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AN INDUCIBLE, SPECIFIC AND DEREPRESSIBLE TRANSPORT OF L-SERINE IN *SACCHAROMYCES CEREVISIAE*

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Both *rho*$^+$ and *rho*$^-$ cells were capable of accumulating L-serine against a concentration gradient; however, the extent of serine accumulation differed between these two strains. About 60% of the total accumulation of serine was reduced in *rho*$^-$ cells which were shown to lack functional mitochondria. The transport of serine was mediated via a specific and an inducible system. It was also derepressible under nitrogen-starved conditions. The derepression of L-serine uptake was also evident under conditions where general amino-acid permease is not expressed.

Introduction

The transport of amino acids in *Saccharomyces cerevisiae* is accomplished by a general amino-acid permease, which has a broad specificity, and by a number of specific transport system having more stringent specificities [1–6]. The number of specific permease(s) in *Saccharomyces* is not yet established, but about ten different transport systems, each of which is specific for one or a small number of amino acids, are described. The specific permeases for arginine, branched-chain amino acids, histidine, lysine, proline, dicarboxylic acids, L-asparagine and methionine are well characterized [1,3–8]. However, little is known about the specificities of the rest of the amino acids, for which it is generally agreed that they may be mediated via general amino-acid permease (GAP) [2]. The transport of L-serine belongs to the category of least-characterized transport systems. There is no report, to our knowledge, where L-serine transport in yeast is characterized. The present paper reports that, in *S. cerevisiae*, L-serine is transported via an inducible, specific and derepressible tranport system. Furthermore, it is demonstrated that *S. cerevisiae* (X-2180) is able to synthesize a specific transport system for accumulating L-serine which could be derepressed under conditions in which general amino-acid permease is not expressed.

Materials and Methods

Yeast strains and growth conditions. Saccharomyces cerevisiae strain IL8-8D, respiratory-competent strain (*rho*$^+$) and respiratory-deficient (*rho*$^-$) strain Bo60-AFI were obtained from Mr. K. Ganesan, India, who had previously obtained them from Drs. P.P. Slominiski and N. Gunge [9,10]. Cultures were grown in yeast extract, peptone and dextrose (YEPD) media containing adenine sulphate (0.003%) for 36 h and then transferred to fresh medium. After 16 h of growth, cells were harvested, washed with sterile distilled water and resuspended in distilled water prior to transport assay. X-2180-A2 (*GAP*$^+$) and X-2180 (*GAP*$^-$) strains of *S. cerevisiae* were obtained from Professor A.H. Rose, University of Bath, U.K. and

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were maintained and grown as described previously [7]. For derepression studies, various strains were either starved of a nitrogen source or grown in media containing a poor nitrogen source (proline, 0.3%). Such cells were then checked for the derepression of L-serine transport.

For induction studies, cells were grown in minimal synthetic growth media containing (w/v) glucose (1%), $(NH_4)_2SO_4$ (0.3%), KH_2PO_4 (0.3%), $MgCl_2$ (0.02%), biotin (10 μg/ml), uracil (0.003%) and $CaCl_2$ (0.025%). Cells were harvested at mid-log phase and preincubated with L-serine (28 mM). In order to check the induction, cells were also grown in L-serine as sole nitrogen source by replacing $(NH_4)_2SO_4$. Cells were harvested after indicated time intervals and resuspended in distilled water or buffer prior to their use.

Transport assay. The amino acids transport assay method was as described earlier [11–16]. Reaction mixture containing cells (1.5–2 mg protein/ml) was suspended in 50 mM citrate buffer (pH 6.0) and incubated for 10 min at 30 °C. The reaction was then initiated by the addition of L-[U-^{14}C]serine (375 μM, 5 μCi/ml). At indicated time intervals, an aliquot of 0.1 ml was transferred to 3 ml of chilled water and filtered rapidly through 0.45 μm Millipore filter discs (Maxflow, Bombay, India). The radioactivity retained on the filter discs was counted in a Packard scintillation counter using toluene-based scintillant. Unless otherwise mentioned, serine transport refers to L-serine transport. Protein was estimated according to Bradford's method [17] using bovine serum albumin as standard.

Oxygen uptake. Oxygen measurements were performed according to Jayakumar et al. [11], using a Clark electrode (YSI model, Ohio, U.S.A.). To assess the effect of inhibitors on oxygen uptake, they were added 10 min prior to actual measurements of oxygen concentration.

Preincubation with oxidizable substrates. In order to ascertain the effect of various oxidizable substrates (acetate, succinate, propionaldehyde and NADH), cells were starved to deplete their endogenous energy pools by aerating the cells for 14–16 h at 30 °C. The starved cells were suspended in 50 mM citrate buffer (pH 6.0), and were incubated with different substrates (5 mM acetate, 5 mM succinate, 5 mM glucose) at 30 °C. At the indicated time intervals, oxygen uptake and transport assay were performed.

Chemicals. L-[U-^{14}C]Serine was procured from Bhabha Atomic Research Center (BARC), Bombay, India. All chemicals used were of Analar grade or of the highest purity available.

Results

Uptake of serine in rho$^+$ and rho$^-$ cells

As compared to *rho*$^+$ cells, the level of accumulation of L-serine was 60% less in *rho*$^-$ cells (Fig. 1). *rho*$^-$ cells have previously been shown to lack functional mitochondria [10]. It therefore became apparent that about 60% of the total accumulation of L-serine was dependent on mitochondrial energy. In both cell types, the incorporation of L-serine into trichloroacetic acid-precipitable proteins was negligible (Fig. 1). An analysis of intracellular product accumulation during serine transport revealed that more than 90% of it could be recovered as free serine, indicating that serine is not significantly metabolized (data not shown). Kinetic data revealed that the apparent K_m values for L-serine in *rho*$^+$ and *rho*$^-$ cells were 166 μM

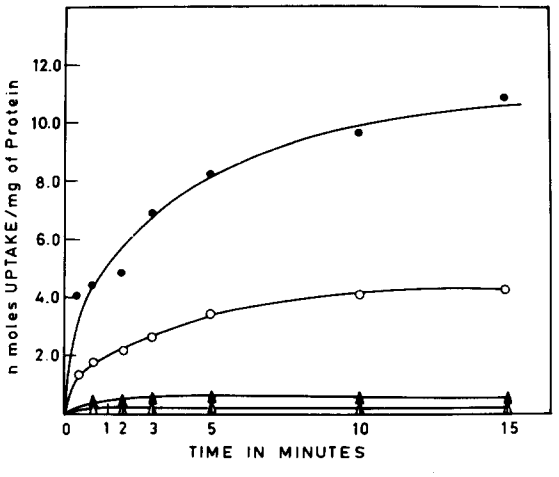

Fig. 1. Uptake of L-[^{14}C]serine in *rho*$^+$ (●———●) and *rho*$^-$ (○———○) cells. Transport of L-serine was assayed as described in Materials and Methods. For determination of L-[^{14}C]serine incorporation into trichloroacetic acid-precipitable proteins, an aliquot of samples was diluted 25-times in 5% trichloroacetic acid, boiled for 15 min and then filtered through millipore filter (0.45 μm): L-[^{14}C]Serine incorporation into trichloroacetic acid-precipitable in *rho*$^+$ (▲———▲) and *rho*$^-$ (△———△) cells.

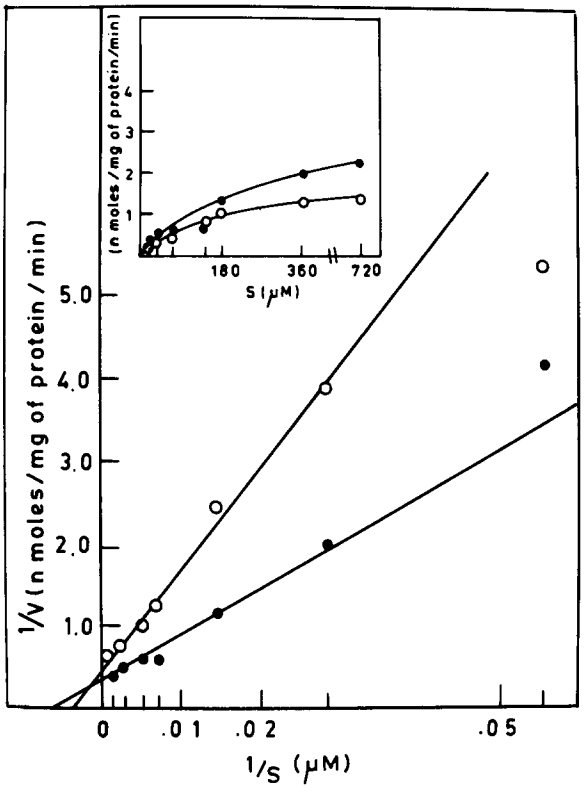

Fig. 2. Double-reciprocal plot of initial uptake of L-[^{14}C]serine in *rho*$^+$ (●——●) and *rho*$^-$ (○——○) cells. Inset shows the *V/S* plots.

and 385 μM, respectively (Fig. 2). The apparent K_m values represent only one system of serine uptake in these strains, since its uptake did not appear to be mediated via general amino-acid permease (see later results; Table IV and Fig. 6).

Effect of respiratory inhibitors on serine uptake

In order to ascertain the contribution of mitochondrial energy in L-serine transport, the effect of respiratory inhibitors was followed in *rho*$^+$ and *rho*$^-$ cells. It was observed that all the inhibitors were quite effective in reducing oxygen uptake and intracellular ATP level (40–98%) (Table I); however, their effect on L-serine accumulation was not of same order in *rho*$^+$ cells (10–50%). In *rho*$^-$ cells other inhibitors, except iodoacetate, a glycolytic inhibitor, had no effect in reducing ATP levels; however,these inhibitors (except arsenate) were able to reduce the uptake of L-serine between 29 and 32% (Table I).

Different ionophores and uncouplers were able to affect L-serine uptake. Similar to respiratory inhibitors, the extent of inhibition by any of the ionophores and uncouplers was not more than 40% in both types of cell (Table II).

Effect of oxidizable substrates on serine uptake

It can be seen from Fig. 3 that, in addition to glucose, the preincubation of *rho*$^+$ cells with acetate and succinate, which are capable of activating electron transfer and oxidative phosphorylation, could result in the enhancement of serine uptake. The preincubation of cells with these substrates was also associated with an increased

TABLE I

EFFECT OF RESPIRATORY INHIBITORS ON L-SERINE UPTAKE, OXIDATION AND CELLULAR ATP LEVEL IN *rho*$^+$ AND *rho*$^-$ CELLS

L-[^{14}C]Serine uptake was measured as described in Materials and Methods. Oxygen uptake and ATP level were measured as described by Jayakumar et al. [11]. For the effect of respiratory inhibitors on transport and cellular ATP level, the cells were preincubated for 10 min with different inhibitors prior to the addition of L-serine and perchloric acid, respectively. Values in parenthesis represent percentage inhibition of the control values. Values represent an average of three or four different sets of experiments. The average variation, in any case, was not more than 10%. n.f., not found.

Additions	Concentration	Serine uptake (nmol/mg protein per 5 min)		Oxygen uptake (μmol/mg protein per min)		Cellular ATP level (nmol/mg protein)	
		rho$^+$	*rho*$^-$	*rho*$^+$	*rho*$^-$	*rho*$^+$	*rho*$^-$
None	—	5.49	2.21	11.67	7.6	6.34	1.78
KCN	10 mM	3.45 (37.1)	1.55 (29.8)	3.79 (68.6)	6.6 (12.3)	0.09 (98.5)	1.75 (1.6)
Iodoacetate	1 mM	4.75 (13.4)	1.50 (32.1)	2.64 (77.3)	4.0 (48.0)	2.25 (64.5)	1.15 (35.3)
Arsenate	25 mM	3.66 (33.3)	1.92 (10.8)	4.37 (62.5)	5.6 (25.7)	3.77 (40.4)	1.78 (n.f.)
Azide	5 mM	2.74 (50.8)	1.53 (30.8)	1.38 (88.1)	2.89 (62.0)	3.90 (38.4)	1.70 (4.4)

TABLE II

EFFECT OF PROTON CONDUCTORS, IONOPHORES AND ATPase INHIBITORS ON THE TRANSPORT OF L-SERINE IN NORMAL *rho*$^+$ AND *rho*$^-$ CELLS

The cells were preincubated for 10 min with these inhibitors prior to the addition of L-[^{14}C]serine. Transport assay was as described in Materials and Methods. Values in parenthesis represent percentage inhibition of the control values. Values represent an average of three or four different sets of experiments. The average variation, in any case, was not more than 10%.

Additions	Concentration	L-Serine uptake (nmol/mg protein per 5 min)	
		rho$^+$	*rho*$^-$
None	—	5.49	2.21
m-ClCCP	50 μM	3.28 (40.2)	1.48 (34.3)
Valinomycin + K^+	40 μM	3.38 (38.4)	1.53 (30.7)
DDA^+	1 μM	3.75 (32.6)	1.43 (35.2)
TPB^-	100 μM	4.4 (19.8)	2.0 (9.5)
DNP	10 μM	3.3 (39.8)	1.6 (27.6)
Dio-9	100 μg/ml	3.41 (37.8)	1.44 (34.8)
DCCD	100 μM	3.73 (32.0)	1.27 (42.5)

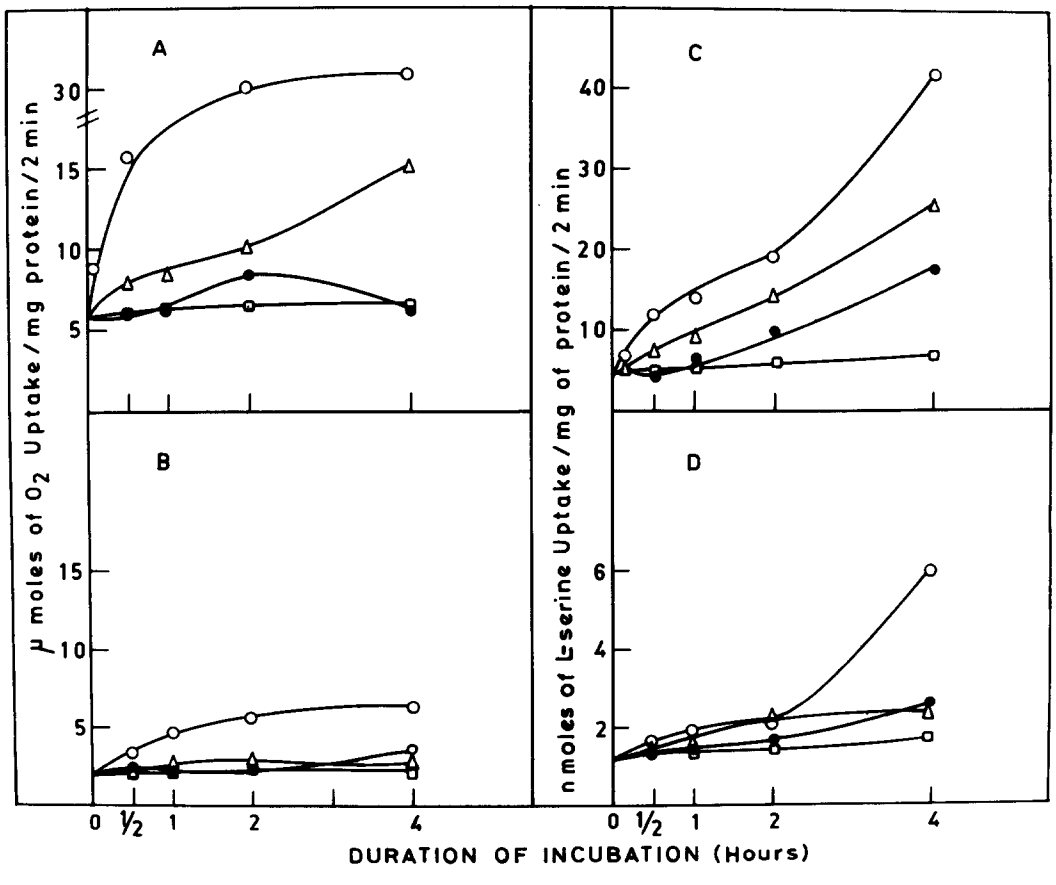

Fig. 3. Effect of oxidizable substrates (glucose (○———○), acetate (●———●) and succinate (△———△)) on the oxygen uptake (A and B) and serine transport (C and D) in *rho*$^+$ (A, C) and *rho*$^-$ (B, D) cells. □———□, the basal level of serine and oxygen uptake in the absence of any oxidizable substrate.

rate of oxygen uptake (Fig. 3A and B). However, under similar conditions, the preincubation of exogenous NADH and propionaldehyde was ineffective in enhancing the transport of L-serine (data not shown). In *rho*$^-$ cells, none of the other substrates, except glucose, could enhance oxygen or serine uptake (Fig. 3B and D).

Serine transport in serine-grown or -preincubated cells

When serine-preincubated *S. cerevisiae* cells were tested for their ability to accumulate L-serine, they exhibited a significant enhancement in the level of its accumulation (Fig. 4A). The apparant K_m for serine uptake in serine-preincubated cells was less than that in the normal-grown cells (for normal cells, 0.40 ± 0.02 mM; serine-preincubated cells, 0.25 ± 0.05 mM) (inset, Fig. 4A). The maximum enhancement of serine uptake was achieved within 30 min. The addition of cycloheximide or ethidium bromide during the preincubation of the cells with serine completely prevented the increase in serine transport (Fig. 4A), which indicated that protein synthesis was probably required for its induction. The continuous presence of the inducer (serine) was also necessary for maintaining the induction phenomenon (Fig. 4B).

When *S. cerevisiae* cells were grown in the presence of serine as a replacement of nitrogen source, the cells did not exhibit this enhancement. The increment in serine uptake in $(NH_4)_2SO_4$-depleted medium was only demonstrable when glucose was also removed from the growth medium (Fig. 5A). Similar results were obtained when exponentially grown cells of *S. cerevisiae* were preincubated with serine in the presence and absence of glucose (Fig. 5A, inset). It seems that, similarly to other eukaryotic systems, the serine-inducible component is also under catabolite repression (Fig. 5A).

Characteristics and specificity of the serine-induced transport system

The preincubation of *rho*$^-$ cells with serine did not result in any enhancement of its transport (Fig. 4A). It is probable that petite mutation has made *rho*$^-$ cells incompetent for the induction of serine transport, or alternatively the decrease in

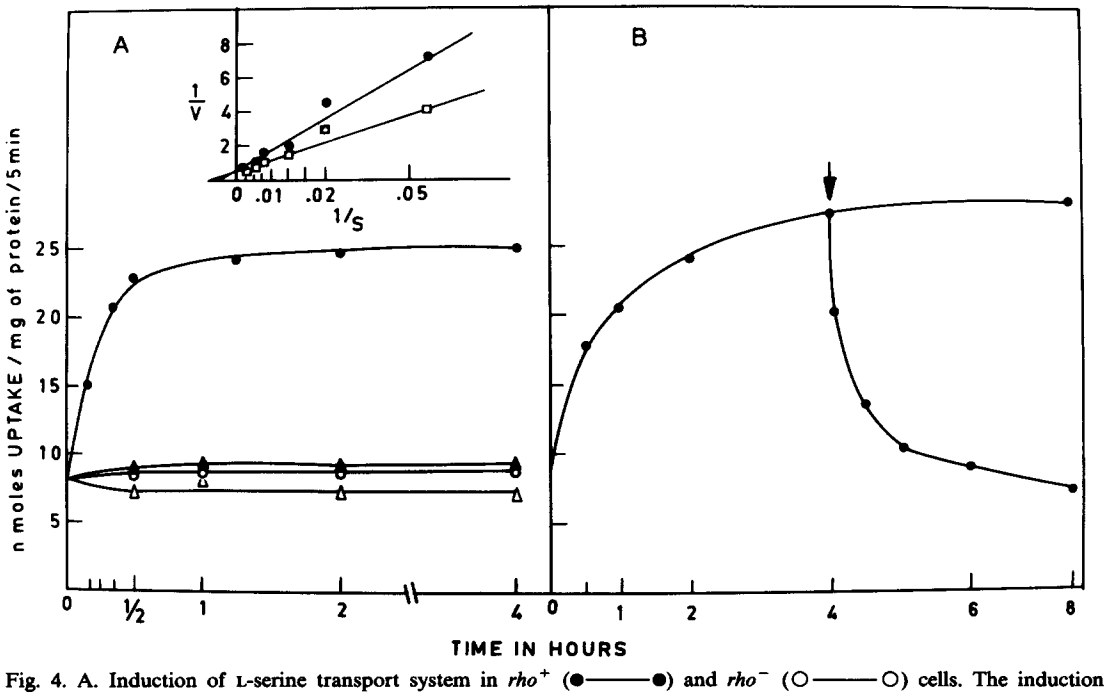

Fig. 4. A. Induction of L-serine transport system in *rho*$^+$ (●———●) and *rho*$^-$ (○———○) cells. The induction of L-serine transport was assayed as described in Materials and Methods. In order to see the effect of drugs, cycloheximide (▲———▲) and ethidium bromide (△———△) were added during the preincubation of cells with L-serine. Inset shows the kinetics of normal (○———○) and induced cells (□———□). B. The arrow shows the time of removal of the inducer (L-serine) from the media.

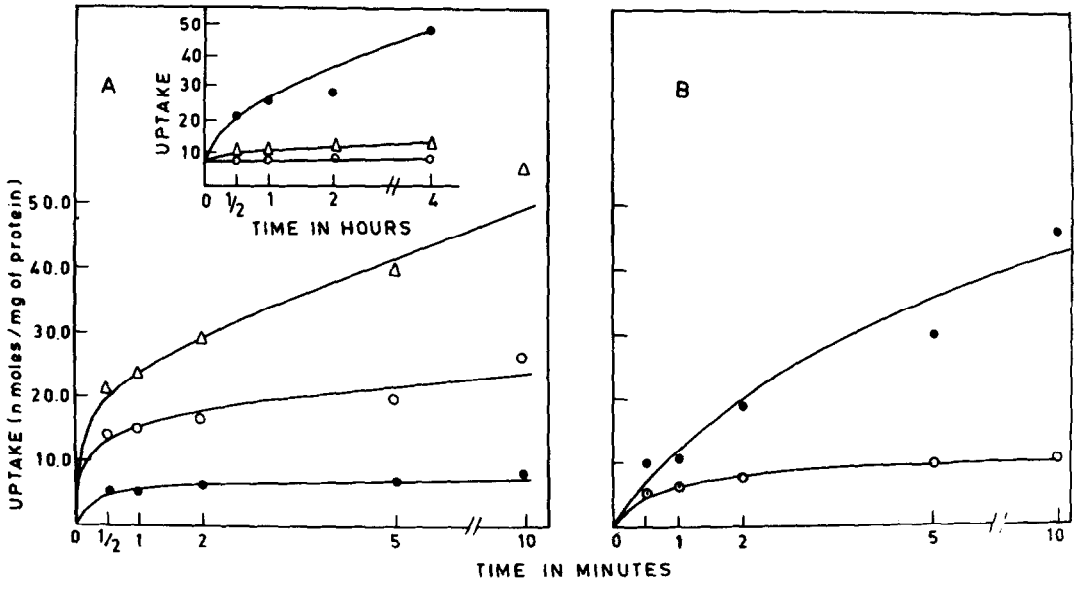

Fig. 5. A. Catabolite repression of L-serine transport in *rho*$^+$ cells. Cells were grown in synthetic growth media containing glucose (1%) and $(NH_4)_2SO_4$ (0.3%) (○———○) or in synthetic growth media containing glucose (1%) and L-serine (0.3%) as a replacement of $(NH_4)_2SO_4$ (●———●) or in synthetic media containing L-serine (0.3%) and neither $(NH_4)_2SO_4$ nor glucose (△———△). After growth, cells were harvested, washed and the transport of L-serine in cells grown differently was then assayed as described in Materials and Methods. The inset shows catabolite repression in preincubated cells. Exponentially grown cells (○———○); cells in a medium containing 28 mM L-serine and 1% glucose (△———△) and cells in a media containing L-serine only (●———●). Values represent 5 min accumulation (nmol/mg protein) of L-serine at the indicated time of preincubation. B. Time-course of L-serine uptake. Cells were grown to mid-log phase in synthetic growth medium, harvested, washed and resuspended in distilled water (○———○) and glucose (●———●). After 4 h of preincubation, the L-serine uptake was assayed in both the sets.

the energy metabolism of *rho*$^-$ cells could also affect their overall inducibility. It is pertinent to mention that certain nuclear genes which determine the surface characteristics have been shown to be regulated by the mitochondria in *S. cerevisiae* cells [18]; however, it is difficult to theorize that the induction of serine was in any way under mitochondrial control. In order to assess the overall specificity of serine induction, cells of various other laboratory strains of *S. cerevisiae* as well as *Candida albicans* were preincubated or grown in serine. While induction was observed in various available *S. cerevisiae* strains, it was not detected in *C. albicans* cells (data not shown).

To determine whether the preincubation of cells with other amino acids might affect serine accumulation, the cells were preincubated with different amino acids and serine uptake was then followed in those cells. The induction of serine transport appeared to be quite specific, since the preincubation with the other amino acids did not result

TABLE III

EFFECT OF AMINO ACIDS ON THE INDUCTION OF L-SERINE IN *rho*$^+$ CELLS

Cells were grown in synthetic minimal media, harvested at mid-log phase, washed and resuspended in sterile distilled water with 0.3% amino acids. The cells were incubated for 4 h at 30 °C before being assayed for L-serine transport, which was measured as described in Materials and Methods. Values represent an average of three or four different sets of experiments. The average variation, in any case, was not more than 10%.

Amino acid	Uptake (nmol/mg protein per 5 min)	% Stimulation
None	6.5	—
Glycine	8.06	24.0
L-Serine	24.35	275.0
L-Arginine	8.14	25.3
L-Leucine	7.47	15.0
L-Lysine	7.50	15.5
L-Alanine	8.14	25.2
L-Glutamic acid	7.11	9.4
L-Methionine	7.18	10.5
L-Cystine	7.71	18.6

in any significant enhancement of serine accumulation (Table III).

Derepression of serine uptake

Cells grown in complete medium were resuspended in nitrogen-free medium (with or without glucose) and incubated at 30 °C for various intervals of time. Fig. 5B depicts the rate and level of accumulation of serine in control as well as in the cells which were preincubated for 4 h in nitrogen-free media. As shown in Fig. 5B, the capacity for serine accumulation increased in nitrogen-starved cells. Such enhancement of amino acid transport in nitrogen-free medium has earlier been termed 'derepression', which was described for general amino acid permease and for certain specific amino acid transport systems of *S. cerevisiae* cells [2,19,20] and *S. chevalieri* [20].

The serine transport system appears to be mediated via a specific permease(s), since its uptake was not significantly reduced by the presence of 10-fold higher concentration of most of other amino acids (Table IV). To ascertain further whether the specific serine transport could also be

TABLE IV

EFFECT OF VARIOUS AMINO ACIDS ON THE TRANSPORT OF L-SERINE IN *rho*$^+$ CELLS

The cells were grown in yeast extract, peptone and dextrose media to exponential phase, washed and incubated with 10-fold higher concentrations of different amino acids for 10 min, prior to the addition of radioactive L-serine. The transport was assayed as described in Materials and Methods. Values represent an average of three or four different sets of experiments. The average variation, in any case, was not more than 10%.

Amino acid	% Inhibition
Glycine	4.5
L-Alanine	6.2
L-Leucine	16.1
L-Phenylalanine	16.9
L-Tryptophan	19.3
L-Cystine	8.9
L-Methionine	29.6
L-Proline	21.3
L-Aspartic acid	14.1
L-Lysine	2.2
L-Histidine	22.7
L-Arginine	13.2
L-Threonine	28.0
D-Serine	6.9

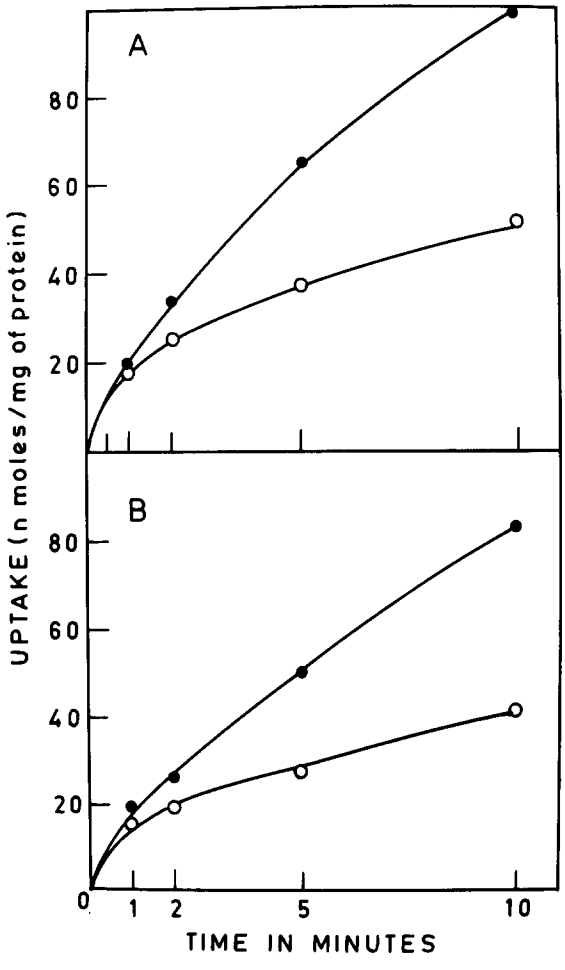

Fig. 6. Uptake of L-serine in X-2180-A2 (*GAP*$^+$) and X-2180 (*GAP*$^-$ mutant) *S. cerevisiae* cells. A. *GAP*$^+$ (X-2180-A2) cells grown in medium containing NH_4^+ (○———○) and in medium containing proline (●———●) as a replacement of NH_4^+. B. *GAP*$^-$ mutant cells were grown in medium containing NH_4^+ (○———○) and medium containing proline (●———●).

derepressed, it was followed in two strains of *S. cerevisiae*, one with general amino-acid permease (*GAP*$^+$), the other without general amino-acid permease (*GAP*$^-$ mutant). There was no significant difference in the rates and levels of accumulation of serine in *GAP*$^+$ and *GAP*$^-$ strains grown in NH_4^+-containing media (Fig. 6). It was interesting to note that the growth of both the strains in proline-containing media (as a replacement of NH_4^+) resulted in an increase in the overall accumulation of serine transport (Fig. 6A and B).

These results indicated that L-serine transport is not mediated via general amino-acid permease and that serine transport can be repressed or derepressed in *S. cerevisiae* cells irrespective of general amino-acid permease's presence or absence.

Discussion

We have attempted to characterize the serine uptake system by using two types of yeast cells, a *rho*$^+$ strain of *S. cerevisiae* which is respiratory-competent [9] and a *rho*$^-$ mutant strain which completely lacked mitochondrial DNA, thereby making it respiratory-deficient [10]. Our results demonstrated that, as compared to *rho*$^+$ cells, 60% of the total transport of serine was reduced in *rho*$^-$ cells. It is quite likely that 60% of the total serine transport in *S. cerevisiae* depends on mitochondrial energy. The results from rho$^+$ cells (Table I) also suggested this, since the accumulation of serine was reduced between 40% and 50% by the respiratory inhibitors in *rho*$^+$ cells. The limited effectiveness of these inhibitors in reducing the uptake of serine could not be attributed to their impermeability in these strains, since under identical conditions they were quite effective in reducing ATP level and oxygen uptake (Table I).

rho$^+$ cells, suspended in 375 μM serine, accumulated about 8–10 nmol serine/mg protein after 10 min. Assuming that 1 mg of cell protein contains 2.0 μl of water [21], the intracellular concentration of serine was 13–15-fold higher than that of external serine present in the medium. Based on the above calculations, it can be concluded that serine was accumulated against a concentration gradient. It was also accumulated against a concentration gradient in *rho*$^-$ cells; however, the gradient was lower as compared to *rho*$^+$ cells.

Although there are several inducible amino acid transport systems in bacteria which have been characterized [22–27], there are not many such systems identified in yeast [12,28]. Our present results indicate that, apart from a very specific constitutive transport system of serine, *S. cerevisiae* cells also have an inducible serine transport permease. The addition of cycloheximide or ethidium bromide during the preincubation of cells with serine prevented the induction of serine uptake, which suggested that the synthesis of a new protein is a prerequisite for the induction of serine transport. These drugs may also increase the internal amino acid pool, which may in turn affect the overall induction.

Out of two types of systems for transporting amino acids in yeast, the general amino-acid permease is known to be repressed when yeast is grown in media containing NH_4^+ [2,29–31]. In addition, some specific transport systems, e.g., proline and dicarboxylic amino acids, also exhibit an 'ammonia effect' [19,20]. In general, however, other types which are known to be specific for a single or a group of amino acids are not repressible [2,4]. Serine transport, in the present case, was found to be repressed in the presence of NH_4^+ and derepressed upon its removal, and yet it does not appear to belong to the general amino-acid permease class. Since general amino-acid permease is known to transport both D- and L-stereoisomers of amino acids, it is important to mention here that a 10-fold concentration of D-serine had no effect on the overall accumulation of serine (Table IV), thereby excluding the possibility of serine being transported via general amino-acid permease. The results in Table IV further demonstrate that the uptake of L-serine is mediated via a specific permease, since 10-fold higher concentrations of various amino acids could not significantly affect its overall accumulation. The rates and the levels of accumulation of L-serine were identical in both general amino-acid *GAP*$^+$ and -minus mutant strains (Fig. 6), which again suggests that serine was not transported via general amino-acid permease. The results presented thus clearly demonstrate that serine transport is quite specific and derepressible under conditions where general amino-acid permease is not expressed.

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