Cloning and sequencing of the *Candida albicans* homologue of *SRB1/PSA1/VIG9*, the essential gene encoding GDP-mannose pyrophosphorylase in *Saccharomyces cerevisiae*

Saradee Warit, Richard. M. Walmsley and Lubomira I. Stateva

Author for correspondence: Lubomira I. Stateva. Tel: +44 161 200 4208. Fax: +44 161 236 0409. e-mail: lubomira.stateva@umist.ac.uk

Two genomic fragments have been isolated from *Candida albicans* which strongly hybridize to *SRB1/PSA1/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/Psalp/Niggp* 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/PSA1/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/PSA1/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 (*PKC1* and *HOG1*) 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regulate cell wall integrity in *Saccharomyces cerevisiae* (Cid et al., 1995; G. Tomlin, S. G. Oliver & L. I. Stateva, unpublished results).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 multicopy 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 *Sau3AI* 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 QIA 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), *T<sub>4</sub>* 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 (α-32P)dCTP (10 mCi ml<sup>−1</sup>, 1 Ci = 3.7 × 10<sup>10</sup>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 25 °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 Analyser software. As an alternative probe for loading and transfer of the total RNA, the gene *TEE3* (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>
</tr>
</thead>
<tbody>
<tr>
<td>S. cerevisiae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7SLU</td>
<td>MATα srb1-1 leu2 ura3</td>
<td>Stateva et al. (1991)</td>
</tr>
<tr>
<td>AH22</td>
<td>MATα leu2-3, leu2-112 his4-519 can1</td>
<td>UMIST collection</td>
</tr>
<tr>
<td>20hPS1-2d MET3-PSA1</td>
<td>MATα leu2 ura3 trp1 ade1 his2 psa1::TRP1 pURA3/MET3-PSA1</td>
<td>Zhang (1997)</td>
</tr>
<tr>
<td>C. albicans</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>E. coli</td>
<td>recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac ['F'proABlacP2AM15 Tn10(Tet^r)]</td>
<td>Bullock et al. (1987)</td>
</tr>
<tr>
<td>XL-1 Blue</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasmids</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>SRBI-4</td>
<td>YCp50 based, SRBI-2 with the ClaI fragment removed</td>
<td>G. Tomlin, Dept of Biomolecular Sciences, UMIST</td>
</tr>
<tr>
<td>SRBI-9b</td>
<td>ClaI-BamHI fragment of SRBI 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>
</tr>
</tbody>
</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 SRBI-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 SRBI-PSA1 cassette were grown in selective SD medium at 30°C with vigorous shaking until the early exponential phase (OD_600 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 SRBI forward (5'-AGCTCAGAGGTCACTATACG-3') and reverse (5'-CGGGATCCATCTACAGACAT-TGATAGCC-3') primers were used to amplify the S. cerevisiae SRBI-PSA1. In 50 μl of PCR reaction, 5 ng DNA template (SRBI-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 SRBI-PSA1/VIG9 putative homologue from C. albicans is unique

In S. cerevisiae the gene SRBI-PSA1 is essential for growth. Speculating that SRBI-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 EcoR I-BglII fragment from the S. cerevisiae SRBI-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 EcoR I 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(bI), 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.

**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 *srbl*-1 mutant 7SLU, and 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 lysis phenotype of the *srbl*-1 mutant 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 maximum stability of the transforming plasmids. The results indicated clearly that the putative *C. albicans* *SRBl* homologues relieve the osmotic shock sensitivity of *S. cerevisiae* *srbl*-1 mutants (data not shown).

To rule out the possibility of allele-specific complementation, the same recombinant plasmids were also transformed into the *srbl* null mutant, 2OhPS1-2d, modified by Zhang (1997); see Table 1 for genotype. The strain contains a wild-type copy of the *SRBl/PSA1* 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/PSA1* 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* *SRBl* gene and to *C. albicans* genomic DNA, but is also able to functionally complement the major phenotypic characteristics of two different *srbl* mutant alleles.

Whilst working with the *S. cerevisiae* 7SLU *srbl*-1 transformants, carrying the putative *SRBl* *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>OD&lt;sub&gt;600&lt;/sub&gt; Minus methionine</th>
<th>OD&lt;sub&gt;600&lt;/sub&gt; 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 ± 0.04</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 2OhPS1-2d *MET3*-PSA1 (see Table 1 for relevant genotype) was used as a control.
† TRA A1, 2 and 3 are three independent transformants of 2OhPS1-2d *MET3*-PSA1 obtained with the *C. albicans* *SRBl/PSA1* homologue isolated from clone A.
‡ TRA B1, 2 and 3 are three independent transformants of 2OhPS1-2d *MET3*-PSA1 obtained with the *C. albicans* *SRBl/PSA1* homologue isolated from clone B.

**DNA sequence and predicted ORF of the *SRBl/PSA1* homologue from *C. albicans* (CaSRBl)**

The sequence of the *SRBl/PSA1* 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 (TATG) 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.
Northern analysis

The expression of CaSRB1 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,
CaSRBI 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 CaSRBI DNA (Fig. 5). This result clearly shows that CaSRBI 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 CaSRBI 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 SRBI/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 SRBI/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 CaSRBI indeed encodes GDP-mannose pyrophosphorylase in C. albicans.

The proteins encoded by SRBI/PSAI/VIG9 in S. cerevisiae and its functional homologue in C. albicans CaSRBI 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 CaSRBI. All the amino acids affected in existing SRBI 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 SRBI/PSAI/VIG9 in S. cerevisiae, the same program recognized only one site in CaSRBI. 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 SRBI mutant phenotypes. Attempts at complementation of a C. albicans CaSRBI 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 CaSRBI 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 phosphogluco 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 SRBI/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 &
Hirshberg, 1992; Herscovics & Orlean, 1993); mannosylation 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 SRE1/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 CaSREBl 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 CaSREBl mutant strains and/or strains expressing the gene under tightly controlled promoters might provide some answers.

ACKNOWLEDGEMENTS

We thank S. Scherer, G. Tomlin and A. Brown for libraries, plasmids and strains, C. James for help with the sequencing, and N. Gow and A. Brown for productive discussions. This work was supported by a Wellcome grant to R. W. and L.-S. and by a scholarship from National Centre for Genetic Engineering and Biotechnology (NCGEB), National Science Technology Development Agency (NSTDA), Ministry of Sciences, Technology and Environment Thailand, and Royal Thai Government to S. W. Patent protection has been sought for this approach under British patent application 9716916.3.

REFERENCES


Received 8 January 1998; revised 15 May 1998; accepted 28 May 1998.