Cloning of a *Candida albicans* peptide transport gene

Munira A. Basrai,¹† Mark A. Lubkowitz,¹ Jack R. Perry,¹ David Miller,¹ Eduardo Krainer,² Fred Naider² and Jeffrey M. Becker¹

Author for correspondence: Jeffrey M. Becker. Tel: +1 615 974 3006. Fax: +1 615 974 4007; e-mail: IN%“JBECKER@UTKVX.UTK.EDU”

¹ Department of Microbiology and Program in Cellular, Molecular, and Developmental Biology, University of Tennessee, Knoxville, TN 37996-0845, USA

² Department of Chemistry, College of Staten Island, City University of New York, Staten Island, New York 10301, USA

A *Candida albicans* peptide transport gene, *CaPTR2*, was cloned from a *C. albicans* genomic library by functional complementation of a peptide transport deficient mutant (strain *ptr2-2*) of *Saccharomyces cerevisiae*. *CaPTR2* restored peptide transport to transformants as determined by uptake of radiolabelled dileucine, growth on dipeptides as sources of required amino acids, and restoration of growth inhibition by toxic peptides. Plasmid curing experiments demonstrated that the peptide transport phenotype was plasmid borne. *CaPTR2* was localized to chromosome R of *C. albicans* by contour-clamped homologous electric field gel chromosome blots. Deletion subclones and frameshift mutagenesis were used to narrow the peptide transport complementing region to a 5.1 kb DNA fragment. DNA sequencing of the complementing region identified an ORF of 1869 bp containing an 84 nucleotide intron. The deduced amino acid sequence predicts a protein of 70 kDa consisting of 623 amino acids with 12 hydrophobic segments. A high level of identity was found between the predicted protein and peptide transport proteins of *S. cerevisiae* and *Arabidopsis thaliana*. This study represents the first steps in the genetic characterization of peptide transport in *C. albicans* and initiates a molecular approach for the study of drug delivery against this pathogen.

Keywords: *Candida albicans*, peptide transport, *Saccharomyces cerevisiae*

INTRODUCTION

Studies from our laboratory and several others have characterized the peptide transport systems of *Candida albicans* and *Saccharomyces cerevisiae* (for reviews see Becker & Naider, 1980; Naider & Becker, 1987). Results from these studies suggested that both yeasts contain peptide permeases with structural requirements that differ from those of bacterial and mammalian peptide permeases (for reviews see Matthews, 1991; Payne, 1980). Though yeasts show optimal growth on peptides containing hydrophobic residues, they lack strict specificity, as indicated by their ability to transport modified peptides (Becker & Naider, 1995). Peptide transport systems in yeasts are

regulated by nitrogen metabolism (Becker & Naider, 1995), and are induced by the addition of micromolar amounts of certain amino acids (Basrai *et al.*, 1992; Island *et al.*, 1991).

In an effort to define peptide transport at the genetic level, *S. cerevisiae* strains resistant to the growth-inhibitory effects of the dipeptide alanyl-ethionine (Ala-Eth) were isolated (Island *et al.*, 1991). These strains defined three complementation groups suggesting that peptide transport in *S. cerevisiae* is mediated by at least three genes, *PTR1*, *PTR2* and *PTR3* (Peptide TRansport). Mutations in *PTR1* and *PTR2* genes make the cells peptide-transport-deficient, whereas mutations in the *PTR3* gene result in an intermediate phenotype characterized by low-level transport of radiolabelled dipeptides. We have cloned and characterized the *S. cerevisiae* *PTR1* (Alagramam *et al.*, 1995) and *PTR2* (Perry *et al.*, 1994) genes which have ORFs of 5850 and 1803 bp, respectively. The deduced amino acid sequence of the *S. cerevisiae* *Ptr2p* suggests a membrane protein with 12 transmembrane domains. *PTR1* was found to be identical to *S. cerevisiae*

† Present address: Department of Molecular Biology and Genetics, Johns Hopkins University, School of Medicine, Baltimore, MD 21205-0831, USA.

Abbreviations: Ala-Eth, alanyl-ethionine; RFLP, restriction fragment length polymorphism.

The GenBank accession number for the nucleotide sequence reported in this paper is U09781.

UBR1, the recognition component of the N-end rule pathway which is a ubiquitin-dependent protein-degradation system (Alagramam *et al.*, 1995; Bartel *et al.*, 1990). Our working hypothesis is that *PTR2* encodes the structural component of the peptide transport system while *PTR1/UBR1* is responsible for regulation.

Current evidence, though not conclusive, suggests the presence of at least two peptide permeases in *C. albicans*. One of the systems mediates transport of di- and tripeptides, whilst the other transports tri- and oligopeptides (Becker & Naider, 1995). A definitive characterization of the number and role of peptide permeases in *C. albicans* and their regulation will require combined genetic and biochemical approaches. Cloning of the genes involved in peptide transport in *C. albicans* will enable the comparative analysis of the structure and physiological role of membrane transporters from different organisms. Previously, peptide conjugates have been used to deliver toxic moieties to *C. albicans* via the peptide transport system (Becker & Naider, 1995). Synthetic analogues of the polyoxins and nikkomycins, which inhibit chitin synthase (Cabib, 1991), have also been designed to enter *C. albicans* through the peptide transport system (Becker & Naider, 1995). Hence, these studies may facilitate the design of an effective and specific anticandidal drug by exploiting the concept of 'illicit transport' (Ames *et al.*, 1973; Fickel & Gilvarg, 1973; Hammond *et al.*, 1987; Higgins, 1987) in this opportunistic pathogen.

The diploid nature of the *C. albicans* genome and the absence of a known sexual cycle have limited genetic studies with this organism. Nevertheless, significant advances have been made in the past few years towards the genetic manipulation of *C. albicans*. Many *C. albicans* genes have been cloned by functional complementation of *S. cerevisiae* mutants, by nucleotide homology, or by conferring a unique phenotype on the cells (reviewed in Kurtz *et al.*, 1990; Scherer & Magee, 1990). Here we report the cloning of a *C. albicans* peptide transport gene by functional complementation of a *S. cerevisiae* peptide transport mutant.

METHODS

Strains, vectors and media. The *S. cerevisiae* strains used in this study were PB1X-9B (MATa *ura3-52 leu2-3,112 lys1-1 his4-38 ptr2-2*), PB3X-5C (MATa *ura3-52 leu2-3,112 lys1-1 his4-38 ptr2-1*), and PB1X-2A (MATa *ura3-52 leu2-3,112 lys1-1 his4-38, PTR2*). These strains were constructed as described previously (Perry *et al.*, 1994). *C. albicans* strain ATCC 18804 (CBS-562) was used for Southern blot analysis.

The genomic *C. albicans* library in the yeast *Escherichia coli* shuttle vector YEp24 was kindly provided by Dr Esther Segal, Dept of Human Microbiology, Tel Aviv University, and Dr Zeev Altboum of the Israel Institute for Biological Research. The library was constructed by partial digestion of DNA from *C. albicans* ATCC 18804 (CBS-562) with *Bam*HI, ligation of 5–15 kb fragments in the tetracycline-resistance gene of YEp24, followed by transformation of *E. coli* strain HB101 to ampicillin resistance (Altboum *et al.*, 1990).

C. albicans and *S. cerevisiae* cells were maintained on YEPD medium containing (w/v): peptone 2%; glucose 2%; yeast

extract 1%; agar (Difco) 2%. The minimal medium (MM) used for most studies was made by adding 10 ml 10 × filter-sterilized YNB without amino acids and without ammonium sulfate (Difco), to 90 ml sterile water containing 2 g glucose, 100 mg allantoin and auxotrophic supplements depending on the strain as follows: histidine (20 mg l⁻¹), uracil (20 mg l⁻¹), lysine (30 mg l⁻¹) and leucine (30 mg l⁻¹) (Sherman *et al.*, 1986). Allantoin, a purine catabolite which can serve as the sole nitrogen source for *S. cerevisiae*, was used in MM as it is neutral with respect to peptide transport activity, whereas other nitrogen sources, such as ammonium sulfate and amino acids, affect peptide transport (Basrai *et al.*, 1992; Island *et al.*, 1987). The mutant strain *S. cerevisiae* PB1X-9B was grown in Complete Medium (CM), which consisted of MM with histidine, uracil, lysine and leucine. *S. cerevisiae* PB1X-9B transformed with YEp24-based plasmids was grown on CM lacking uracil (CM-Ura) and those transformed with pRS201-based plasmids were grown on CM lacking leucine (CM-Leu) medium. Dipeptide medium [His-Lys, Lys-Met, Lys-Lys, His-Leu, Leu-Met, Leu-Leu, or Lys-Leu (80 µM)] consisted of MM supplemented with the auxotrophic requirements minus the amino acid components of the added peptides. For example, the dipeptide (Lys-Leu) medium for the growth of *S. cerevisiae* PB1X-9B transformed with the *PTR2* gene on a YEp24 plasmid contained MM supplemented with Lys-Leu and histidine. To determine the auxotrophic markers, 'drop-out' plates were made which consisted of MM with all the auxotrophic supplements except the one being tested. *E. coli* cells used for transformation were grown in LB medium (Sambrook *et al.*, 1989).

Enzymes, chemicals and reagents. L-Leucyl-L-[³H]leucine, Ala-Eth and oxalysine-containing peptides were synthesized by standard solution-phase techniques (Becker & Naider, 1977; Naider *et al.*, 1983) and have been described previously (Basrai *et al.*, 1992; Island *et al.*, 1991). Eth and the dipeptides were obtained from Sigma. Oxalysine, a toxic analogue of lysine, was a gift from Hong-Long Zhang of Shanghai Institute of Materia Medica, Academia Sinica, Shanghai 20021, China. Polyoxin D was purchased from Calbiochem; restriction endonucleases, T4 DNA ligase, T4 DNA polymerase and alkaline phosphatase were purchased from BRL, New England BioLabs or Promega, and were used according to the manufacturers' specifications.

DNA manipulations. Small-scale plasmid DNA preparations from *E. coli* transformants were as described in Sambrook *et al.* (1989), except that cultures were grown in modified Terrific Broth containing 50 µg ampicillin ml⁻¹. Large amounts of plasmid DNA were obtained using the pZ523 column chromatography protocol (5Prime → 3Prime, Inc.). Plasmid DNA from *S. cerevisiae* transformants was obtained as described by Sherman *et al.* (1986). Whole-cell DNA from *S. cerevisiae* was obtained as described by Ausubel *et al.* (1990). All agarose gels were prepared in 1 × TAE buffer (40 mM Tris acetate and 1 mM EDTA, pH 8.0).

E. coli cells were transformed using the Hanahan procedure described in Sambrook *et al.* (1989). Yeast transformations were done using the procedure described by Gietz *et al.* (1991) and plates were incubated at 30 °C for 4 d or longer.

For Southern analyses whole-cell DNA was digested with restriction enzymes and electrophoresed on 0.9% agarose gels (1–1.5 V cm⁻¹ for 14–18 h). Lambda DNA digested with *Hind*III was used as a size marker. Southern blotting was done as described in Sambrook *et al.* (1989). A PCR-amplified 980 bp fragment internal to *CaPTR2* (see Fig. 1b) was labelled with [α -³²P]dCTP using the random primer labelling kit from USB to a specific activity of 6–8 × 10⁸ c.p.m. per µg DNA. PCRs were carried out according to Innis & Gelfand (1990) for 35

cycles with a final MgCl_2 concentration of 1 mM with denaturation at 95 °C for 5 s, annealing at 50 °C for 5 s, and elongation at 72 °C for 1 min. Primers used in the PCR reaction were (P15) 5'CCAAAACAGATTCTCCAT3' and (P19) 5'GT-TTACAAGCTGCCAGTG3'. The blot containing the probe was incubated for 18 h at 65 °C in a rotary oven. The blot was washed twice in $1 \times \text{SSC}$ with 0.1% SDS at 55 °C for 15 min followed by two washes in $0.1 \times \text{SSC}$ with 0.1% SDS at 65 °C for 15 min. Autoradiography was performed at -70 °C in Kodak film holders using Kodak XAR-5 X-Ray film placed between two DuPont Cronex Lighting-Plus intensifying screens.

For plasmid-curing experiments, *S. cerevisiae* transformants were grown nonselectively in YEPD broth for about 40 generations. Cells were then plated on YEPD plates to obtain isolated colonies which were picked, washed with water and resuspended to 1×10^7 cells ml^{-1} . Three microlitres of the cell suspension was spotted as described in growth assays on appropriate media and checked for the auxotrophic markers, sensitivity to ethionine and Ala-Eth, and the ability to grow on dipeptides.

Several subclones were generated from plasmid pMB3 (Fig. 1a) to determine the minimum *C. albicans* DNA fragment that could functionally complement the *S. cerevisiae ptr2* mutation (Fig. 1b). Plasmids pMC1, pMC2 and pMC7 were derived from pMB3 by ligation of insert DNA into plasmid pRS201 (Sikorski & Hieter, 1989). Plasmid pMC1 contained a 3.2 kb *HindIII* fragment of the insert DNA and pMC2 contained a 2.9 kb *HindIII* fragment (with 348 bp of flanking vector DNA). Plasmid pMC7 contained a 2.1 kb *PstI-HindIII* fragment from pMB3 ligated to the *PstI-HindIII* site of pRS201. Plasmids pMC9 and pMC11 contain frameshift mutations at the *BglII* site and *KpnI* site, respectively, and were generated by cleavage with the appropriate restriction enzyme, conversion to blunt ends with T4 DNA polymerase and ligation. Plasmid pMC12 was constructed by deletion of 2.4 kb *NheI-KpnI* fragment from pMB3 while plasmid pMC13 was constructed by deletion of the 1.1 kb *MluI-BamHI* fragment from pMC12. The constructed plasmids were then used to transform strain PB1X-9B. A two-step selection was done in which transformants were first plated on CM-Leu medium for constructs with *LEU2* marker (pRS201) or CM-Ura for constructs with *URA3* marker (YEp24). *LEU*⁺ and *URA*⁺ transformants were then plated on dipeptide medium containing Lys-Leu with other auxotrophic requirements minus the amino acid components of the dipeptide.

The entire 5.1 kb subclone corresponding to pMC13 that contained *CaPTR2* was sequenced. Sequencing was done using the fluorescent dideoxy-terminator method of cycle sequencing on an Applied Biosystems 373A automated DNA sequencer, following the manufacturer's protocols (McCombie *et al.*, 1992; Smith *et al.*, 1986). Both strands were sequenced using primers to fully confirm the order of bases. The DNA sequencing for this analysis was done at the DNA analysis facility of Johns Hopkins University, School of Medicine.

Growth and transport assays. Growth assays to determine the phenotype of the cells was done as described by Island *et al.* (1991). Briefly, 3 μl of culture from a suspension of 1×10^7 cells ml^{-1} was applied to the surface of the medium and plates were incubated at 30 °C for 48 h or longer.

Uptake of L-leucyl-L-[³H]leucine was determined as described by Basrai *et al.* (1992). The final concentration of the components in the uptake assay was: glucose (2%, w/v), 4 mM sodium citrate/potassium phosphate buffer (pH 5.5), and L-leucyl-L-[³H]leucine (10 μM ; 20 mCi mmol^{-1} ; 740 MBq mmol^{-1}). Competition experiments were done in the presence of either 1.0 mM L-leucine or 1.0 mM L-leucyl-L-leucine. There was no peptide

adsorption to the cell surface or sticking to filters since at 0 °C the counts were at background level. Each point on the uptake curve represents the mean of three independent determinations whose values did not vary more than 15% from the mean value. The uptake results, calculated on the basis of 50% counting efficiency (determined using L-[³H]lysine as a standard, and the specific activity of the peptide), are expressed as nmol peptide uptake per mg cell dry weight.

Sensitivity assays. Sensitivity to ethionine, Ala-Eth, oxalysine and oxalysine-containing peptides was determined as described by Island *et al.* (1987). Zones of inhibition were measured after 24 h incubation at 30 °C. Each test comprised at least three independent assays and the results represented in the Tables are means of the values obtained. Maximum variation between the zones of inhibition measured for each test was ≤ 2 mm. A value of 7 mm for the diameter of zone of inhibition represents a minimal growth inhibition value as the disk diameter was 6 mm.

Sensitivity to polyoxin D was determined using a liquid assay as described by Cabib (1991). Briefly, cells were grown overnight in YNB supplemented with lysine (30 mg ml^{-1}) and leucine (30 mg ml^{-1}) to a density of approximately 1×10^7 cells ml^{-1} . The cultures were diluted to 1×10^6 cells ml^{-1} in fresh growth medium with or without 750 μg polyoxin D ml^{-1} and incubated at 30 °C for 5 h. Appropriate dilutions of the cultures were plated on CM-Ura medium, incubated at 30 °C for 36 h and colonies were counted.

RESULTS

Cloning of a *C. albicans* peptide transport gene

We screened a *C. albicans* genomic library in YEp24 to determine if the *ptr2* mutation in *S. cerevisiae* could be functionally complemented by a *C. albicans* gene. The host for transformation was the *S. cerevisiae ptr2-2* mutant, strain PB1X-9B, which is resistant to the toxic dipeptide Ala-Eth, fails to accumulate radiolabelled dileucine at wild-type levels, and is unable to use dipeptides to satisfy its auxotrophic requirement for amino acids. A two-step selection was utilized in which *S. cerevisiae* transformants were first plated to select for uracil prototrophs. The *URA3*⁺ transformants were then plated and scored for the ability to grow on dipeptide medium: MM containing histidine and the dipeptide Lys-Leu in place of the lysine and leucine. This strategy precluded the complementation of the amino acid auxotrophies by homologous *C. albicans* *LYS* and *LEU* genes, and provided strong selective pressure for isolation of peptide transport genes. Histidine in the dipeptide medium served to supplement for the *his4-38* mutation in PB1X-9B and induce the peptide transport system (Basrai *et al.*, 1992; Island *et al.*, 1987). Approximately 15000 *S. cerevisiae* *URA3*⁺ transformants were obtained after transformation of strain PB1X-9B with 1.5 μg DNA from the *C. albicans* library. A portion of each of seven pools, each representing approximately 2200 independent *URA3*⁺ transformants, was plated on dipeptide medium. Several hundred *S. cerevisiae* *PTR2*⁺ colonies from each pool were obtained on dipeptide medium after incubation for 3–4 d at 30 °C. Amino acid requirements of these colonies when checked using 'drop out' plates corresponded to the expected auxotrophies.

Plasmid DNA was isolated from a representative from each of the seven pools of the *S. cerevisiae* *PTR2*⁺

Fig. 1. Restriction enzyme map and subcloning of *C. albicans* insert DNA in plasmid pMB3. (a) The restriction sites were mapped by using single and double restriction enzyme digests. The thick line represents the YEp24 vector sequences. The genes *tet*, *amp*, and *ori* are derived from plasmid pBR322 which encode for tetracycline resistance, ampicillin resistance and the origin of replication for pBR322, respectively. The *URA3* and 2μ are derived from *S. cerevisiae* and encode the *URA3* gene, and the 2μ sequence, respectively. Restriction enzymes are abbreviated as B, *Bam*HI; P, *Pst*I; M, *Mlu*I; H, *Hind*III; R, *Eco*RV; D, *Nde*I; K, *Kpn*I; G, *Bgl*II; N, *Nhe*I; I, *Hinc*II. (b) Plasmids containing deletions, subclones or frameshift mutations were derived from plasmid pMB3 as described in Methods, and tested for their ability to complement the *S. cerevisiae ptr2-2* mutation in strain PB1X-9B. Ability of each plasmid to functionally complement the *ptr2-2* mutation is represented as a plus (+) or lack of complementation as a minus (-). Frameshift mutations at the *Bgl*II site in plasmid pMC9, and at the *Kpn*I site in plasmid pMC11, are indicated by a triangle with +4 to denote the frameshift after the site was filled in with Klenow. The PCR-generated probe used in the Southern blots is marked as such.

transformants and was used to transform *E. coli* HB101. Restriction enzyme analysis of the plasmids from the *E. coli* transformants showed that two different plasmids, pMB3 and pMB5, were isolated, with inserts of 8.7 kb and 11.2 kb, respectively. A partial restriction enzyme map of the 8.7 kb insert in plasmid pMB3 was determined (Fig. 1a). The 8.7 kb insert of pMB3 was also present in plasmid pMB5 (data not shown). Plasmids were amplified and used to transform the mutant strain *S. cerevisiae* PB1X-9B. Both plasmids, pMB3 and pMB5, were capable of complementing the *ptr2-2* mutation in strain PB1X-9B. It was thus concluded that the 8.7 kb DNA fragment present in plasmid pMB3 probably contained the entire *C. albicans CaPTR2* gene. Deletion and frameshift analyses were used to localize the *PTR2* complementing region to a 5.1 kb insert contained in plasmid pMC13 (Fig. 1b).

Fig. 2. Transport of L-leucyl-L-[³H]leucine in *S. cerevisiae* transformants. *S. cerevisiae ptr2* gene deletion strain PB1X-2AΔ transformed with plasmids (YEp24) (▽) or (pMB3) (●), and the wild-type PB1X-2A transformed with (YEp24) (○) grown overnight in CM-Ura medium were harvested in the exponential phase of growth ($1-2 \times 10^7$ cells ml⁻¹), washed and resuspended to 1.5×10^8 cells ml⁻¹ in 2% glucose. Cell suspension (500 μl) was added to an equal volume of reaction mixture containing L-leucyl-L-[³H]leucine (10 μM; specific activity 20 mCi mmol⁻¹; 740 MBq mmol⁻¹) as described in Methods. At various time points, 180 μl portions were removed, placed on filters and radioactivity was measured by liquid scintillation counting. Each point represents the mean of three values whose values do not vary more than 15% from the mean value.

Plasmids pMB3 and pMC13 were able to functionally complement the *ptr2* mutation in PB3X-9C, an independently isolated *ptr2-1* mutant, and PB1X-2AΔ, a *PTR2* deletion strain.

A plasmid-curing experiment showed that the primary *S. cerevisiae* transformants (obtained after transformation with the *C. albicans* library) and secondary *S. cerevisiae* transformants (obtained after transformation with a plasmid isolated from the primary transformants and shuttled through *E. coli*) had lost both the *URA3* marker and the *PTR*⁺ phenotype. This suggested that the phenotype of the *S. cerevisiae* transformants carrying plasmid pMB3 was due to a plasmid-borne gene and not due to a reversion of the *ptr2-2* allele.

Transport of L-leucyl-L-[³H]leucine in *S. cerevisiae* transformants

Neither strain PB1X-9B nor PB1X-2AΔ accumulated radioactivity from labelled dileucine (Island *et al.*, 1991, Perry *et al.*, 1994). PB1X-2AΔ carrying plasmid YEp24 was also unable to transport dileucine (Fig. 2). In contrast, both the wild-type strain PB1X-2A carrying the YEp24 shuttle vector and PB1X-2AΔ transformed with pMB3 showed similar high levels of dipeptide uptake (Fig. 2). The uptake of radioactive dileucine was competed by unlabelled dileucine but not by leucine (data not shown). These results show that pMB3 restored peptide transport

Table 1. Sensitivity of *S. cerevisiae* transformants to ethionine, oxalysine, Ala-Eth and oxalysine-containing peptides

Disk sensitivity assays were done as described in Methods using CM-Ura medium. *S. cerevisiae* strains PB1X-2AΔ (Δ PTR2::LEU2) and PB1X-2A (PTR2), which also have other auxotrophic requirements, are described in Methods. The plasmids used to transform the strains are given in parentheses. YEp24 plasmid is a shuttle vector which contains the *S. cerevisiae* URA3 gene as the selectable marker. pMB3 is plasmid YEp24 containing the *CaPTR2* gene.

Transformant	Zone of inhibition (mm)*						
	E	AE	O	OG	OLG	O(L) ₂ G	O(L) ₃ G
PB1X-2A(YEp24) [PTR2]	50	45	None	35	24	None	None
PB1X-2AΔ(YEp24) [ΔPTR2]	47	None	None	None	None	None	None
PB1X-2AΔ(pMB3) [ΔPTR2]	47	41†	None	20†	24†	None	None

* Ethionine or Ala-Eth (0.38 μmol), oxalysine or oxalysine-containing peptides (0.25 μmol) were spotted on a disk (diameter 6 mm). E, ethionine; AE, Ala-Eth; O, oxalysine; OG, Oxa-Gly; OLG, Oxa-Leu-Gly; O(L)₂G, Oxa-(Leu)₂-Gly; O(L)₃G, Oxa-(Leu)₃-Gly. None, no growth inhibition. Each test comprised three independent assays and the results represented in the Table are means of the values obtained. Maximum variation between the zones of inhibition for each test was ≤ 2 mm.

† The outline of the zone of inhibition was not very sharp and a hazy zone of inhibition was observed (see Fig. 3).

function to the mutant strain, and that dileucine enters through a transport system distinct from the amino acid permease(s). The initial rate of peptide transport in strain PB1X-2AΔ(pMB3) was equal to the wild-type level, but the total accumulation was lower upon longer incubation of the cells.

Dipeptides as growth substrates for *S. cerevisiae* transformants

Growth assays showed that the mutant strains PB1X-9B (*ptr2-2*), PB3X-9C (*ptr2-1*) and PB1X-2AΔ(Δ PTR2) with or without the YEp24 vector were unable to use the dipeptides Lys-Leu, Lys-Met, Lys-Lys, His-Leu, Leu-Leu, or His-Lys to satisfy the auxotrophic requirements for amino acids. *S. cerevisiae* strains PB3X-9C, PB1X-9B or PB1X-2AΔ transformed with plasmid pMB3 were able to use all the dipeptides mentioned above as substrates to satisfy corresponding amino acid auxotrophies in appropriately supplemented media (data not shown). The wild-type strain, PB1X-2A, with or without the vector YEp24, was able to grow on all of the dipeptide substrates tested.

Sensitivity of *S. cerevisiae* transformants to toxic compounds

The mutant strain PB1X-9B is sensitive to the toxic amino analogues ethionine and oxalysine, but is resistant to the toxic peptides Ala-Eth and oxalysine-containing di- and tripeptides (Island *et al.*, 1991; Perry *et al.*, 1994). For disk sensitivity assays, lysine was added to the growth medium to supplement auxotrophic requirements. Lysine reverses the toxicity of oxalysine (Table 1) by preventing this

amino acid from entering the yeast (Basrai *et al.*, 1992). Disk sensitivity assays showed that the resistance of the deletion mutant PB1X-2AΔ to Ala-Eth, Oxa-Gly and Oxa-Leu-Gly was reversed by transformation with plasmid pMB3 (Table 1). However, the outlines of the zones of inhibition were not very sharp and growth could be observed close to the boundary of the zone for Ala-Eth (Fig. 3). No toxicity was observed for oxalysine-containing tetra- or pentapeptides toward the *S. cerevisiae* wild-type or any transformant tested. However, *C. albicans* ATCC 18804 exhibited sensitivity to oxalysine-containing di-, tri-, tetra- and pentapeptides (data not shown).

S. cerevisiae PB1X-2A(YEp24) was sensitive (8% of cells were viable), whereas PB1X-2AΔ(YEp24) was relatively resistant (80% of cells viable) to the toxic effect of the peptidyl nucleoside polyoxin D (750 μg ml⁻¹) after a 5 h incubation. Under similar conditions *S. cerevisiae* PB1X-9B(pMB3) showed an intermediate phenotype between the deletion strains and the wild-type strains, as 32% of the cells were viable after incubation with the drug.

Southern blotting and chromosome mapping of *CaPTR2*

Southern blotting was done to establish whether the cloned fragment of DNA originated from *C. albicans* genomic DNA, and to determine if there were other homologous genes in *C. albicans*. Whole-cell DNA was isolated from *C. albicans* ATCC 18804 and digested with enzymes (*Pst*I, *Hind*III, and *Mlu*I/*Hinc*II) predicted to yield one band on the basis of the restriction map of pMB3 (Fig. 1a). DNA was resolved by electrophoresis, and

Fig. 3. Sensitivity of *S. cerevisiae* transformants to Eth and Ala-Eth. Disk sensitivity assays were done in CM-Ura medium as described in Methods. (a) Eth or (b) Ala-Eth (0.38 μ moles) was applied to disks placed on a lawn of cells. Plate (a), *S. cerevisiae* PB1X-2A(YEp24); plate (b), *S. cerevisiae* PB1X-2A Δ (YEp24); plate (c), *S. cerevisiae* PB1X-2A Δ (pMB3).

Fig. 4. Southern blots of whole-cell DNA from *C. albicans* probed with a PCR-amplified *C. albicans* CaPTR2 internal probe. Whole-cell DNA isolated from *C. albicans* ATCC 18804 was digested with *Pst*I (lane 1), *Hind*III (lane 2) and *Mlu*I/*Hinc*II (lane 3), and separated on a 0.9% agarose gel. The blot was probed with a 32 P-labelled 980 bp PCR-amplified internal fragment. The location of the probe is indicated in Fig. 1(b).

Southern blotting was done as described in Methods. As seen in Fig. 4, the PCR-derived probe (as described in Fig. 1b) produced the expected bands for all digests (7.9 kb for *Pst*I digests, 3.1 kb for *Hind*III digests, and 3.8 kb for *Mlu*I/*Hinc*II double digests) along with a second band in the *Hind*III digest (5.4 kb). The presence of the unexpected band can be explained by multiple copies or restriction fragment length polymorphism (RFLP) within this diploid organism. The CaPTR2 probe did not hybridize to whole-cell DNA from *S. cerevisiae* under the same stringency conditions (data not shown). The CaPTR2 gene was mapped to chromosome R that contains the rDNA-containing linkage group (Wickes *et al.*, 1991) by Dr B. B. Magee of University of Minnesota (personal communication; data not shown).

Nucleotide and deduced amino acid sequences of CaPTR2

The dideoxynucleotide chain-termination method was used to determine the nucleotide sequence of both strands of the complementing region in the plasmid pMC13.

Sequence analysis revealed the presence of ORFs of 488 amino acids (nucleotides 1–1425) and 148 amino acids (nucleotides 1510–1953, Fig. 5) separated by 84 nucleotides. The highly conserved consensus sequences of a type II intron, consisting of a 5' splice site (GTATGT), a 3' splice site (TAG), and a branch point (TACTAAC), were identified in the region separating the two ORFs (Rymond & Rosbash, 1992). The subclone pMC13, which contains both exons and the intron, functionally restored the PTR2 phenotype to mutants, while the subclone, pMC1, which contains the first exon, the intron and 59 nucleotides of the second exon, did not (Fig. 1). Furthermore, exon 2 encodes a peptide of 148 residues which showed 25.5% identity and 59.1% similarity to the last 148 amino acids of SacPtr2p, implicating a highly conserved region of the protein that is presumably necessary for function. Therefore, based upon this sequence analysis and subcloning data, we propose that the CaPTR2 gene contains a type II intron spanning 84 nucleotides and separating two ORFs of 475 and 148 amino acid residues (Fig. 5). In contrast, no intron was found in the SacPTR2 gene (Perry *et al.*, 1994).

A search of the GenBank database with the BLAST algorithm (Altschul *et al.*, 1990) showed that three proteins identified as peptide transporters had sequence similarity to CaPtr2p. CaPtr2p exhibited 30% identity and 56.9% similarity to SacPtr2p from *S. cerevisiae* (Perry *et al.*, 1994), 32% identity and 54.8% similarity to AtPtr2p from *Arabidopsis thaliana* (Steiner *et al.*, 1994), and 24.1% identity and 51.4% similarity to PepT1p from rabbit intestines (Fei *et al.*, 1994). These sequences were compiled under the Pileup program (Feng & Doolittle, 1987) of the Genetics Computer Group (GCG) database (Devereux *et al.*, 1984) and conserved sequences identified (Fig. 6).

DISCUSSION

The data presented in this paper show that a *C. albicans* gene, CaPTR2, has been cloned which functionally complements *S. cerevisiae* peptide transport mutants of the PTR2 complementation group. CaPTR2 complements two independently isolated ptr2 mutants as well as a *S. cerevisiae* strain which is deleted in the PTR2 gene. Due to

	TGATTAAATTAACCTTAATAGAAAATAGCTGCATATTTTCTTTTTCACAAATGGTTGGGTTTTTCATCTAAAGTGA	-712
	AAATTAATGAATCAATCACTCTTCAATGAACTCAAGAAAAAAATTTAATAAGGGTAAATCCGACATATTCGAA	-633
	AAAAAAACAGAGCGCTCCGATTAATTTCTTTCTTAATCCCGGATGATATGCAAGATAGCAAAACAAAGCATATCT	-554
	ACCAATAGTGTGAGAGCGGGGTGGAAGAGGGGGAGTAGCTCAGACATAGATAGGTCATAGAGCACTTAGAGGATAA	-475
	TGGAAGGGAGCGGATCAAAAAATTAAGAACCAAGTTGACGTGTGAGTTTGGTGATTTTTTGGGGCTAGTTTGGGGG	-396
	AATTCTTATTCGCAATTGGTTTTCACAGATTTTTCACAGACACCAAAAAATTTAATCAATTTTACAAACCGGGT	-317
	TACTAAAACTCAGAACATCTGTTGGAATGATAAATCATATTATACACATTTTCAACCATTCCTTTTACTATTGTGCACATT	-238
	ATTTCCAATTAAATCATATATCTTTTAAAAAAAACAAAAATTTGATGCCATATCCCTCATTAATGTGAAAAATTT	-159
	TCGTTGCCATATAAATTTGCCAACACATCTCTGATATACATACTAATTTGGTTTATTTTATTCTTTTGTGAAAAAC	-80
	ATTGTTCTTATGCTTTCAGCCCTCTTAAGTTTCTTACAACCTGAATTAATATACCATTTTGTAAATTTTATAACCAA	-1
1	ATG GTA TCT GAC TTT GAA AAT GAA AAA CAA CCA GAT GTT GTT CAA GTT CTA ACC GAT	60
	M V S S D F E N E A A K Q P D V V Q V L T D	
21	GAA AAA AAT ATT TCC TTG GAC GAT AAA TAT GAC TAT GAA GAC CCT AAG AAC TAC AGT ACA	120
	E K N N I T S L L D D K Y D D Y E D C C T A A K N Y A S T A	
41	AAC TAT GTT GAT GAC TAC AAT CCC AAA GGG TTA AGG AGA CCA ACT CCA CAA GAA TCT AAA	180
	N Y V D D Y N P K G L A R R P T P Q E S K	
61	TCT TTG AGA AGA GTT ATT GGT AAC ATA AGA TAC AGT ACA TTT ATG CTT TGT ATT TGT GAA	240
	S L R R A R V I G T N I R Y A T A F T M L C I T C E	
81	TTT GCT GAA CGT GCT TCA TAT TAT TCT ACC ACT GGT ATT CTT ACT AAT TAT ATT CAA AGA	300
	F A E R A S Y Y S T T G I L T N Y I Q R	
101	AGA ATT GAT CCT GAC TCA CCA CAT GGT TGG GGT GCA CCA CCA CCA GGA AGT CCA GAT GCT	360
	I D P C T S P H G G T G A P P P G S P D A	
121	TCA GCT GGT GCT TTG GGT AAA GGT TTA CAA GCT GCC AGT GCC TTG ACC AAT CTT TTA ACA	420
	S A G A L L G K G L Q A S A L T N L L T	
141	TTT TTA GCT TAT GTA TTG CTT TTA ATT GGT AGT TAT TTA GGT GAT AGC ACA ATT GGA AGA	480
	F L A A Y V F T I G G Y L A S A I T A C A I G R	
161	TGG AAA GCT ATT CAG TGG GGG GTA TTT TTT GGA TTT GTT GCC CAT TTG TTT TTT ATT TTT	540
	N K A A I Q W G V F F V A H L F F I F	
181	GCT AGT ATC CCA CAG GCA ATT GAA AAT GCC AAT GCT GGG TTG GGA TTA TGT GTT ATT CCA	600
	A S I P Q A I E A A N A G L G L C V I A	
201	ATT ATA ACT TTG TCA GCA GGA CTG GGA TTA ATG AAG CCT AAC TTG TTA CCT CTT GTT TTA	660
	I I T L S A C G L G L M A A C C T N L L P L V L	
221	GAT CAA TAC CCT GAA GAA AGA GAT ATG GTA AAA GTG TTA CCA ACA GGT GAA CTG ATT ATT	720
	D Q Y P E A R D M V K V L L P T G E L I I	
241	TTG GAT AGA GAG AAA AGT TTG AGT AGA ATC ACA AAC GTA TTT TAT CTT GCA ATT AAT ATT	780
	L D R E E K S L S R I T N V F A L A I N I	
261	GGT GCC TTT TTG GCA ATT GCT ACT TCG TAT TGT GAA AGA AGA GTT GGG TTT TGG CTT GCA	840
	G A F L Q I A T S Y C E R R V G F W L A	
281	TTT TTT GTT CCT ATG ATA TTG TAC ATA ATT GTA CCA ATT TTT TTA TTT ATT GTG AAA CCT	900
	F F V P M I L Y I I V P A C A T T F I V I K P	
301	AAA CTT AAG ATT AAG CCA CCA CAA GGT CAA GTC ATG ACC AAT GTC GTC AAG ATT TTA GCA	960
	K L K I K P P Q Q V Q V M T N V V K I L A	
321	GTT TTG TTT TCT GCA NAT TTC ATC AAG AGA TTG TGG AAT GGA ACA TTT TGG GAT CAT GCA	1020
	V L F S G N F I K R L L W N A G A T F W D H A	
341	AGA CCT TCA CAT ATG GAA GCC AGA GGG ACT ATT TAC TAC AAT AGT AAA AAG AAA AGT GCC	1080
	R P S C A T A R G G T T Y N S K K K S	
361	ATT ACT TGG TCT GAC CAA TGG ATA TTA GAT ATC AAG CAA ACA TTT GAT TCC TGC AAA ATT	1140
	I T W S D Q W I L D I K Q T F D S C K I	
381	TTT CTT TAC TAT ATT ATT TCT AAT TTG GCC GAT AGT GGA TTA GGA TCA GTA GAA ACT TCC	1200
	F L Y Y I I F N L A D S G L G S V E T S	
401	TTA ATT GGT GCT ATG AAA TTA GAC GGA GTT CCA AAT GAT CTT TTT AAT AAT TTT AAT CCA	1260
	L I G A M K L D G V P N D L F N N F N P	
421	TTG ACC ATT ATC ATT TTG ATT CCG ATC CTT GAA TAC GGA CTC TAC CCA TTG TTG AAC AAA	1320
	L T I I I L I P I L E A Y G L C P L L N K	
441	TTC AAG ATT GAC TTT AAA CCA ATA TGG AGA ATC TGT TTT GGA TTT GTT GTT TGT TCC TTT	1380
	F K I D F K P I W R I C F T G F V V C S F	
461	TCA CAA ATT GCC GGG TTT GTT TTA CAA AAA CAA GTT TAT GAG CAA GTA TGT AAA TTA TCA	1440
	S Q I I G G F V L Q K Q V Y E E	
	GAG GTT TTT TTT ACA CTC ATT GTA TAG TTC TTT TCA AGA TTC CGT TGA TCA CTA A TCC	1500
	TTT TTT TAG TCA CCA TGT GGA TAC GCT ACC AAC TGT GAT AGT CCA GCT CCA ATC ACT	1560
476	TTT TTT TAG TCA CCA TGT GGA TAC GCT ACC AAC TGT GAT AGT CCA GCT CCA ATC ACT	
	S P C G Y Y A T N C D S P A P I T	
493	GCC TGG AAA GCT TCA TCT CTT TTC ATA TTA GCC GCC GCT AGT GAA TGT TGT GCT TAT ACC	1620
	A W K A S L F I L A C A G E C W A Y T	
513	ACT GCT TAT GAA TGT GCA TAT ACC AGA TCA CCA CCA GCA TTT AAA AGT CTC GTA TAT GCC	1680
	T A Y E L A Y T R S P P A L K S L V Y A	
533	TTA TTT TTA GTA ATG TCT GCT TTC TCC GCT GCA TTG AGT CTT GCC ATA ACT CCA GCT TTA	1740
	L F L V M N S A F S L L L A I T P A A L	
553	AAA GAC CCT AAT TTA CAT TGG GTA TTC CTT GCA ATT GGT CTT GCT GGA TTC CTT TGT GCC	1800
	K D P N L H W V F L A I G G A T T C L C A	
573	ATT GTT ATG TTG GCT CAA TFC TGG AAT TTG GAT AAA TGG ATG GAA AAT GAA ACA AAT GAA	1860
	I V M L A Q T N W N L D K W M E N E A T N E	
593	AGA GAA AGA TTG GAT AGA GAA GAA GAG GAA GCC AAC AGA GGA ATC CAC GAT GTT GAT	1920
	R E R L D R E E E A A N R G I H D V D	
613	CAT CCA ATT GAA GCA ATT GTA TCT ATC AAG TCA TGA TTATATATTTATATATAAGATTACATTACTC	1987
	H P I E A I V S I K S END	
	TTTCGAATTAATGACATTTGCCAACATACATCTTCTTCTAATTAGTTTATGTGAAAAATTAATCTAGTATAAGCTGCTT	2066
	TTTCTTGTATGAGAGAACTCTTAATGGCATGTTTATCAATGAAGTAGGCAAGAAATGTAAATCAATATCT	2145
	CTGCAAAATTTTATCTTAAAAAATGCTTTTATTAATCAATCAAAATAGCCAAACCTTTTATGTCGTCGTATAT	2224
	TCTATAAAGTTTAAATACGATATCTTTCTTTTAAATCCCTTTTTCGATCTGGTTTCTTATTTATGTTTTCACCT	2303
	TTTATTTGCTGTCAACGAGAACCTCGGCAAACTTAATTTTTCACAGTGAAAAATACCAACAATATCATTTGTT	2382
	TTAAAGTTGTTAATAAACTCCCTTTTCTTGGCGAGTCCAAATGCACATCAGGGAAAGCTGTCCATTTTCTTATTT	2461

Atptr2	MSII	EQITKSDSD	FIISEDQSYL	SKEKADGSA	TINQADEQSS	44	
Sacptr2	MLNHPQSGSD	DAQDEK QGD	FFVIEEE	..KTQAVTLKD	SVYTDVANS	45	
Captr2	MVSS	DFENEKQPDV	QVQLTDE	K	NISLDDKYDY	32	
Rabbitptr							
Consensus	-----M-S-	D-Q-EK----	F-V-EDE----	K-----	-I--DD-----S		
Atptr2	TDELQKSMST	GVLVNGDLYP	SPTEELATL	PSVCGTIPWK	AFIILIVELC	94	
Sacptr2	TERYNLSPTF	..EDEDDE	FTTEEDMQTL	RHVGGKIPMR	CWLIAIVELS	91	
Captr2	EDPKNYSNY	VDVYNPKGLR	RPTPEKSKL	RRVIGNIRYS	TFMLCICFPA	82	
Rabbitptr							
Consensus	TD--N-S-S-	-----N-----	-PTEEE-K-L	R-VGG-IP-S	-P-I-IVE-C		
Atptr2	ERFAYIGLTV	PFQNYMQF		..GPKDATP	GALNIGETGA	129	
Sacptr2	ERFSYGLSA	PFQNYMEY		..GPNDSPK	GVLNLSAQGA	126	
Captr2	ERASYYSTTG	ILTYITQRII	DPDSPHGWA	PPGSPDASA	GALGKGLQAA	132	
Rabbitptr	ERFSYGMRA	LLILYF			NFIGMDNLS	52	
Consensus	ERFSYIGLTA	P-QNYMQ-	-----	---GP-DA--	GALGLG-QGA		
Atptr2	DGLSNFFTFM	CVVTFVGAAL	IADQFLGRYN	TIVCSAVIYP	IGILILITCTA	179	
Sacptr2	TGLSYFFQFM	CVVTFVVDSD	ITLQNVFQYV	TICCGTAIYI	AGIFILITPIS	176	
Captr2	SALTNLLTFL	AVVFLIGGY	LGDSTIGRMK	AIQWGVFPF	VAHLFFIFAS	182	
Rabbitptr	TVIYHTFVAL	CYLPFLGAL	AVCMGLGKFK	TYVLSIVYT	IGQAVTSLS	102	
Consensus	TGLSNFFTF-	CVVTFV-G--	IAD-PLG-Y-	TIV-G--IYF	IGILIL--TS		
Atptr2	IP.SVIDAGK	S.....M	GGFVSLIII	GLGTGGIKSN	VSLPMAQLP	220	
Sacptr2	IP.SVGNRDS	A.....	GGFVSLIII	GLGTGGIKSN	VSLPMAQLP	217	
Captr2	IP.QALINANA	G.....L	GLCVTAIITL	SAGLGLMKPM	LLPLVLQDYP	224	
Rabbitptr	VNELTDNRND	GTPDPLPVHV	AVCMGLGKFK	ALGTGGIKPC	VSAFGGDQFS	152	
Consensus	IP-SV-NA--	G-----	GG-VIA-ILI	GLGTG-IPN	-SPL-ADQLP		
Atptr2	KIPPYVTKTK	NGSKVIVDPV	VTTSRAYMIF	YVTVNGVSL	VLATTSLES	269	
Sacptr2	KRPKSIKVLK	SGERVIVDSN	ITLQNVFQYV	YFMVNGVSL	LMATTELEY	266	
Captr2	EERDMVKVLP	TGELITLDRE	KSLSRITNVF	YLAINIGAFI	QIATSYCER	273	
Rabbitptr							
Consensus	K--P-VKVLK	-GE-VIVD--	-TLSR-PMIF	YLAINVSL-	--ATT-LER-		
Atptr2	TK	GFVYAVLPL	CVFVPLIIL	AVSKTAPTST	LLPVPVSLFV	311	
Sacptr2	HK	GFVYAVLPL	CVFVPLIIL	IPGKQY	..IQRPIDG	302	
Captr2	RV	GFVYAVLPL	CVFVPLIIL	PIVKKPL	..K	IKPQGGQVMT	313
Rabbitptr	QCGIIVKQA	CYPLAFGIPA	ILMAVSLIVF	IIGSGMY	..K	KFKPGQNLIS	233
Consensus	-----K	GFVLA-L-P-	-LF-I-LI-L	IIGK--Y-K	I-PP-G----		
Atptr2	..LVKCSLL	KTNLISKKFN	HLALL		..LERYVKDQW	345	
Sacptr2	..KVIKSKFKV	CMILTQKFD	FNAAKPS		..VHPKQYPM	337	
Captr2	..NVVKILAVLF	SGNFIRKLN	GTFWDHARPS	HMEARGTIYI	NSKKKSAITM	363	
Rabbitptr	..KVVKICIFA	IKNRFR	HRSKQPKRA	HMLDA		..KEYK	268
Consensus	..VVKC-SFL-	..NLINKNKN	-----AKPS	H-----	-----K-W		
Atptr2	DDLFIDELKR	ALRACKTFLF	YPIYWCYGO	MTNNLSQAG	QM.....QTGN	391	
Sacptr2	NDKPVDEIKR	ALRACKTFLF	YPIYWCYGO	MTNNLSQAG	MM.....ELHG	393	
Captr2	SDQWILDIKQ	TPDSCIKPLY	YIIFNLADSG	LGSVETSLIG	AM.....KLGD	409	
Rabbitptr	DERLIAQIKM	VTVPLFLYIP	LPFWALDVG	QGSRTWLQAT	TMSGRTGLAS	318	
Consensus	DD-FIDEIKR	ALRACK-F-F	YPI-W--YGO	MGS---SQAG	M-----L-G		
Atptr2	VSNDLQAFD	SIALIIFIPI	CDNIYPLLR	KYNIPFKPII	RITLGFPMFT	441	
Sacptr2	IPNDFLQAFD	SIALIIFIPI	FEKIVYVFT	RY..TLPKPI	KIFFGPMFGS	432	
Captr2	VPNDFLNNFN	PLTIIILIFI	LEYGLVPLN	KFKIDFKPIW	RICFPGVVC	459	
Rabbitptr	IOPDMQTVN	TILLIILVPI	MDAVVYPLIA	KGLNFTSLK	KMTIGHPLAS	368	
Consensus	..PNDFLQAF-	SIA-II-IPI	---VYPL-R	KY-IPFKPI-	-ITFGPMFAS		
Atptr2	ASMIYAAVLQ					451	
Sacptr2	FAHTMAAVLQ					442	
Captr2	FSQIAGFVLQ					469	
Rabbitptr	MAFVAAAILQ	VEIDKTLFV	PKANEVQIKV	LVNGSENHII	SLPGQVTILN	418	
Consensus	F-MI-AAVLQ	-----	-----	-----	-----		
Atptr2							
Sacptr2							
Captr2							
Rabbitptr	QMSQTNEFMT	FNEDTLTSIN	ITSGSQVTMI	TPSLEAGQRH	TLVWMAPNMY	468	
Consensus							
Atptr2				..AKIYQRG	PCYANF	464	
Sacptr2				..SFVYKAG	PWYNBP	455	
Captr2				..KQVYEQS	PCGYIA	482	
Rabbitptr	RVVNDGLTQK	SKDGKENGIRF	VNTYSQPINV	TMSGKVYEHI	ASYNASEYQF	518	
Consensus				..KVYE-G	PCYN		
Atptr2							
Sacptr2							
Captr2							
Rabbitptr	FTSGVGQFTV	SSAGISEQCR	RDPESPYLEF	GSAYTYLITS	QATGCPQVTE	568	
Consensus							
Atptr2	TDTCVSNDIS	VWQIPAYVL	IAFSEIFASI	TGLEFAPTKA	PPSMKSIITA	514	
Sacptr2	LGHNTPMHVH	VCWQIPAYVL	ISFSEIFASI	TGLEFAYSKA	PASMSFIMS	505	
Captr2	TNCDSPAPIT	AWKASSLFI	AAAGECWAYT	TAYELAYTRS	PPALKSLVYA	532	
Rabbitptr	FEDIFPNTMN	MAWQIPQYFL	ITSEGVFISI	TGLEFSYSQA	PPSMKSLVLA	618	
Consensus	-----PN-I-	VWQIPAYVL	IAF-EIFASI	TGLEFAY-KA	PPSMKS-I-A		
Atptr2	LFLFTNAFGA	ILSICISSTA	VNPKLTWMT	GIATVAFIAG	IMFWCFPHY	564	
Sacptr2	IFLLTNAFGS	AIGCALSPVT	VDPKTLWLT	GLAVACFISG	CLFMLCFPKY	555	
Captr2	IFLVMSAFSA	ALSLAITPFI	KDPLNHWPL	AIGLAGFLCA	IVMLQAFMNL	562	
Rabbitptr	GMLLTAVAGN	ITVLITVAGG	IKNLQWAEYI	LFA..ALLLV	CVIPALMARF	587	
Consensus	IFLLTNAFGA	--SLAISPA-	VDPKLTW--T	G-AVA-F--G	--FWACF--Y		
Atptr2	DA.MEDBQNG	LE.FKRNDAI	TKKDKVEKH	DSYSHADESQ	YNLEKANC*	610	
Sacptr2	ND.YEEDHNA	MD.YEEDPE	VDNPIAPKA	NDIEILEPME	SLRSTPKY*	601	
Captr2	DKWMENETNE	RERLDREDEE	EANRGTMDVD	HPTEATVSIR	S*	623	
Rabbitptr	ITYVNPAAIE	AQF..REDEK	PEPKKNLDY	PSLAPVSQTO	M.....	706	
Consensus	D--ME-E-NE	-E-----EDE-	-KNP---DV-	-SIE-----Q	S-----		

Fig. 6. A GenBank comparison using the GCG program Pileup was done for the peptide transport proteins Ptr2p of *C. albicans*, *S. cerevisiae* and *A. thaliana* along with the Ptp1p from rabbit intestine. Conserved residues are denoted as 'consensus'.

the fact that CaPtr2p shows high similarity to SacPtr2p and contains multiple hydrophobic domains typical of transporter proteins (Grenson, 1992; Higgins, 1992), we propose that CaPtr2p is a membrane-bound structural component of peptide transport in *C. albicans*. However, CaPTR2 did not fully restore peptide transport activity to *S. cerevisiae* mutants. As seen in Fig. 2, the level of accumulation of radiolabelled dipeptide in *S. cerevisiae* PB1X-2AΔ(pMB3) was somewhat lower than in the wild-type strain *S. cerevisiae* PB1X-2A(YEp24). We also observed that transformants with the plasmid pMB3 showed decreased sensitivity to Ala-Eth, Oxa-Gly, Oxa-Leu-Gly and polyoxin D when compared to transformants with *SacPTR2* (data not shown). Interestingly, the 8.7 kb fragment from pMB3 did not complement *ptr2* mutants when expressed on a low-copy-number plasmid (data not shown). These results indicate either a sub-optimal expression of CaPTR2 in the heterologous system, or different structural specificities of the proteins encoded by the CaPTR2 gene as compared to the *S. cerevisiae* PTR2 gene. Several *C. albicans* genes, such as *ERG16* and *ERG7*, are reported to be expressed poorly in *S. cerevisiae* (Kelly *et al.*, 1990; Kirsch *et al.*, 1988). Additionally, it is possible that the product encoded by CaPTR2 is unable to interact efficiently with other requisite components of the *S. cerevisiae* peptide transport system to restore full peptide transport activity. This hypothesis is based on studies with *S. cerevisiae* in which at least three genes, *PTR1*, *PTR2* and *PTR3*, are involved in peptide transport. A mutation in either *PTR1* or *PTR2* renders the cells totally incapable of peptide transport (Island *et al.*, 1991). The mechanistic role of Ptp1p in peptide transport at this point is not fully understood but is postulated to be involved in regulation of *PTR2* expression (Alagramam *et al.*, 1995).

Southern blot analyses demonstrated each of the predicted bands as well as an additional band in the *Hind*III digest. The unexpected band can be explained by RFLP between the two chromosomal homologues or alternatively by multiple copies of CaPTR2 on the R chromosome. We favour the former explanation because all other digests produced their respective single bands. Both *Mlu*I and *Pst*I restriction sites reside well outside the ORF and we believe it unlikely that two enzymes would produce the same band in multiple copies of a gene. RFLP has been demonstrated previously at the *EXG1* (Chambers *et al.*, 1993) and *URA3* loci (Kelly *et al.*, 1987) in *C. albicans*.

The nucleotide sequence from pMC13 predicts a protein of 623 amino acids with a molecular mass of 70 kDa and a pI of 6.4. Computer-assisted analysis of the structure revealed an amino terminus of 63 hydrophilic residues followed by 12 hydrophobic segments of approximately 20 amino acids in length, with intervening hydrophilic segments and a carboxy terminus consisting of 43 hydrophilic residues. An almost identical hydrophathy plot was seen in peptide transporters in *S. cerevisiae* and *A. thaliana* (Steiner *et al.*, 1994). Furthermore, computer-assisted predictions of protein secondary structure revealed that the hydrophobic regions (putative transmembrane domains) consisted of β -sheet-forming regions

rather than α -helices. Predicted β -sheet formation in membrane proteins has been reported previously in the *S. cerevisiae* proteins Ptr2p (Perry *et al.*, 1994) and Dal5p, an allantoin permease (Rai *et al.*, 1988).

The existence of multiple peptide permeases based on growth and toxicity studies has been reported previously in *C. albicans* (Becker & Naider, 1995; McCarthy *et al.*, 1985; Payne *et al.*, 1991), while *S. cerevisiae* has been shown by genetic experiments to possess only a di-tripeptide transporter (Perry *et al.*, 1994). The fact that PB1X-9B transformed with pMB3 is resistant to oxalysine-containing tetra- and pentapeptides, whereas the wild-type *C. albicans* 18804 is sensitive to toxic tetra- and pentapeptides, suggests that CaPtr2p is only capable of transporting di- and tripeptides, although a size limitation may be imposed on CaPtr2p due to its expression in the *S. cerevisiae* heterologous host. Therefore, more genetic studies must be undertaken to determine whether multiple *C. albicans* peptide transport genes exist. Cloning of a *C. albicans* oligopeptide transporter gene by functional expression in *S. cerevisiae* is currently being attempted in our laboratory.

A BLAST search identified significant similarity between CaPtr2p and the peptide transporters SacPtr2p of *S. cerevisiae*, AtPtr2p of *A. thaliana*, and PepT1p of rabbit intestine (Fig. 6). In contrast, neither the *S. cerevisiae* STE6 gene, which exports the peptide mating pheromone α -factor from MATa cells (Kuchler *et al.*, 1989; McGrath & Varshavsky, 1989), nor the OPP operon of *Salmonella typhimurium* (Hiles *et al.*, 1987), which encodes the peptide transport system of enteric bacteria, hybridized to *C. albicans* DNA as determined by Southern blotting (data not shown). Furthermore, a BLAST search revealed no similarity between CaPtr2p and a *C. albicans* multiple-drug-resistance protein product containing 12 transmembrane domains (Ben-Yaacov *et al.*, 1994). This analysis has led us to the identification of a new family of predominately eukaryotic peptide transporters (Steiner *et al.*, 1994).

In summary, we report here the identification, cloning, and sequencing of a *C. albicans* gene that can functionally complement a *S. cerevisiae* peptide transport mutant. We have demonstrated that *S. cerevisiae* is a model recipient for peptide transporters and could provide an invaluable tool to evaluate peptide transport in other fungi and from complex organs, such as the intestine and the kidney, under controlled conditions. Such studies should prove invaluable in the rational design of peptide-based drugs.

ACKNOWLEDGEMENTS

This work was supported by a grant from the American Cancer Society, BE-39B. We gratefully acknowledge Dr Beatrice Magee of the University of Minnesota for chromosomal mapping of the CaPTR2 gene, Dr Esther Segal and Dr Zeev Altboum for providing the *C. albicans* genomic library, Roxanne Ingersoll for sequencing, and Kim Caldwell for helping with the Southern blotting. We thank Larry Zhang for peptide synthesis, Michael Craig and Keith Henry for technical assistance, and Stevan Marcus, Guy Caldwell, Henry-York Steiner, David Barnes and Kumar Alagramam for helpful suggestions.

REFERENCES

- Alagramam, K., Naider, F. & Becker, J. M. (1995). A recognition component of the ubiquitin system is required for peptide transport in *Saccharomyces cerevisiae*. *Mol Microbiol* **15**, 225–234.
- Altboum, Z., Gottlieb, S., Lebens, G. A., Polacheck, I. & Segal, E. (1990). Isolation of the *Candida albicans* histidinol dehydrogenase (HIS4) gene and characterization of a histidine auxotroph. *J Bacteriol* **172**, 3898–3904.
- Altshul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. (1990). Basic local alignment search tool. *J Mol Biol* **215**, 403–410.
- Ames, B. N., Ferro-Luzzi Ames, G., Young, J. D., Tsuchiya, D. & Lecocq, J. (1973). Illicit transport: the oligopeptide permease. *Proc Natl Acad Sci USA* **70**, 456–458.
- Ausubel, F. M., Brent, R., Kingston, R., Moore, D. D., Seidman, J. G., Smith, J. A. & Struhl, K. (1990). *Current Protocols in Molecular Biology* 2. New York: Wiley Interscience.
- Bartel, B., Wunning, I. & Varshavsky, A. (1990). The recognition component of the N-end rule pathway. *EMBO J* **9**, 3179–3189.
- Basrai, M. A., Zhang, H.-L., Miller, D., Naider, F. & Becker, J. M. (1992). Toxicity of oxalysine and oxalysine-containing peptides against *Candida albicans*: regulation of peptide transport by amino acids. *J Gen Microbiol* **138**, 2353–2362.
- Becker, J. M. & Naider, F. (1977). Peptide transport in yeast: uptake of radioactive trimethionine in *Saccharomyces cerevisiae*. *Arch Biochem Biophys* **178**, 245–255.
- Becker, J. M. & Naider, F. (1980). Transport and utilization of peptides by yeast. In *Transport and Utilization of Amino Acids, Peptides and Proteins by Microorganisms*, pp. 257–279. Edited by J. W. Payne. London: John Wiley.
- Becker, J. M. & Naider, F. (1995). Fungal peptide transport as a drug delivery system. In *Peptide Based Drug Design: Controlling Transport and Metabolism*, pp. 369–384. Edited by M. Taylor & G. Amidon. Washington, DC: American Chemical Society.
- Ben-Yaacov, R., Knoller, S., Caldwell, G. A., Becker, J. M. & Koltin, Y. (1994). *Candida albicans* gene encoding resistance to benomyl and methotrexate is a multidrug resistance gene. *Antimicrob Agents Chemother* **38**, 648–652.
- Cabib, E. (1991). Differential inhibition of chitin synthetases 1 and 2 from *Saccharomyces cerevisiae* by polyoxin D and nikkomycins. *Antimicrob Agents Chemother* **35**, 170–173.
- Chambers, R. S., Broughton, M. J., Cannon, R. D., Carne, A., Emerson, G. W. & Sullivan, P. A. (1993). An α - β -(1,3)-glucanase of *Candida albicans*: purification of the enzyme and molecular cloning of the gene. *J Gen Microbiol* **139**, 325–334.
- Devereux, J., Haeberli, P. & Smithies, O. (1984). A comprehensive set of sequence analysis programs for the VAX. *Nucleic Acids Res* **12**, 387–395.
- Fei, Y.-J., Kanai, Y., Nussberger, S., Ganapathy, V., Leibach, F. H., Romero, M. F., Singh, S. K., Boron, W. F. & Hediger, M. A. (1994). Expression cloning of a mammalian proton-coupled oligopeptide transporter. *Nature* **368**, 563–566.
- Feng, D.-F. & Doolittle, R. F. (1987). Progressive sequence alignment as a prerequisite to correct phylogenetic trees. *J Mol Evol* **25**, 351–360.
- Fickel, T. E. & Gilvarg, C. (1973). Transport of impermeant substances in *Escherichia coli* by way of oligopeptide permease. *Nature* **241**, 161–163.
- Gietz, D., Andrew, J., Woods, R. & Schiestl, R. (1991). Improved methods for high efficiency transformation of intact yeast cells. *Nucleic Acids Res* **20**, 1425.
- Grenson, M. (1992). Amino acid transporters in yeast: structure,

- function and regulation. In *Molecular Aspects of Transport Proteins*, pp. 219–245. Edited by J. J. H. M. De Pont. Amsterdam: Elsevier.
- Hammond, S. M., Claesson, A., Jansson, A. M., Larsson, L.-G., Pring, B. G., Town, C. M. & Ekstrom, B. (1987). A new class of synthetic antibacterials acting on lipopolysaccharide biosynthesis. *Nature* **327**, 730–732.
- Higgins, C. F. (1987). Synthesizing designer drugs. *Nature* **327**, 655–656.
- Higgins, C. F. (1992). ABC transporters: from microorganisms to man. *Annu Rev Cell Biol* **8**, 67–113.
- Hiles, I. D., Gallagher, M. P., Jamieson, D. J. & Higgins, C. F. (1987). Molecular characterization of the oligopeptide permease of *Salmonella typhimurium*. *J Mol Biol* **195**, 125–142.
- Innis, M. A. & Gelfand, D. H. (1990). Optimizations of PCR. In *PCR Protocols: a Guide to Methods and Applications*, pp. 3–12. Edited by M. A. Innis, D. H. Gelfand, J. J. Sninsky and T. J. White. New York: Academic Press.
- Island, M. D., Naider, F. & Becker, J. M. (1987). Regulation of dipeptide transport in *Saccharomyces cerevisiae* by micromolar amino acid concentrations. *J Bacteriol* **169**, 2132–2136.
- Island, M. D., Perry, J. R., Naider, F. & Becker, J. M. (1991). Isolation and characterization of *S. cerevisiae* mutants deficient in amino acid-inducible peptide transport. *Curr Genetics* **20**, 457–463.
- Kelly, R., Miller, S. M., Kurtz, M. B. & Kirsch, D. R. (1987). Directed mutagenesis in *Candida albicans*: one step gene disruption to isolate *URA3* mutants. *Mol Cell Biol* **7**, 199–207.
- Kelly, R., Miller, S. M., Lai, M. H. & Kirsch, D. R. (1990). Cloning and characterization of the 2,3-oxidosqualene cyclase-encoding gene of *Candida albicans*. *Gene* **87**, 177–183.
- Kirsch, D. R., Lai, M. H. & O'Sullivan, J. (1988). Isolation of the gene for cytochrome P450 L1A1 (lanosterol 14 α -demethylase) from *Candida albicans*. *Gene* **68**, 229–237.
- Kuchler, K., Sterne, R. E. & Thorner, J. (1989). *Saccharomyces cerevisiae* *STE6* gene product: a novel pathway for protein export in eukaryotic cells. *EMBO J* **8**, 3973–3984.
- Kurtz, M. B., Kelly, R. & Kirsch, D. R. (1990). Molecular genetics of *Candida albicans*. In *The Genetics of Candida*, pp. 21–73. Edited by D. R. Kirsch, R. Kelly & M. B. Kurtz. Boca Raton, FL: CRC Press.
- McCarthy, J. P., Nisbet, L. J., Boehm, J. C. & Kingsbury, W. D. (1985). Multiplicity of peptide permeases in *Candida albicans*: evidence from novel chromophoric peptides. *J Bacteriol* **162**, 1024–1029.
- McCombie, W. R., Heiner, C., Kelley, J. M., Fitzgerald, M. G. & Gocayne, J. D. (1992). Rapid and reliable fluorescent cycle sequencing of double stranded templates. *DNA Seq* **2**, 289–296.
- McGrath, J. P. & Varshavsky, A. (1989). The yeast *STE6* gene encodes a homologue of the mammalian multidrug resistance P-glycoprotein. *Nature* **340**, 400–404.
- Matthews, D. M. (1991). *Protein Absorption: Development and Present State of the Subject*. New York: Wiley-Liss.
- Naider, F. & Becker, J. M. (1987). Peptide transport in *Candida albicans*: implications for the development of antifungal agents. *Curr Top Med Mycol* **2**, 170–198.
- Naider, F., Shenbagamurthi, P., Steinfeld, A. S., Smith, H. A., Boney, C. & Becker, J. M. (1983). Synthesis and biological activity of tripeptidyl polyoxins as antifungal agents. *Antimicrob Agents Chemother* **24**, 787–796.
- Payne, J. W. (1980). Transport and utilization of peptides by bacteria. In *Microorganisms and Nitrogen Sources*, pp. 211–256. Edited by J. W. Payne. New York: John Wiley.
- Payne, J. W., Barrett-Bee, K. J. & Shallow, D. A. (1991). Peptide substrates rapidly modulate expression of dipeptide and oligopeptide permeases in *Candida albicans*. *FEMS Microbiol Lett* **79**, 15–20.
- Perry, J. R., Basrai, M. A., Steiner, H.-Y., Naider, F. & Becker, J. M. (1994). Isolation and characterization of a *Saccharomyces cerevisiae* peptide transport gene. *Mol Cell Biol* **14**, 104–115.
- Rai, R., Genbauffe, F. S. & Cooper, T. G. (1988). Structure and transcription of the allantoate permease gene (*DAL5*) from *Saccharomyces cerevisiae*. *J Bacteriol* **170**, 266–271.
- Rymond, B. C. & Rosbash, M. (1992). Yeast pre-mRNA splicing. In *The Molecular and Cellular Biology of the Yeast Saccharomyces*, pp. 143–192. Edited by E. W. Jones, J. R. Pringle & J. R. Broach. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989). *Molecular Cloning: a Laboratory Manual*, 2nd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- Scherer, S. & Magee, P. T. (1990). Genetics of *Candida albicans*. *Microbiol Rev* **54**, 226–241.
- Sherman, F., Fink, G. R. & Hicks, J. B. (1986). *Methods of Yeast Genetics*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- Sikorski, R. S. & Hieter, P. H. (1989). A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. *Genetics* **122**, 19–27.
- Smith, L. M., Sander, J. Z., Kaiser, R. J., Hughes, P., Dodd, C., Connel, C. R., Heiner, C., Kent, S. B. & Hood, L. E. (1986). Fluorescence detection in automated DNA sequence analysis. *Nature* **321**, 674–679.
- Steiner, H.-Y., Song, W., Naider, F., Zhang, L., Stacey, G. & Becker, J. M. (1994). An *Arabidopsis* peptide transporter is a member of a new class of membrane transport proteins. *Plant Cell* **6**, 1289–1299.
- Wickes, B., Staudinger, J., Magee, B. B., Kwon-Chung, K. J., Magee, P. T. & Scherer, S. (1991). Physical and genetic mapping of *Candida albicans*: several genes previously assigned to chromosome 1 map to chromosome R, the rDNA containing linkage group. *Infect Immun* **59**, 2480–2484.

Received 14 October 1994; accepted 12 January 1995.