae srbl-1, both clones A and B were able to grow normally in minimal selective media for maximum stability of the transforming plasmids. The strain containing only pRS415, they were all able to grow without the addition of 10% sorbitol to the medium (examples are shown in Fig. 2). A mitotic stability test was performed on three transformants of each type. It confirmed that their newly acquired feature co-segregated in all cases with the plasmid-borne nutritional marker LEU2.

The ability of the putative C. albicans SRBl homologues to complement the lytic phenotype of the srbl-1 mutant strain was also tested. The standard lysis test, as described in Methods, was performed with two different transformants of each type and the original mutant. All strains were grown in minimal selective media for stationary phase, irrespective of the type of medium (rich or minimal), or the temperature (4, 22 or 30°C). In all cases tested, the percentage of viable cells decreased very rapidly and was almost zero after only 3 d (data not shown).

To rule out the possibility of allele-specific complementation, the same recombinant plasmids were also transformed into the srbl-1 null mutant, 20hPS1(−), modified by Zhang (1997); see Table 1 for genotype. The strain contains a wild-type copy of the SRBI/PSAI gene under the control of the tightly regulated MET3 promoter (Mountain et al., 1991). As a result, the strain grows as wild-type in the absence of methionine, but the cells quickly stop growing and lose viability upon addition of 2 mM methionine to the medium (Zhang, 1997). Transformants of this strain carrying the putative C. albicans SRBl/PSAI homologues were able to grow normally in methionine-supplemented medium and reached cell densities comparable to those of the control cultures without methionine (Table 2).

On the basis of these results, it was concluded that the two C. albicans clones A and B contain a common 2.5 kb XbaI–PstI fragment, which not only uniquely hybridizes to the S. cerevisiae SRBI gene and to C. albicans genomic DNA, but is also able to functionally complement the major phenotypic characteristics of two different srbl1 mutant alleles.

Whilst working with the S. cerevisiae 7SLU srbl-1 transformants, carrying the putative SRBI C. albicans homologues, an additional phenotype was observed. The transformants carrying the recombinant plasmids, but not the cloning vector, lost viability very rapidly in stationary phase, irrespective of the type of medium (rich or minimal), or the temperature (4, 22 or 30°C). In all cases tested, the percentage of viable cells decreased very rapidly and was almost zero after only 3 d (data not shown).

Table 2. Complementation of the lethality of srbl null mutants by C. albicans SRBl homologues

<table>
<thead>
<tr>
<th>Strain</th>
<th>Minus methionine</th>
<th>Plus methionine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control*</td>
<td>1.2 ± 0.05</td>
<td>0.3 ± 0.03</td>
</tr>
<tr>
<td>TRA A1†</td>
<td>1.2 ± 0.03</td>
<td>1.2 ± 0.06</td>
</tr>
<tr>
<td>TRA A2†</td>
<td>1.18 ± 0.07</td>
<td>1.28 ± 0.01</td>
</tr>
<tr>
<td>TRA A3†</td>
<td>1.22 ± 0.03</td>
<td>1.11 ± 0.05</td>
</tr>
<tr>
<td>TRA B1‡</td>
<td>1.02 ± 0.04</td>
<td>1.22 ± 0.05</td>
</tr>
<tr>
<td>TRA B2‡</td>
<td>1.18 ± 0.01</td>
<td>1.01 ± 0.08</td>
</tr>
<tr>
<td>TRA B3‡</td>
<td>1.22 ± 0.02</td>
<td>1.05 ± 0.04</td>
</tr>
</tbody>
</table>

* Strain 20hPS1-2d MET3-PSAI (see Table 1 for relevant genotype) was used as a control.
† TRA A1, 2 and 3 are three independent transformants of 20hPS1-2d MET3-PSAI obtained with the C. albicans SRBl/PSAI homologue isolated from clone A.
‡ TRA B1, 2 and 3 are three independent transformants of 20hPS1-2d MET3-PSAI obtained with the C. albicans SRBl/PSAI homologue isolated from clone B.

DNA sequence and predicted ORF of the SRBI/PSAI homologue from C. albicans (CaSRBl)

The sequence of the SRBI/PSAI homologues represented by both clones A and B was determined, as described in Methods. A single ORF, encoding a putative
product of 362 amino acids, was identified. The first
ATG of this ORF is 77 bp downstream from a TATAA
box. The 3' region of the gene contains two in-frame
stop codons 21 nucleotides downstream of the final
codon and a consensus site for transcription termination
(TATA) 137 nucleotides downstream of the final codon.
No polyadenylation consensus sequences could be
identified within the sequenced regions.

The predicted protein sequence of this new gene (called
CaSRBl) has an estimated molecular mass of about
40 kDa (39961 Da for clone A; 39989 Da for clone B). A
comparison between the protein sequences of the two
Candida clones, together with that of S. cerevisiae
Srblp, is given in Fig. 3. The following points are
noteworthy. First, there is only one significant difference
between the two C. albicans clones: at position 288, the
encoded amino acid is Gln in clone A and Arg in clone
B. Second, there is a very high percentage of identity
(82%) and homology (90%) between the C. albicans
and S. cerevisiae sequences. Third, the amino acid at
position 254 in the C. albicans sequence is not present in
the corresponding position of the S. cerevisiae protein.

Fig. 4 shows the hydropathy profile of the predicted
CaSRBl protein. The profile suggests that, like the S.
cerevisiae counterpart (Hashimoto et al., 1997), the
protein from C. albicans is soluble and does not have
hydrophobic stretches long enough to be considered
potential transmembrane domains or signal sequences
for secretion.

Fig. 3. Comparison of the deduced amino acid sequences of the two putative C. albicans SRBl/PSA1 (Ca.Ap and Ca.Bp)
and S. cerevisiae SRBl/PSA1 (Sc.Srblp). Amino acids given in bold correspond to differences, asterisks to identities and
dots to similarities in the deduced C. albicans and S. cerevisiae protein sequences. The amino acids in boxes (positions 254
and 288) correspond to an insertion in the Candida protein in comparison to the S. cerevisiae counterpart, and to the
only difference between the two C. albicans protein sequences, respectively.
GDP-mannose pyrophosphorylase gene from *C. albicans*

Fig. 5. Northern analysis of total mRNA from *C. albicans* SC5314 after induction of hyphae by serum (a) and pH (e), and during growth in the yeast form in rich medium (c). Panels (a), (c) and (e) show, from top to bottom: ethidium bromide staining pattern, hybridization results with probe A, hybridization results with probe B and (a and e only) hybridization with TEF3 as a probe (T). Times of induction and growth and conditions of controls are shown above the tracks. Panels (b), (d) and (f) show levels of expression after quantification of the Northern results, as described in Methods. Levels at all time points are expressed relative to that taken 30 min after start of experiments.

**Northern analysis**

The expression of CaSRBI was investigated during yeast-to-hyphal transition and also under conditions of yeast-only growth. The dimorphic switch was induced by either addition of serum or pH change, and both combined with a higher growth temperature (37 °C). At a pH of 4.5 and a temperature of 25 °C in minimal medium, and also in rich medium at the same temperature, *C. albicans* grows purely as a yeast. When the pH and the temperature are increased to 6.5 and 37 °C respectively, or when 10% serum is added to the rich medium at 37 °C, the cells undergo a morphogenic switch, forming first germ tubes, then hyphae. Under the conditions of our experiments, the first germ tubes were clearly visible 30 min after induction (either method) and the hyphal transition was completed after 4 h (data not shown). The pattern of expression of the 'control' probe in our experiments, the gene TEF3 (Swoboda *et al.*, 1994), was consistent with published results (Swoboda *et al.*, 1994). Since neither actin (Paranjape & Datta, 1991), nor indeed TEF3 or any other *Candida* gene (Swoboda *et al.*, 1994), could be used as a classic internal loading mRNA control in this experiment,
CaSRBl mRNA levels were quantified relative to the major rRNA species (see Methods).

Total RNA isolated from C. albicans growing in either yeast or hyphal form, as well as during the transition between the two forms, showed one distinct band when probed with the CaSRBl DNA (Fig. 5). This result clearly shows that CaSRBl is an expressed gene. RNA quantification, however [Fig. 5(b) and (f) for serum and pH induction, and (d) for the yeast-only growth in rich medium], suggests that the gene might be also regulated. The different induction conditions give slightly different results, although both show a high level of expression early after induction and a considerable reduction after hyphal transition (four- to fivefold, 6 h after induction). In the experiments where the transcription levels of CaSRBl were determined after growth in the yeast form in rich medium (Fig. 5c, d), and in minimal medium (data not shown), the pattern of expression was different from that observed under conditions of hyphal induction: there was no reduction in expression levels after 6 h. Taken together, these experiments demonstrate that similar to the situation in S. cerevisiae (Benton et al., 1996), in C. albicans this gene might be also regulated, with levels of expression particularly high during periods of intense cell wall synthesis as in germ tube formation seen during the morphological switch, and bud initiation during growth in the yeast form.

**DISCUSSION**

We have cloned and sequenced the functional C. albicans homologue of SRBl/PSAI/VIG9. This gene is unique and essential for growth in S. cerevisiae. The data presented here (Fig. 1) suggest that it is most likely unique in the C. albicans genome, although at present it is not known whether the gene is also essential for growth in this species.

Two positive clones were identified out of a fosmid library which, according to our data, appear to define the two copies of this gene in the C. albicans diploid genome. They have almost identical nucleotide and thus amino acid sequences (Fig. 3). The only significant difference is an amino acid polymorphism at position 288 (Gln in clone A, Arg in clone B). Significantly, however, both copies of the Candida gene are able to complement, in S. cerevisiae, the characteristic phenotypes conferred by two different srbl1 mutant alleles, including that of the null allele (Fig. 2, Table 2, and lysis data not shown), and have similar patterns of expression under conditions of hyphal induction by pH and serum (Fig. 5). Given that SRBl/PSAI is an essential gene for growth in S. cerevisiae, the ability of the C. albicans homologues to give functional complementation of its null allele is strong evidence that CaSRBl indeed encodes GDP-mannose pyrophosphorylase in C. albicans.

The proteins encoded by SRBl/PSAI/VIG9 in S. cerevisiae and its functional homologue in C. albicans CaSRBl appear to be highly conserved. This is shown by the high percentages of identity and homology between their amino acid sequences (Fig. 3), as well as their almost identical hydropathy profiles (Fig. 4, and Hashimoto et al., 1997). The protein from C. albicans contains an additional amino acid (glycine, position 254), not present in its S. cerevisiae counterpart, although we have no data on its functional or structural significance. We can only speculate that it might be partially responsible for the low survival of the S. cerevisiae 7SLU strains transformed with CaSRBl.

All the amino acids affected in existing SRBl mutant alleles (G. Tomlin, S. G. Oliver & L. I. Stateva, unpublished results) are, however, conserved in the C. albicans homologues, giving indirect proof of their functional importance. Interestingly, there appears to be little conservation of the glycosylation capacity of the proteins. Whilst a computer search identified five potential sites for glycosylation in SRBl/PSAI/VIG9 in S. cerevisiae, the same program recognized only one site in CaSRBl. However, our functional complementation data indicate that the glycosylation pattern, if different in vivo, does not affect the function of the protein in a heterologous system.

Outside the sequence of the ORF there is little similarity between the genes from S. cerevisiae and C. albicans. The putative regulatory sites are, however, located in a similar position relative to the first and the final codons of the ORF, and the C. albicans sequences do appear to be sufficient for function in S. cerevisiae, as evidenced by the complementation of different SRBl mutant phenotypes. Attempts at complementation of a C. albicans CaSRBl mutant with the S. cerevisiae gene will show whether the opposite is also true, although to date heterologous gene expression in Candida has met with little success.

Taken together, all our data suggest strongly that CaSRBl is the C. albicans gene encoding GDP-mannose pyrophosphorylase and we are currently developing a biochemical analysis to prove this unambiguously.

The gene encoding GDP-mannose pyrophosphorylase in S. cerevisiae may be considered as a morphogene, given the plethora of cell-wall-related phenotypes associated with the srbl-1 mutation (G. Tomlin, S. G. Oliver & L. I. Stateva, unpublished results). There are other microbial mutants with altered morphology which also have defects in genes determining metabolic functions [e.g. glucose-6-phosphate dehydrogenase and phosphoglucomutase in Neurospora crassa (Brody & Tatum 1966, 1967); NADP-linked glutamate dehydrogenase and phosphoglucose isomerase in S. cerevisiae (Wilkinson et al., 1996; Dickinson & Williams, 1987)].

One conclusion from these data is that many genes make an indirect contribution to morphogenesis acting via a series of pleiotropic effects. In the case of SRBl/PSAI/VIG9, these pleiotropic effects are determined by the demand for GDP-mannose, either directly or via the dolichol pathway linking a number of basic cellular processes which contribute to the biogenesis of a functional cell envelope [N- and O-linked glycosylation of proteins (Kukuruzinska et al., 1987; Abeijon &
Hirschberg, 1992; Herscovics & Orlean, 1993; mannossylation of lipids in the Golgi (Lester & Dickson, 1993) and the synthesis of GPI anchors attached to the C-terminus of some proteins (Orlean, 1990). This high demand probably accounts for the high levels of expression of the gene in both S. cerevisiae and C. albicans (our Northern data; Benton et al., 1996; Zhang, 1997). In S. cerevisiae, the expression levels of SRB1/PSA1 vary and are tightly regulated with the cell cycle, with a peak of expression in G1 at START (Benton et al., 1996). Our data indicate that in C. albicans as well, the levels of expression of CaSRB1 are not constant. There are clear changes in mRNA levels following the switch to hyphal growth. Our data also suggest that there might be some periodicity in the rate of mRNA synthesis during growth in the yeast form. It will be interesting to discover how this regulation is achieved. Our future studies on CaSRB1 mutant strains and/or strains expressing the gene under tightly controlled promoters might provide some answers.

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