Biogenesis of *Candida albicans* Can1 permease expressed in *Saccharomyces cerevisiae*

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Abstract The *Candida albicans CAN1* gene, encoding a high-affinity permease for arginine, lysine and histidine, was tagged at its C-terminus with a c-*myc* epitope and introduced into strains of *Saccharomyces cerevisiae* lacking basic amino acid permeases. The expression levels of Ca-Can1p were influenced by the available nitrogen source, being almost negligible when cells were grown in the presence of ammonia. Ca-Can1p was shown to follow the secretory pathway in *S. cerevisiae*. Ca-Can1p activity was not detected in a secretion-defective *sec1-1* mutant grown at a non-permissive temperature. Shr3p, an ER protein that participates in the biogenesis of amino acid permeases was also required for the functional expression of Ca-Can1p. The *shr3* mutation does not affect the affinity for substrate but does decrease the number of Can1p molecules reaching the plasma membrane.

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Key words: Basic amino acid permease; Secretory pathway; Heterologous expression; (*Candida albicans*); (*Saccharomyces cerevisiae*)

1. Introduction

The amino acid permease Can1 from *Candida albicans* (Ca-Can1p) can be functionally expressed in *Saccharomyces cerevisiae* [1]. Ca-Can1p actively transports lysine, arginine and histidine across the plasma membrane [1,2]. Its protein sequence (571 amino acids) is strongly homologous to two permeases specific for basic amino acids of *S. cerevisiae*, Sc-Can1p (57.3% identity, 75.5% similarity) and Sc-Lyp1p (49.7% identity, 73.3% similarity) and to the lysine-specific permease LysP of *Escherichia coli* [3]. The Ca-Can1p differs from Sc-Can1p in its substrate specificity and affinity to substrates: Ca-Can1p transports all three basic amino acids with high affinity ($K_{T,Arg} = 18$ µM, $K_{T,Lys} = 12$ µM, $K_{T,His} = 37$ µM) [4] whereas the Sc-Can1 system is specific for arginine and lysine [4] and prefers arginine to lysine ($K_{T,Arg} = 10$ µM, $K_{T,Lys} = 180$ µM) [5].

In *S. cerevisiae*, secreted and plasma membrane proteins reach their destination via the secretory pathway, i.e. they move in transport vesicles from the endoplasmic reticulum (ER) where they are synthesized, to the Golgi and finally to the plasma membrane. Many genes encoding components participating in the sorting, post-translational modification and intracellular transport through the secretory pathway have been characterized [6]. Mutations in these secretory (*sec*) genes exhibit phenotypes of a general nature; they are non-specific and affect a great number of different exported proteins. Re-cently, the existence of a specific transport molecular chaperone in the secretory pathway has been discovered. This non-essential integral ER membrane protein, Shr3p, is necessary for the in vivo localization of amino acid permeases to the plasma membrane of *S. cerevisiae* [7]. Shr3p is required for the packaging of amino acid permeases into ER derived COPII transport vesicles [8].

Using *S. cerevisiae* strains defective in the uptake of basic amino acids, we show that the functional heterologous expression of *C. albicans* Can1 permease depends upon an operational secretory pathway and requires the Shr3 chaperone.

2. Materials and methods

2.1. Strains, plasmids, media and cultivation

S. cerevisiae strains and plasmids used in this study are listed in Tables 1 and 2, respectively. Two types of minimal media were used: YNB-NH₄ consisted of the yeast nitrogen base w/o amino acids (6.7 g/l) and 2% glucose; YNB-Pro was prepared from yeast nitrogen base w/o amino acids and ammonium sulphate (1.7 g/l) supplemented with proline (1 g/l) as a source of nitrogen, and 2% glucose. Cells were cultivated aerobically at 30°C unless otherwise specified.

2.2. Disruption of the SHR3 gene

The strain HS 100-3C was transformed with a linear *EcoRI-SalI* fragment of DNA derived from pPL288 containing *shr3Δ5::hisG-URA3-hisG* [9,8] to create AM 8. AM 8 was propagated on media containing 5-fluoro-orotic acid [10] to attain the unmarked deletion of *shr3Δ* resulting in AM 8-1. Correct disruption and deletion were checked by Southern blots. The 650 bp fragment *PstI-ClaI* containing a part of the *SHR3* gene (410 bp) was excised from pPL210 [7], labelled with digoxigenin and used as a probe for Southern blots.

2.3. Transport measurements

The uptake of amino acids was measured as described previously using ¹⁴C-labelled amino acids [11]. In all experiments, cells from early exponential phase of growth were used (OD₇₀₀ ≈ 0.5). If the transport was measured in strains with functional Gap1 permease, its transport activity was saturated by addition of 1 mM citrulline to the cell suspension 1 min before addition of the radioactively labelled substrate.

2.4. Epitope tagging

The *C. albicans* amino acid permease Can1 was tagged at its C-terminus with the c-*myc* epitope using yeast multi-copy vector YEp-*myc*181 and yeast centromeric vector YCp*myc*111 [12]. Plasmid pCA2-4 containing the *C. albicans CAN1* gene was first digested with *NsiI*, the ends were made blunt using the Klenow fragment of DNA polymerase I, and finally digested *BglII*. In this way we generated a 2.9 kb fragment with *BglII* site in the promoter region and a blunt end at 3′-region of *CAN1* gene. This fragment with the last 30 nucleotides and stop-codon of *CAN1* gene truncated was inserted into vectors YEp*myc*181 and YCp*myc*111 that had previously been linearized with *XmaI* (filled in to form blunt ends by treatment with the Klenow fragment of DNA polymerase I) and subsequently digested with *Bam*HI (compatible with *BglII*), to create pCAmyc181 and pCA-myc111, respectively. These constructs encode a Ca-Can1p with the last 10 amino acid residues of the native *C. albicans* Can1p (AWDKF-WANVA) replaced by one arginine (R) and 11 amino acids (EQKLI-SEEDLN) of the c-*myc* epitope.

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2.5. Yeast cell extracts and Western immunoblotting

Cell extracts were prepared by alkaline lysis as described previously [13]. Protein samples in $2\times$ sample buffer ($2\times$SB: 4% SDS; 0.1 M Tris-HCl, pH 6.8; 4 mM EDTA; 20% glycerol; 2% 2-mercaptoethanol; 0.2% bromphenol blue; mixture of proteinase inhibitors described below) were heated for 10 min at 37°C and proteins were separated on 10% SDS-polyacrylamide gel, transferred to nitrocellulose membrane and treated with monoclonal anti-c-*myc* 9E10 antibody, 1:200. Primary antibody was detected by chemiluminiscence (ECL, Amersham) using HRP-conjugated antimouse IgG secondary antibody, 1:2000 (Sevac, Czech Republic).

2.6. Plasma membrane preparation

Partially purified plasma membranes were prepared according to Zinser et al. [14] with some modifications. Yeast cells in the exponential phase of growth were harvested by centrifugation, washed twice by distilled water and suspended in breaking buffer (50 mM Tris-HCl, pH 7.5; 250 mM sucrose; 5 mM EDTA; 0.5 ml/1 g wet weight of cells). All subsequent steps were carried out at 4°C. Chilled glass beads (0.45 mm diameter) were added to the meniscus and the cells were broken by vigorous vortex mixing (30 s pulses repeated 6 times). A mixture of proteinase inhibitors (final concentration 1 mM phenylmethylsulfonyl fluoride and 1 µg/ml each of leupeptin, antipain, pepstatin and chymostatin) was added before and between each two pulses. The resulting homogenate was diluted 3-fold with breaking buffer, a mixture of proteinase inhibitors was added again, unbroken cells and large debris were removed by centrifugation at 3500 rpm for 5 min and then at 4000 rpm for 5 min (Sorvall RC-5B, rotor SS34). The plasma membrane-enriched fraction was collected by centrifugation for 40 min at 14000 rpm. This membrane pellet was resuspended in glycerol medium (50 mM phosphate buffer, pH 6.3; 20% glycerol; 1 mM EDTA) containing the mixture of proteinase inhibitors and applied to a discontinuous gradient made using 1 volume 53% (w/w) and 2 volumes 43% (w/w) sucrose. The plasma membranes were recovered at the 43/53% sucrose interface after centrifugation in a swinging bucket rotor (SW28) at $100000\times g$ for 4 h (Beckman L8-55), and diluted 3-fold with ice-cold water. Membranes were sedimented at $80000\times g$ for 20 min (SW28, Beckman L8-55), resuspended in glycerol medium, applied to a second identical gradient and centrifuged as described for the first gradient centrifugation step. The purified plasma membranes withdrawn from the 43/53% sucrose interface were sedimented after dilution as described above, and resuspended in glycerol medium containing the mixture of proteinase inhibitors. Protein concentrations were determined by the method of Bradford [15]. The plasma membrane ATPase activity was estimated according to Sychrova and Kotyk [16] after each purification step. Aliquoted membranes were frozen in liquid nitrogen and stored at -80°C. For SDS-polyacrylamide electrophoresis, plasma membranes (12 mg of proteins per lane) were dissolved in $4\times$ sample buffer and heated at 37°C for 1 h.

3. Results and discussion

3.1. Epitope tagging

To study the properties of heterogously expressed *C. albicans* Can1 permease in *Saccharomyces cerevisiae* we tagged the Can1p with c-*myc* epitope. The tagging was accomplished using two *S. cerevisiae-E. coli* shuttle vectors YEp*myc*181 and YCp*myc*111 that allowed the in-frame fusion of the *C. albicans CAN1* gene with a sequence encoding the epitope of the

human c-*myc* protein [12]. The c-*myc* epitope tagged *Ca-CAN1* gene (*Ca-CAN1*[myc]) was introduced into the *S. cerevisiae* strain HS 60-2B (*can1 lyp1 ura3Δ leu2*) on either multicopy or centromeric vector (pCA*myc*181 or pCA*myc*111, respectively). First, the uptake of 100 µM lysine into the transformed cells was measured to check the activity of the tagged *Ca-CAN1*[myc] constructs. The initial rates were 7.5 nmol (mg dry mass)$^{-1}$ min^{-1} and 1.4 nmol (mg dry mass)$^{-1}$ min^{-1} in strains harbouring multi-copy or centromeric plasmids, respectively (Fig. 1a). The amount of Ca-Can1p[myc] permease in the cells was estimated using Western blots. Immunoreactive signals were detected in total protein extracts of cells transformed with the epitope tagged *Ca-CAN1*[myc] constructs but not in cells containing either the untagged *Ca-CAN1* or YEp*myc*181. The relative amounts of Ca-Can1p[myc] observed on Western blots corresponded to initial rates of lysine uptake (Fig. 1). In the case of cells transformed with the centromeric vector, the weak signal observed on Western blots was accompanied by low transport activity. As usual for highly hydrophobic proteins, the immunodetected Can1p[myc] had an apparent mass smaller (≈ 58 kDa; Fig. 1b) than that one calculated from the amino acid sequence (≈ 63 kDa). An unidentified polypeptide with an apparent molecular mass of about 110 kDa was detected (also observed by Hein et al. [17]). The presence of this crossreacting protein seems to be strain and media dependent, as it appears only in strains of *S. cerevisiae* derived from Σ1278b and only in cells grown on minimal media [18].

To verify that the replacement of last 10 amino acid residues of the Ca-Can1p by c-*myc* epitope did not change the permease transport activity and/or substrate specificity, we compared transport activities of Ca-Can1p[myc] permease with untagged Ca-Can1p. Both *Ca-CAN1*[myc] construct and untagged *Ca-CAN1* gene were cloned into multi-copy vector YEp352 with *URA3* marker gene and expressed in the strain HS 100-3C which lacks Gap1 transport activity in contrast to strain HS 60-2B that was originally used. Both tagged and untagged permeases transported all three substrates, but in case of Can1p[myc] the initial rates of uptake were a bit lower, e.g., the initial uptake rate measured using 100 µM lysine into the HS 100-3C [pCA*myc*352] was 10.2 nmol (mg dry mass)$^{-1}$ min^{-1} whereas in HS 100-3C [pCA2-4ΔBE] it was 19.7 nmol (mg dry mass)$^{-1}$ min^{-1}. However, the decrease of the initial rate of lysine uptake is probably not caused by the change of tagged permease affinity to its substrates as the detailed kinetic analysis showed that the K_T for lysine uptake did not differ for both Can1p[myc] and Can1p ($K_{T,Can1p-myc} = K_{T,Can1p} = 13$ µM).

3.2. Ca-Can1p[myc] transport activity in the presence of ammonia

It is known that the expression levels and activities of sev-

Table 1
Strains of *S. cerevisiae* used in this work

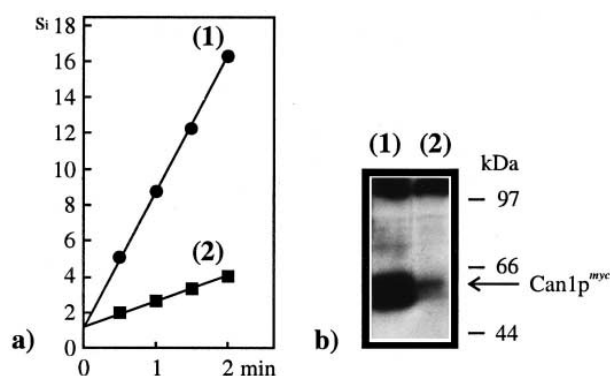| Strain	Genotype	source or Reference
HS 100-3C	*MATa can1 gap1 lyp1 ura3Δ*	[11]
HS 60-2B	*MATa can1 lyp1 leu2 ura3Δ*	this work
HS 35-4A	*MATα can1 lyp1 sec1-1 ura3Δ*	this work
PLAS 1-6A	*MATα ura3-52 his4Δ29*	[7]
PLAS 23-4B	*MATα ura3-52 his4Δ29 shr3-23*	[7]
AM 8	*MATa can1 gap1 lyp1 ura3Δ shr3Δ5::hisG-URA3-hisG*	this work
AM 8-1	*MATa can1 gap1 lyp1 ura3Δ shr3Δ*	this work

Fig. 1. Uptake of 100 μM lysine in the presence of 1 mM citrulline (a) and Western blotting protein analysis (b) in *S. cerevisiae* strain HS 60-2B (*can1 lyp1 ura3Δ leu2*) transformed with *C. albicans CAN1*^{myc} construct in multi-copy vector (pCA*myc*181; 1) or centromeric vector (pCA*myc*111; 2). s_i, internal concentration of lysine in nmol (mg dry mass)^{−1}.

Fig. 2. Immunodetected amounts of Can1p^{myc} in cells of strain HS 100-3C [pCA*myc*352] grown in minimal media containing either proline (YNB-Pro) or ammonia (YNB-NH$_4$) and corresponding initial rates (J_0 in nmol (mg dry mass)$^{-1}$ min^{-1}) of 100 μM lysine uptake.

eral amino acid permeases in *S. cerevisiae*, probably including *S. cerevisiae* Can1p, are regulated in accordance to the nitrogen sources available in the growth medium [19]. To estimate if heterologously expressed Ca-Can1p, under control of its own promoter is influenced by the presence of ammonia in the growth medium, the transport activity and the amount of Ca-Can1p^{myc} was determined in cells grown in minimal media containing either ammonium ions or proline as sole nitrogen sources. Fig. 2 shows that initial rates of lysine uptake and also the amount of permease molecules in cells grown in YNB-NH$_4$ is lower compared to cells grown in YNB-Pro. Apparently, Ca-Can1p expression is inhibited by the presence of ammonia. In addition, we observed that after the cells were transferred from YNB-NH$_4$ to 1% glucose (i.e., without any source of nitrogen), the initial rate of lysine uptake increased about 5-fold within 3 h, while the high lysine uptake activity in cells from YNB-Pro slowly decreased after cells were transferred to 1% glucose (data not shown).

3.3. Biogenesis of the heterologous permease

The biogenesis of heterogously expressed Ca-Can1p in *S. cerevisiae* was studied and compared with the biogenesis of the endogenous *S. cerevisiae* Lyp1 system [11] which shares high sequence similarity with Ca-Can1p. First, the transport activities of both of these permeases were measured in temperature sensitive *sec1-1* mutants (Fig. 3a,b). At non-permissive temperature the secretory vesicles of *sec1-1* cells cannot fuse with the plasma membrane [20]. HS 35-4A (*can1 lyp1 sec1-1*
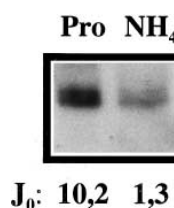
ura3Δ) cells transformed with pCA2-4ΔBE were grown at 25°C in the presence of ammonia (YNB-NH$_4$) to reduce the transport activity of Ca-Can1p. Exponential phase cultures were transferred to medium without ammonium ions (YNB-Pro) and divided into two parts: one part incubated at permissive (25°C) and the other part shifted to the restrictive (37°C) temperature. Uptake of 100 μM lysine was measured at 40 min intervals during 2 h. At 25°C, the transport activity continuously increased due to the derepression of the Ca-Can1 permease in the absence of ammonia. At 37°C, the *sec1-1* secretory block caused a decrease in lysine uptake. After this 2-h incubation, at the non-permissive temperature, the protein-synthesis inhibitor cycloheximide was added, and half of the cells were transferred to the permissive temperature. The lysine uptake activity, negligible at the restrictive temperature, rapidly increased after cells were transferred to the permissive temperature, even though protein synthesis was blocked (Fig. 3a). Similar results were obtained when this experiment was repeated using cultures of HS 35-4A [pLY7-9] cells (Fig. 3b). These results suggest, that both heterologous Ca-Can1p and endogenous Sc-Lyp1p follow the secretory pathway.

In a similar experiment the amount of Ca-Can1p^{myc} was determined using Western blots of proteins isolated from cells of HS 35-4A [pCAmyc352] pregrown in YNB-NH$_4$ and incubated at permissive or restrictive temperatures in YNB-Pro. Surprisingly, the amount of immunodetected Can1p^{myc} in cells incubated at the permissive temperature was lower than that in cells incubated at the restrictive temperature (Fig. 3c) in spite of the fact that the initial rate of transport was almost negligible at the restrictive temperature. This phenomenon could be explained if the Ca-Can1p^{myc} accumulated in secretory vesicles incubated at 37°C turnover at rates appreciably slower than the Ca-Can1p^{myc} in the plasma membrane of cells incubated at 25°C. If purified plasma membranes were ana-

Table 2
List of plasmids

Plasmids	Composition	Source or reference
YEp352	5.2 kb, *URA3*, 2μ, *Amp*^R, *ORI*	[22]
pCA2-4	5.6 kb fragment containing *C. albicans CAN1* in YEp352	[1]
pCA2-4ΔBE	3.4 kb fragment containing *C. albicans CAN1* in YEp352	[1]
pLY7-9	5.2 kb fragment containing *LYP1* in pFL61	[11]
YEp*myc*181	5.8 kb, *LEU2*, 2μ, *Amp*^R, *ORI*	[12]
YCp*myc*111	6.1 kb, *LEU2*, *CEN4*, *ARS1*, *Amp*^R, *ORI*	[12]
pCA*myc*181	2.9 kb *Bgl*II-*Nsi*I fragment containing c-*myc*-tagged *C. albicans CAN1* in YEp*myc*181	this work
pCA*myc*111	2.9 kb *Bgl*II-*Nsi*I fragment containing c-*myc*-tagged *C. albicans CAN1* in YCp*myc*111	this work
pCA*myc*352	3.9 kb *Pst*I-*Nsi*I fragment containing c-*myc*-tagged *C. albicans CAN1* in YEp352	this work
pPL210	1.4 kb *Acc*I fragment containing *SHR3* in pRS316	[7]
pPL288	5.7 kb *Eco*RI-*Sal*I fragment containing *shr3Δ5::hisG URA3 hisG* in pBSII SK(+)	[8]

Fig. 4. Uptake of 100 μM histidine in the presence of 1 mM citrulline into strains PLAS 1-6A (*SHR3*; squares) and PLAS 23-4B (*shr3*; circles) both transformed either by pCA2-4ΔBE (full lines) or by YEp352 without any insert (dashed lines). s_i, internal concentration of histidine in nmol (mg dry mass)$^{-1}$.
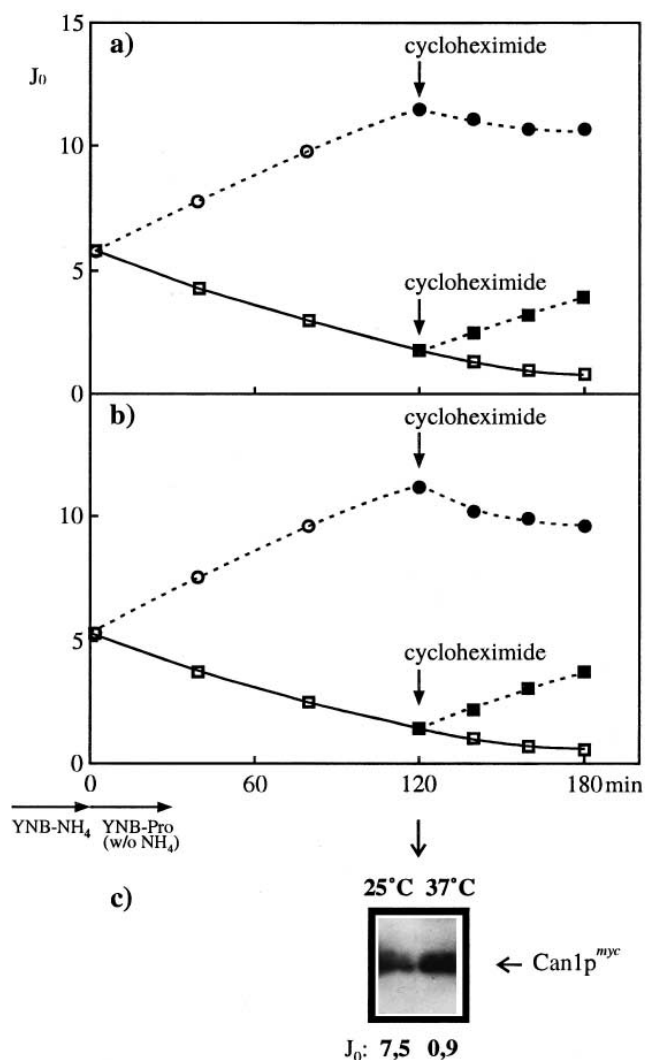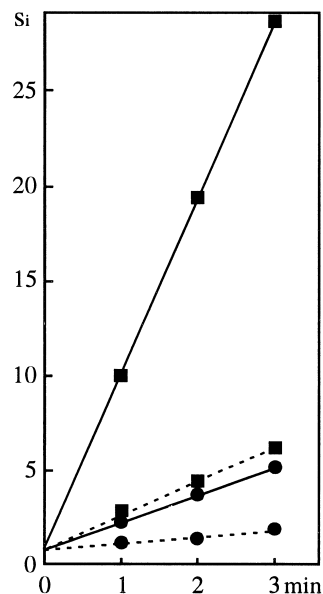

Fig. 3. Uptake of 100 mM lysine in HS 35-4A (*can1 lyp1 sec1-1 ura3Δ*) transformed with pCA2-4ΔBE (a), or pLY7-9 (b), and amounts of Can1p^{myc} in HS 35-4A [pCAmyc352] determined by immunoblotting (c). Cells were harvested from YNB-NH₄ and resuspended in YNB-Pro. After 2-h incubation at permissive (25°C, dashed lines) or restrictive (37°C, full lines) temperatures: (i) in (a) and (b), the protein-synthesis inhibitor cycloheximide (0.4 mM) was added and a half of the cells was transferred from restrictive to permissive temperature; (ii) in (c), total protein extracts were prepared from HS 35-4A [pCAmyc352]. J_0, initial rate of uptake in nmol (mg dry mass)$^{-1}$ min^{-1}.

lysed using Western blots, a weak signal was observed on Western blots of membranes from cells incubated 2 h at per-

missive temperature and no immunoreactive signal was detected in plasma membranes from cells incubated 2 h at restrictive temperature (data not shown).

shr3 mutants defective in the biogenesis of amino acid permeases on the ER level [7] were used to find out whether the Ca-Can1p is recognized and influenced by Shr3p in *S. cerevisiae*. First, the *C. albicans CAN1* gene in YEp352 was expressed in strains PLAS 1-6A (*SHR3* wild type) and PLAS 23-4B (*shr3* mutant). Unfortunately, these strains are wild-type for Can1, Gap1 and Lyp1 permeases. To be sure that only the uptake mediated by Ca-Can1p is measured, histidine was used in transport assays. In *S. cerevisiae*, histidine is transported by Gap1 permease and by a histidine-specific system (Hip1) which transports histidine with high affinity but low capacity [21]. In our measurements, histidine uptake through the Gap1 system was out saturated by the inclusion of 1 mM citrulline, and the rate of histidine uptake via Hip1p was estimated in cells transformed with plasmid YEp352 without any insert (Fig. 4). Transformed PLAS 1-6A and PLAS 23-4B cells were grown in YNB-Pro medium supplemented with histidine (15 μg/ml). Before histidine uptake measurements were made cells were washed and pre-incubated in minimal medium without histidine for 30 min. The observed de-

Table 3
Uptake of 100 μM lysine, arginine and histidine

Strain		Lysine	J_0 Arginine	Histidine	K_T Lysine	J_{max} Lysine
HS 100-3C [pCAmyc352]	(*SHR3*)	10.2	19.4	11.9	13	18
AM 8-1 [pCAmyc352]	(*shr3*)	2.1	3.6	2.5	13	4
HS 100-3C [pLY 7-9]	(*SHR3*)	14.7				
AM 8-1 [pLY 7-9]	(*shr3*)	2.6				

J_0, initial rate of transport in nmol (mg dry mass)$^{-1}$ min^{-1}.
K_T, half-saturation constant in μM.
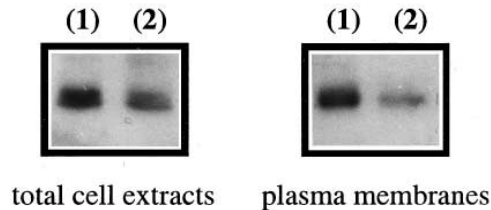J_{max}, maximum rate of transport in nmol (mg dry mass)$^{-1}$ min^{-1}.

Fig. 5. Western blots of protein extracts from total cells and isolated plasma membranes of strains HS100-3C (*SHR3*; 1), and AM 8-1 (*shr3*; 2) both transformed with pCA*myc*352.

crease in the uptake activity into *shr3* mutants ($\approx 20\%$ of the *SHR3* strain; Fig. 4) suggests that the heterologous Ca-Can1p is recognized by Shr3p. To verify this observation a *shr3* null mutant strain also carrying mutations in *CAN1*, *GAP1* and *LYP1* (AM 8-1) was constructed. This strain was transformed with multi-copy vectors containing either the *Sc-LYP1* gene or the *Ca-CAN1*[myc] construct. Transport activities of both permeases in *shr3* mutant were measured. The initial rates of the amino acid transport in these strains with disrupted *shr3* gene were about 5 times lower compared to the uptake in similarly transformed *SHR3* wild-type strains (HS 100-3C) (Table 3). A detailed kinetic analysis showed that the K_T of lysine uptake mediated by *C. albicans* Can1 system did not change, whereas the maximum velocity decreased about 5 times compared to the *SHR3* cells (Table 3). This data suggests that the absence of Shr3p influences the amount of permease molecules in the plasma membrane but not the permease substrate affinity. These results were confirmed using Western blots (Fig. 5) of total cell extracts and isolated plasma membranes (the ATPase activity in purified plasma membranes was about 4 μmol P_i min^{-1} (mg protein) $^{-1}$ for the *shr3* mutant and 3 μmol P_i min^{-1} (mg protein) $^{-1}$ for the *SHR3* strain). The decreased amount of Can1p[myc] in total cell extracts of the *shr3* mutants indicates that Can1p accumulated in the ER is probably degraded.

In conclusion, heterologous amino acid permease Can1p follows in *S. cerevisiae* the secretory pathway and its amount in the plasma membrane is influenced by the amino acid permease specific transport chaperone Shr3p analogously as *S. cerevisiae* own amino acid transport system Lyp1.

References

[1] H. Sychrová, M.R. Chevallier, Curr. Genet. 24 (1993) 487–490.
[2] H. Sychrová, J.-L. Souciet, Yeast 10 (1994) 1647–1651.
[3] H. Sychrová, A. Matijèková, J.-L. Souciet, M.-R. Chevallier, Folia Microbiol. 39 (1994) 547–551.
[4] M. Grenson, M. Mousset, J.M. Wiame, J. Bechet, Biochim. Biophys. Acta 127 (1966) 325–338.
[5] J. Horák, Biochim. Biophys. Acta 864 (1986) 223–256.
[6] N.K. Pryer, L.J. Wuestehube, R. Schekman, Annu. Rev. Biochem. 61 (1992) 471–516.
[7] P.O. Ljungdahl, C.J. Gimeno, C.A. Styles, G.R. Fink, Cell 71 (1992) 463–478.
[8] M.J. Kuehn, R. Schekman, P.O. Ljungdahl, J. Cell Biol. 135 (1996) 585–595.
[9] E. Alani, L. Cao, N. Kleckner, Genetics 116 (1987) 541–545.
[10] J.D. Boeke, F. Lacroute, G.R. Fink, Mol. Gen. Genet. 197 (1984) 345–346.
[11] H. Sychrová, M.R. Chevallier, Yeast 9 (1993) 771–782.
[12] P. Reisdorf, A.C. Maarse, B. Daignan-Fornier, Curr. Genet. 23 (1993) 181–183.
[13] S. Silve, C. Volland, C. Garnier, R. Jund, M.R. Chevallier, R. Haguenauer-Tsapis, Mol. Cell Biol. 11 (1991) 1114–1124.
[14] E. Zinser, G. Daum, Yeast 11 (1995) 493–536.
[15] M.M. Bradford, Anal. Chem. 72 (1976) 248–254.
[16] H. Sychrova, A. Kotyk, FEBS Lett. 183 (1985) 21–24.
[17] C. Hein, J.-Y. Springael, C. Volland, R. Haugenauer-Tsapis, B. André, Mol. Microbiol. 18 (1995) 77–87.
[18] Matijèková, A. and Sychrová, H. (1997) Folia Microbiol., in press.
[19] J.R. Daugherty, R. Rai, H.M. El Berry, T.G. Cooper, J. Bacteriol. 175 (1993) 64–73.
[20] P.J. Novick, C. Field, R. Schekman, Cell 21 (1980) 205–215.
[21] M. Crabeel, M. Grenson, Eur. J. Biochem. 14 (1970) 197–204.
[22] J.E. Hill, A.M. Myers, T.J. Koerner, A. Tzagoloff, Yeast 2 (1986) 163–167.