*Biochimica et Biophysica Acta*, 978 (1989) 203–208
Elsevier



BBA 74293

# Transport of L-lysine in the fission yeast *Schizosaccharomyces pombe*

Hana Sychrová, Jaroslav Horák and Arnošt Kotyk

*Department of Membrane Transport, Institute of Physiology, Czechoslovak Academy of Sciences, Prague (Czechoslovakia)*

(Received 5 July 1988)

Key words: L-Lysine; Amino acid transport; Yeast; (*S. pombe*)

**Systems of L-lysine transport in *Schizosaccharomyces pombe* are not constitutive, as at no phase of growth in a rich medium is lysine taken up. Transport activity appears only after preincubation of harvested cells with glucose or another suitable source of energy. If cycloheximide is added during this preincubation no transport systems are synthesized. After removal of glucose, the activity of the transport system decays with a half-time of 13 min. The transport of L-lysine into *S. pombe* cells from the stationary phase of growth preincubated for 60 min with 1% D-glucose is mediated by at least two systems, the high-affinity one with a $K_t$ of 26 μmol/l and $J_{max}$ of 4.95 nmol/min per mg dry wt., the low-affinity one with a $K_T$ of 1.1 mmol/l and $J_{max}$ of 11.8 nmol/min per mg dry wt. The transport of lysine mediated by these two systems proceeds uphill. The high-affinity system has a pH optimum at 4.0–4.2, the accumulation ratio is highest at a cell density 2–5 mg dry wt. per ml and decreases with increasing lysine concentrations. Lysine accumulated by this system does not exit from cells. The only potent competitive inhibitors are L-arginine, L-histidine and D-lysine. The other amino acids tested do not behave as competitive inhibitors. Of the various metabolic inhibitors tested, the most potent were proton conductors and antimycin A.**

## Introduction

Transport of amino acid in yeasts is an active, practically unidirectional, process operating against substantial concentration gradients. Yeasts generally possess two types of transport system for amino acids: specific systems for a single one or a few structurally related amino acids and a general system which mediates the uptake of most amino acids [1].

The fission yeast *S. pombe* is one of the genetically best characterized eukaryotic microorganisms, but its biochemistry and physiology, including membrane transport, lag behind that of *Saccharomyces cerevisiae*. The only systematic paper on amino acid transport in *S. pombe* is the one by Fantes and Creanor [2] who, using mutants resistant to the arginine analogue L-canavanine showed that arginine was transported by at least two systems. The first system (defective in can1.1 strains) mediated the uptake of L-arginine, L-canavanine and probably also L-lysine and L-ornithine. The system operated in the wild-type strain, with either ammonium or glutamate as the nitrogen source during growth. The second system (presumably specific only for arginine) was absent in cells grown on ammonium as the sole nitrogen source.

Recently, Coddington and Schweingruber [3] investigated uptake of tyrosine (presumed to be a substrate of the general amino-acid transport system) and arginine (supposedly transported by a specific system) into wild-type and acid-phosphatase-deficient mutants (pho1) of *S. pombe*. While the uptake of tyrosine was lost in the pho1 mutant, arginine uptake was not significantly affected.

Lysine being another important amino acid from the point of view of specific transport systems, we report here on the kinetic characterization of its transport in the wild-type strain of *S. pombe*, designated 972 $h^-$.

## Methods

### *Microorganism and its growth*

*S. pombe* (972 $h^-$) was grown in a YPD medium containing (w/v) 1% yeast extract, 1% bacto-peptone and 1% D-glucose. The pH of the medium was adjusted to 4.5 with HCl before autoclaving. To inoculate the

Abbreviations: CCCP, carbonylcyanide *m*-chlorophenylhydrazone; DCCD, dicyclohexylcarbodiimide; DES, diethylstilbestrol.

Correspondence: H. Sychrová, Institute of Physiology, Czechoslovak Academy of Sciences, Vídeňská 1083, 142 20 Prague 4, Czechoslovakia.

culture (100 ml) 3 ml of an overnight preculture were used. Cells were grown in flasks on a reciprocal shaker (1.6 Hz) at 29 °C, harvested by centrifugation, washed twice with distilled water and suspended in distilled water.

*Preincubation*

Suspension of yeast cells (5–6 mg dry wt./ml) was incubated for 60 min with D-glucose (usually 1%) at 30 °C aerobically in a Dubnoff-type water bath, shaken at 1.5 Hz. Then the cells were washed and resuspended in distilled water or in a buffer containing 50 mmol/l phthalic acid/triethanolamine (pH 4.2).

*L-Lysine uptake*

Cells were incubated again at 30 °C in a Dubnoff-type incubator in the presence of [$^{14}$C]labelled L-lysine (approx. 130 Bq per nmol of lysine) and another amino acid or inhibitor, if necessary. Samples (0.2 ml) were withdrawn at 30-s intervals, filtered through a membrane filter (0.85 μm pore diameter; Synpor, Czechoslovakia) and washed twice with 2 ml of ice-cold water. The pellet with filter was transferred to a vial with 8 ml of a toluene-ethanol scintillation coctail. Radioactivity was counted in a Beckman liquid scintillation spectrometer 9800.

*Chemicals*

L-Amino acids, D-lysine and iodoacetamide were purchased from Serva (F.R.G.), cycloheximide and phenylmethylsulfonyl fluoride were from Fluka (Switzerland), dicyclohexylcarbodiimide and sodium orthovanadate were from BDH (U.K.); other inhibitors were from Sigma, (U.S.A.). L-[U-$^{14}$C]Lysine (8 MBq/ml) was obtained from the Institute for Research, Production and Uses of Radioisotopes, Czechoslovakia. All other chemicals were obtained from local commercial sources and were of the highest purity.

## Results

*Transport activity during growth*

Aliquots of 8 ml (during the early exponential phase) or 4 ml (during later phases of growth) were removed from a growing culture at 2-h intervals between 10 and 36 h of growth. The samples were centrifuged and resuspended (a) in a fresh growing medium, (b) in distilled water. The uptake of 50 μmol/l lysine was then examined and none was found. The same was true if the resuspended samples were aerated for a further 2 h in water and then the uptake of lysine was measured.

However, if the resuspended samples were incubated with glucose (or another suitable source of energy, see below) and then lysine added, it was taken up avidly. The rate of lysine uptake after preincubation of cells differed, depending on the phase of growth at which the

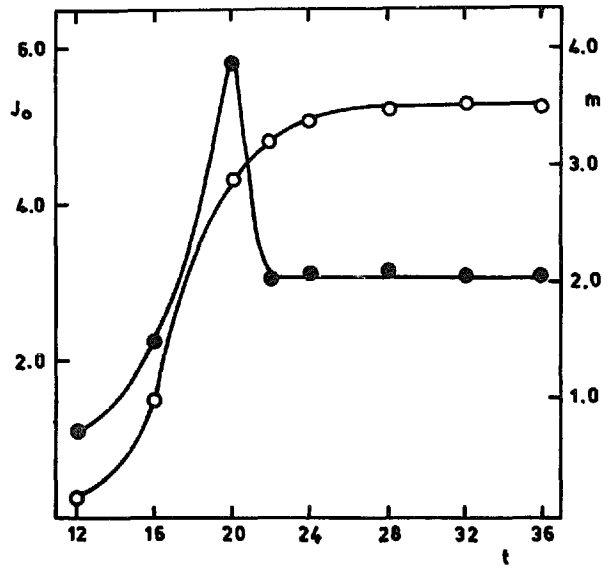


Fig. 1. Initial rate of transport of L-lysine (50 μmol/l) in cells harvested during the growth of a culture in 1% YPD medium and preincubated for 60 min with 1% D-glucose prior to measuring the uptake. $J_0$, ●, initial rate of uptake in nmol/min per mg dry wt.; $t$, time of growth in h; $m$, ○, cell mass in mg dry wt. per ml.

cells were harvested, peaking at 20 h of growth and attaining a constant value as the stationary phase was reached (Fig. 1). For this reason, all the subsequent experiments were done with stationary cells (28 h) after preincubation with glucose.

*Conditions of preincubation*

The extent to which the transport activity was stimulated by preincubation with a source of energy depended on a number of factors.

*Character of 'substrate'.* Table I shows D-glucose to be the most suitable substrate for promoting the appearance of lysine-transport activity, followed by maltose, sucrose and fructose. Ethanol, galactose, trehalose and glycerol had no stimulatory effect (contrast this with *S. cerevisiae*, e.g., Refs. 4 and 5). Lysine itself, when present during preincubation at 0.5 mmol/l, had no effect whatsoever.

*Concentration of 'substrate'.* At 0.1% concentration, the effect of glucose was only about one-tenth of the optimum at or above 1% concentration, indicating that the effect is not one of a trigger but rather of continuous presence (Table I).

*Length of preincubation.* The functioning L-lysine transport system appears after a considerable period of preincubation with 1% glucose. At 15 min, only 2% of the maximum is present, at 30 min, 7%, at 60 min, 35%, at 120 min the maximum is reached and longer preincubation with glucose brings about a decrease of activity (32% down at 150 min).

*Presence of inhibitors.* Judging from earlier experience with *S. cerevisiae* [6] it was assumed that the increase of transport activity following preincubation with glucose

TABLE I

*Initial rate of uptake of L-lysine (50 μmol/l) into the cells preincubated 60 min with different sources of energy*

Initial rate in nmol/min per mg dry wt.

| Substrate during preincubation | Concentration (% w/v) | Initial rate of uptake |
|---|---|---|
| None | – | 0 |
| Glucose | 0.1 | 0.30 |
| | 1.0 | 3.15 |
| | 3.0 | 3.14 |
| | 5.0 | 3.16 |
| + cycloheximide (0.4 mmol/l) | 1.0 | 0.22 |
| + $NH_4Cl$ (1 mmol/l) | 1.0 | 0.15 |
| + $NH_4Cl$ (0.1 mol/l) | 1.0 | 0 |
| Maltose | 1.0 | 3.05 |
| Fructose | 1.0 | 2.59 |
| Sucrose | 1.0 | 2.67 |
| Ethanol, Galactose, Trehalose, Glycerol | 1.0 | 0 |

was of proteosynthetic nature. Application of 0.4 mmol/l cycloheximide bore this out (Table I). Likewise, addition of $NH_4^+$ to glucose during preincubation (assumed to repress the synthesis of amino-acid transport systems, e.g., Ref. 6) had a negative effect (Table I).

*Stability of the 'induced' lysine transport*

If, after 60 min of preincubation with glucose, cells were centrifuged, washed and further incubated in distilled water, their lysine-transporting activity (for 50 μmol/l lysine) kept slightly rising for 5–10 min but then decreased sharply with a half-time of 13 min.

To elucidate the nature of this activity–decay process, cycloheximide (blocking protein synthesis on eukaryotic ribosomes) or phenylmethylsulfonyl fluoride (stopping the action of serine proteinases) were added to the suspension after preincubation with glucose and washing (Table II). Clearly, glucose stimulated the appearance of transport systems, as noted before, and cycloheximide had at first no effect, presumably because the transport protein precursor had already been synthesized and its movement from the endoplasmic reticulum to the plasma membrane is not inhibited by cycloheximide. However, once there (presumably after the 10-min period or thereabouts) it was subject to degradation just like without glucose (portion D). Phenylmethylsulfonyl fluoride retarded the degradation, so it could be concluded that the loss of activity after termination of exposure to glucose was proteolytic in nature.

The lysine transport activity could be partly preserved not only by phenylmethylsulfonyl fluoride but, almost equally, also by iodoacetamide (2 mmol/l). If both inhibitors were added together, the preservation of transport activity was nearly additive. In contrast, other inhibitors of proteinases (e.g., pepstatin A, EDTA) slightly enhanced the diminution of the initial transport rate of L-lysine, whereas 4-hydroxymercuribenzoic acid and *N*-ethylmaleimide fully suppressed lysine transport within 20 min of incubation in their presence [7].

*Kinetic analysis of L-lysine transport*

The uptake of L-lysine tested at external concentrations ranging from 0.01 to 5 mmol/l was linear with time for only 2 min and after about 10–12 min, the amount of intracellular lysine attained a constant value. A similar qualitative observation was made by Johnston and Coddington [8,9]. We showed that the uptake was active, and that concentrations of lysine in cells after 12 min were higher than the ones in external medium. The accumulation ratio decreased with increasing external concentration of L-lysine from 160 : 1 for 0.01 mmol/l lysine to 4 : 1 for 5 mmol/l lysine. Intracellular lysine is not significantly metabolized (measured by the production of $CO_2$ in a Warburg vessel – results not shown) or incorporated into proteins (cycloheximide added together with lysine had no effect on the uptake).

The efflux of lysine (accumulated in 12 min from 50 μmol/l) from the cells was no more than 3% of the total accumulated lysine, regardless of whether the preloaded cells were resuspended in water, nonlabelled lysine (1 mmol/l) or 2,4-dinitrophenol (0.5 mmol/l).

The Lineweaver-Burk plot of L-lysine uptake into *S. pombe* is clearly biphasic, composed of a high-affinity component with a half-saturation constant $K_T$ of 26 ± 8 μmol/l and maximum rate $J_{max}$ of 4.95 ± 1.4 nmol/min per mg dry wt. and a low-affinity component with a $K_T$ of 1.05 ± 0.05 mmol/l and $J_{max}$ of 11.8 ± 1.6 nmol/min

TABLE II

*Dependence of the initial rate of L-lysine uptake on the conditions of incubation after pretreatment with glucose*

Cells were preincubated for 60 min with 1% glucose, resuspended in distilled water and divided in five portions (A–E). A, control without any addition; B, supplemented with 1% glucose; C, with 1% glucose and cycloheximide (0.4 mmol/l); D, with cycloheximide (0.4 mmol/l) alone and E, with phenylmethylsulfonyl fluoride (1 mmol/l). Each portion was divided into three parts and incubated at 30 °C. At time zero, and after 10 and 20 min of incubation, labelled lysine (50 μmol/l) was added to the three parts of each portion and the uptake of lysine was measured for 2 min.

| Incubation (min) | Initial rate of uptake (nmol/min per mg dry wt.) | | | | |
|---|---|---|---|---|---|
| | A | B | C | D | E |
| 0 | 3.37 | 3.23 | 3.33 | 3.30 | 3.31 |
| 10 | 1.81 | 5.07 | 5.17 | 1.78 | 2.53 |
| 20 | 0.48 | 6.40 | 4.64 | 0.51 | 1.05 |

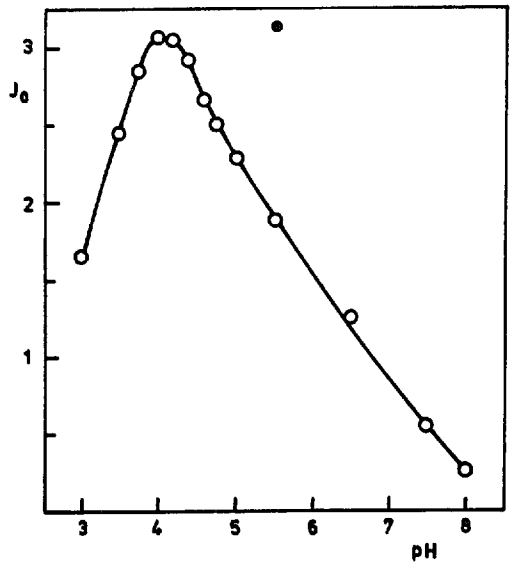


Fig. 2. Initial rate of transport ($J_0$, in nmol/min per mg dry wt.) of L-lysine (50 μmol/l) as a function of pH. The uptake was measured in a phthalic acid/triethanolamine buffer (at 0.05 mol/l); ●, cells resuspended in water.

per mg dry wt., determined from eight separate experiments. The second (low-affinity) transport activity representing under our conditions (external concentration of lysine 50 μmol/l) less than 15% of the total activity was not studied further.

TABLE III

*Inhibition of the initial rate of uptake of L-[$^{14}$C]lysine (50 μmol/l) by amino acids at 500 μmol/l added simultaneously $I_{50}$ represents the concentration of amino acid corresponding to 50% inhibition of L-lysine uptake*

| Amino acid | Inhibition (%) | $I_{50}$ (mmol/l) |
|---|---|---|
| Glycine | 31 | > 5.0 |
| L-Alanine | 27 | |
| L-Valine | 19 | |
| L-Leucine | 47 | 0.70 |
| L-Isoleucine | 16.5 | > 5.0 |
| L-Proline | 0 | |
| L-Phenylalanine | 34 | 1.75 |
| L-Tyrosine | 10 | |
| DL-Tryptophan | 13 | |
| L-Serine | 23 | 2.85 |
| L-Threonine | 25 | > 5.0 |
| L-Histidine | 87 | 0.09 |
| L-Arginine | 98.5 | 0.06 |
| L-Glutamic acid | 20.5 | > 5.0 |
| L-Aspartic acid | 26 | |
| L-Glutamine | 25 | 3.75 |
| L-Asparagine | 18 | |
| 2-Aminoisobutyric acid | 0 | |
| L-Cysteine | 30 | 2.75 |
| D-Lysine | 60 | 0.40 |

*Effect of pH and of temperature*

The uptake of L-lysine showed a pH optimum at pH 4.0–4.2 (Fig. 2). Detailed analysis showed that $K_T$ did not change over the range examined, while the $J_{max}$ decreased toward alkaline values of pH.

The Arrhenius plot of log $J_0$ vs. $1/T$ was biphasic with a transition point at 27 °C and activation energies of 39 and 127 kJ per mol above and below the transition point, respectively.

*Specificity of the high-affinity system*

Table III indicates that, except for L-proline and 2-aminoisobutyric acid (which are not taken up by the present strain of *S. pombe* below 1 mmol/l, unpublished data), all amino acids display a certain degree of inhibition with respect to L-lysine uptake.

The inhibition by L-arginine, L-histidine and D-lysine was competitive with $K_{i(Arg)} = 19.5$ μmol/l and $K_{i(His)} = 138$ μmol/l, indicating that the high-affinity system for L-lysine is one for basic amino acids. Inhibition by the other amino acids with the lowest values of $I_{50}$ (L-phenylalanine, L-leucine and L-cysteine, table III) was different from pure competitive inhibition. Partial inhibition by most amino acids suggests that we are dealing here with competition for energy rather than for the carrier itself (see Ref. 10).

The uptake of L-lysine was also inhibited by ammonium ions (Table IV).

*Effect of inhibitors of metabolism and transport*

Of the variety of inhibitors tested (Table V), uranyl nitrate predictably inhibited the uptake of L-lysine (see Ref. 11) while 2,4-dinitrophenol, sodium azide and CCCP blocked it completely through their protonophoric action. The effect of antimycin A suggested that mitochondrial oxidation (ATP?) is required for efficient uptake.

TABLE IV

*Inhibition of the initial rate of uptake of L-lysine (50 μmol/l) by ammonium ions*

A, $NH_4Cl$ in different concentrations was added together with lysine; B, $NH_4Cl$ (0.05 mg/ml) was added 1 or 5 min before lysine.

| A | Concentration of $NH_4Cl$ (mg/ml) | Inhibition (%) |
|---|---|---|
| | 0 | 0 |
| | 0.05 | 20.5 |
| | 0.1 | 26.8 |
| | 2.5 | 46.5 |
| | 5.0 | 63.0 |
| B | Incubation with $NH_4Cl$ (min) | Inhibition (%) |
| | 0 | 20.5 |
| | 1 | 27.7 |
| | 5 | 48.5 |

TABLE V

*Inhibition of the initial rate of uptake of [$^{14}$C]-L-lysine (50 μmol/l) by metabolic inhibitors and inhibitors of transport*

$I_{50}$ represents the concentration of inhibitor corresponding to 50% inhibition of lysine uptake. The inhibitors were added to the suspension 1 min before labelled lysine. Abbreviations: CCCP, carbonylcyanide *m*-chlorophenylhydrazone; DES, diethylstilbestrol; DCCD, dicyclohexylcarbodiimide.

| Inhibitor | Concentration (μmol/l) | Inhibition (%) | $I_{50}$ (mmol/l) |
|---|---|---|---|
| $Na_2HAsO_4$ | 2500 | 11 | – |
| Vanadate | 500 | 2 | – |
| 2-Deoxy-D-glucose | 5000 | 0 | – |
| Iodoacetamide | 500 | 0 | – |
| Uranyl nitrate | 100 | 40 | 0.280 |
| | 500 | 58 | |
| | 1000 | 60 | |
| 2,4-Dinitrophenol | 1 | 33 | 0.003 |
| | 10 | 82 | |
| | 50 | 99 | |
| | 100 | 100 | |
| Sodium azide | 10 | 30 | 0.019 |
| | 20 | 52 | |
| | 50 | 98 | |
| | 100 | 100 | |
| Antimycin A | 0.1 [a] | 79 | 0.05 [a] |
| | 1.0 | 90 | |
| | 5.0 | 91 | |
| | 10.0 | 92 | |
| DES | 200 | 28 | |
| CCCP | 5 | 2 | 0.008 |
| | 7 | 12 | |
| | 10 | 92 | |
| | 20 | 94 | |
| | 50 | 98.5 | |
| DCCD | 100 | 0 | – |

[a] In μg/ml.

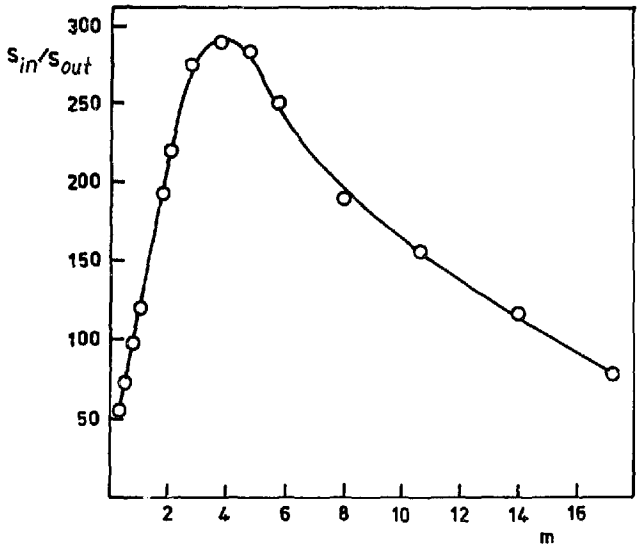


Fig. 3. Dependence of the accumulation ratio ($s_{in}/s_{out}$) of L-lysine (initial concn. 50 μmol/l) on the density of yeast suspension (*m*, mg dry wt. per ml).

*Accumulation ratio and suspension density*

As described by Janda and Kotyk [12] and Kotyk [13] suspension density plays a role in the capacity of various metabolic and transport processes of yeast. The high-affinity system transporting lysine in *S. pombe* is no exception. The accumulation ratio of lysine showed a maximum at about 2.5–5.0 mg dry wt. per ml (Fig. 3), somewhat lower than in the case of sugars in *Lodderomyces elongisporus* or *Rhodotorula gracilis*, or in the case of L-proline in *S. cerevisiae* [13].

## Discussion

The transport of L-lysine in *S. pombe* shows some features that are similar to those found in *S. cerevisiae* and other yeasts and some that are unique.

(1) Transport of lysine in *S. pombe* is active and unidirectional, in analogy with the transport of amino acids in other yeasts [1].

(2) The high-affinity system for L-lysine transport of *S. pombe* is similar in its $K_T$ (26 μmol/l) to the highly specific systems for transport of lysine in *S. cerevisiae* ($K_T$ of 25 μmol/l [14]) and in *S. lipolytica* ($K_T$ of 20 μmol/l [15]). On the other hand, Foury and Goffeau [16] estimated a $K_T$ for lysine transport in *S. pombe* to be 250 μmol/l. However, they used the mutant strain C0B5 and different experimental conditions.

(3) Transport of lysine under our conditions is inhibited by ammonium ions similarly as in *S. cerevisiae*, where the systems for amino-acid transport and for ammonium transport are supposed to compete for the energy source [17]. Moreover, ammonium ions also inactivate and repress the synthesis of GAP1 (general amino acid permease) and PUT4 (proline permease) in *S. cerevisiae* [18–20].

But system I of Fantes and Creanor [2], which is supposed to transport all basic amino acids in *S. pombe*, operates normally in the wild-type strain whether ammonium ions are present or not.

(4) Preliminary measurement of membrane potential and ΔpH across the plasma membrane showed that the protonmotive force was sufficient for producing the accumulation ratios of lysine found. Unfortunately, there is no direct evidence of the role of the proton gradient in lysine uptake in this yeast. No changes in the extracellular pH were found if lysine (50 μmol/l) was added to the cell suspension, which might be due to the small capacity of the transport system. Also, the depolarization of the plasma membrane during lysine transport could not be demonstrated because of the short period of lysine uptake (about 10 min) and the much longer time (at least 60 min) needed for measurement of the membrane potential with tetraphenylphosphonium ions.

However, the effect of proton conductors (Table V) and analogous findings in *S. cerevisiae* [21] and also in

*S. pombe* [9,16] show that the proton gradient across the plasma membrane appears to be the driving force for lysine transport. The lack of effect of DCCD may be due to its inavailability at the principal site of its action, the mitochondrial ATP synthase or plasma membrane ATPase.

(5) Unlike in the other yeast species examined so far, the sytems for L-lysine transport in *S. pombe* are not constitutive. In *S. cerevisiae*, two systems (CAN1, LYP1) transport lysine and both of them are constitutive [14,22]. Systems for lysine transport in *S. lipolytica* [15] and *C. albicans* [23] are also constitutive.

The inability of stationary-phase cells to transport nitrogenous compounds was observed previously in *S. cerevisiae* [24,25] and, for xanthine, also in *S. pombe* [26]. The inability of exponential-phase cells of *S. pombe* to transport amino acids, in agreement with Johnston and Coddington [8,9], could have several reasons. (1) Osmotic shock if the harvested cells are washed with water; (2) presence of some inhibitory agent in the growth medium; (3) high intracellular reserves of free amino acids (*trans*-inhibition?); (4) shortage of energy necessary for transport; and (5) absence of the appropriate transport systems. The first four possibilities may be excluded on the basis of the following observations. (1) Transport was not measurable, regardless of whether the cells were resuspended in the growth medium or in water, and starvation of cells (to deplete the amino-acid reserves) did not bring about an increase in transport activity. (2) During the exponential phase of growth the cells have enough energy at their disposal. (3) The conditions usually used for 'energization' of yeast cells (15-min preincubation with 0.1% glucose, e.g. Ref. 27) did not lead to any significant increase of transport activity. Our results show that transport systems for L-lysine in *S. pombe* are synthesized only under certain conditions (absence of nitrogen source?) in the presence of a sufficient source of energy. When the source of energy is exhausted, transport activity sharply decreases because of rapid degradation of proteins involved in the transport. Systems transporting amino acids in *S. cerevisiae* are supposed to be more stable (Horák, unpublished data).

(6) The uptake of lysine in *S. pombe* is strongly and competitively inhibited by L-arginine, L-histidine and D-lysine. In *S. cerevisiae* [22] and *S. lipolytica* [15] histidine is not transported by the same systems as lysine and arginine are. In *C. albicans* there exist specific systems for both lysine and/or arginine and systems for transport of histidine and other basic amino acids [23]. As far as substrate specificity is concerned, it is possible that our high-affinity transport system for lysine is very similar to the system for arginine described by Fantes and Creanor [2]. In their work, inhibition of arginine uptake by lysine was observed but histidine was not tested.

The absence of lysine-transport activity without preincubation of the cells with glucose is the major feature differing from other yeasts. Nonconstitutive systems seem to be common in *S. pombe*, the uptake of L-leucine, L-proline, L-glutamic acid and 2-aminoisobutyric acid is also measurable only after preincubation of cells with glucose (Synchrová, H. et al., unpublished data). The reasons for this phenomenon remain to be elucidated.

## References


1 Horák, J. (1986) Biochim. Biophys. Acta 864, 223–256.
2 Fantes, P.A. and Creanor, J. (1984) J. Gen. Microbiol. 130, 3265–3273.
3 Coddington, A. and Schweingruber, M.E. (1986) Curr. Genet. 11, 113–117.
4 Kotyk, A. and Michaljaničová, D. (1979) J. Gen. Microbiol. 110, 323–332.
5 García, J.C. and Kotyk, A. (1988) Folia Microbiol. 33, 281–284.
6 Kotyk, A., Horák, J. and Knotková, A. (1982) Biochim. Biophys. Acta 698, 243–251.
7 Sychrová, H., Horák, J. and Kotyk, A. (1988) in 14th International Congress of Biochemistry, Abstr. Tu:061.
8 Johnston, P.A. and Coddington, A. (1982) Mol. Gen. Genet. 185, 311–314.
9 Johnston, P.A. and Coddington, A. (1983) Curr. Genet. 7, 299–307.
10 Kotyk, A. (1979) in Vth Winter School of Biophysics of Membrane Transport, Vol. 2, pp. 49–67, Agricultural University Press, Wroclaw.
11 Kotyk, A., Říhová, L. and Ponec, M. (1971) Folia Microbiol. 16, 445–450.
12 Janda, S. and Kotyk, A. (1985) Folia Microbiol. 30, 465–473.
13 Kotyk, A. (1987) Yeast 3, 263–271.
14 Grenson, M. (1966) Biochim. Biophys. Acta 127, 339–346.
15 Beckerich, J.M. and Heslot, H. (1978) J. Bacteriol. 133, 325–338.
16 Foury, F. and Goffeau, A. (1975) J. Biol. Chem. 250, 2354–2362.
17 Roon, R.J., Levy, J.S. and Larimore, F.S. (1977) J. Biol. Chem. 252, 3599–3604.
18 Courchesne, W.E. and Magasanik, B. (1983) Mol. Cell. Biol. 3, 672–683.
19 Grenson, M. (1983) Eur. J. Biochem. 133, 135–139.
20 Grenson, M. (1983) Eur. J. Biochem. 133, 141–144.
21 Eddy, A.A. (1982) Adv. Microb. Physiol. 23, 11–76.
22 Grenson, M., Mousset, M., Wiame, J.M. and Bechet, J. (1966) Biochim. Biophys. Acta 127, 325–338.
23 Rao, L.R.S., Prasad, D.S. and Prasad, R. (1986) Biochim. Biophys. Acta 856, 237–243.
24 Kotyk, A. and Říhová, L. (1972) Biochim. Biophys. Acta 288, 380–389.
25 Woodward, J.R. and Cirillo, V.P. (1977) J. Bacteriol. 130, 714–723.
26 Seipel, S. and Reichert, U. (1980) FEBS Lett. 115, 289–292.
27 Ramos, E.H., De Bongioanni, L.C., Claisse, M.L. and Stoppani, A.O.M. (1975) Biochim. Biophys. Acta 394, 470–481.