Characterization of *AAT1*: a Gene Involved in the Regulation of Amino Acid Transport in *Saccharomyces cerevisiae*

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A new class of *Saccharomyces cerevisiae* mutants (*aat1* – amino acid transport) has been identified. These mutants are unable to grow on rich medium or on minimal medium supplemented with certain amino acids (isoleucine, methionine, phenylalanine, tyrosine or valine). This phenotype is directly linked to the presence of the *leu2* allele in these strains: *aat1 LEU2* organisms grow normally on all media tested. Leucine uptake through the leucine-specific permease is inhibited to <35% of wild-type levels in *aat1* cells preincubated in nonpermissive media, and the activity of the general amino acid permease is also low in these conditions. *aat1* cells are therefore unable to grow on rich media because they cannot take up enough leucine to supplement their auxotrophic requirement.

INTRODUCTION

The yeast *Saccharomyces cerevisiae* is able to utilize many nitrogenous compounds as its principal nitrogen source for growth (Large, 1986). A complex system of regulation (nitrogen catabolite repression) has evolved which allows the utilization of preferred nitrogen sources first; these are usually compounds that are readily metabolized to ammonia in the cytoplasm, e.g. glutamine (reviewed in Cooper, 1982*a*; Large, 1986). Although the phenomenon of nitrogen catabolite repression was described a long time ago (Hierholzer & Holzer, 1963; Middlehoven, 1964), the molecular details of the mechanism are still under investigation. When a good nitrogen source is available, yeast will repress many pathways of amino acid catabolism, e.g. asparagine degradation (Dunlop *et al.*, 1980), allantoin degradation (Bossinger *et al.*, 1974), the activity of the catabolic NAD-linked glutamate dehydrogenase (Roon & Even, 1973), and the uptake of amino acids into the cell by the general amino acid permease (GAP) (Horak, 1986). Nitrogen control of the amino acid permeases has been shown to involve at least three gene products: Np1 and 2 (formally Mut2 and Mut4), which are involved in permease inactivation in ammonia, and Npr1 (nitrogen permease reactivator protein; Vandenbol *et al.*, 1987), which is essential for reactivation of transport activity in non-repressing media.

While it is obviously an advantage for the cell to shut down import and metabolism of poor nitrogen compounds when a more favourable one is available, it is also imperative that the cell have a mechanism to allow import of an essential nutrient. Therefore strains auxotrophic for a particular amino acid must be able to take up that amino acid when a preferred compound is being used as nitrogen source and nitrogen catabolite repression is in effect. This is accomplished by utilizing at least two different transport systems for most amino acids (Cooper, 1982*b*). The first, mentioned above, is the catabolic GAP transporter; this is a low-affinity, high-capacity system that is relatively non-specific and can import an amino acid which is in high concentration in the medium when needed as a nitrogen source (i.e. in the absence of nitrogen repression). There are also anabolic transport systems in which amino acids are brought in principally as precursors for macromolecule biosynthesis. These transporters are high-affinity,

Abbreviation: GAP, general amino acid permease.

low-capacity systems that are specific for one, or a small group of amino acids and are responsible for uptake of an amino acid that cannot be synthesized *de novo* in the cell. These systems are not subject to nitrogen catabolite repression. Many are controlled by feedback inhibition or by transinhibition of structurally related compounds (Horak, 1986).

This dual set of transport systems allows yeast to grow on preferred nitrogen sources and also supplies required nutrients for growth. There is at least one known example where this type of regulation does not appear to work. Proline auxotrophs (*pro1*, *pro2*, *pro3*; Brandriss, 1979) are unable to grow on rich (YPD) medium, even when 0.1% proline is added to the medium. This phenotype has not been investigated in detail, however, as both the specific proline transport system (*PUT4*) and GAP are subject to nitrogen catabolite repression (Lasko & Brandriss, 1981; Horak, 1986); a probable explanation is that proline auxotrophs are unable to take up sufficient proline for growth in rich medium.

This paper describes the isolation and characterization of a group of mutants unable to grow on rich media or on minimal media supplemented with certain amino acids. This particular mutant phenotype (*aat1* – amino acid transport) can only be observed in a strain auxotrophic for leucine and has been shown to be caused by the inhibition of leucine uptake under certain growth conditions.

METHODS

Strains and plasmids. The following strains of *Saccharomyces cerevisiae* were used: SEY2101 (*MAT α ura3-52 leu2-3,112 suc2-9 ade2-1 gal2*), SEY2102 (*MAT α ura3-52 leu2-3,112 his4- Δ 519 gal2*) (Emr *et al.*, 1986), JGY1001 (as SEY2102 *aat1-1*), JGY1002 (as SEY2101 *aat1-2*) and BC14 (*MAT α ade1 gap1-1*), which was kindly supplied by B. Magasanik (Massachusetts Institute of Technology). The plasmid pZUC13 (*LEU2*; G. Ammerer, unpublished results) was transformed into yeast using standard methods (Sherman *et al.*, 1986).

Media and growth conditions. Standard yeast media as described by Sherman *et al.* (1986) were used for all procedures. Minimal media (Wickerham, 1946) are identified as follows: MA, minimal-glucose-ammonia; MP, minimal-glucose-proline. Supplements were added at the following concentrations (mg l⁻¹) when required: histidine 20; leucine, 30; adenine, 30; and uracil, 30. Minimal media containing a nitrogen source other than ammonia were made as follows: Bacto yeast nitrogen base without amino acids and without ammonia (1.7 g l⁻¹), glucose (2.0%, w/v) and nitrogen source (0.2%, w/v); the medium was buffered to pH 5.5 after nitrogen source addition.

Growth measurements. The doubling times of cultures in balanced growth were determined by repeated dilution of exponentially growing cultures into fresh glucose minimal medium containing 0.2% of the specified nitrogen source and supplementary amino acids as required. The increase in optical density at 600 nm of cultures aerated at 30 °C was followed for 2–4 d to ensure that a balanced growth rate was observed.

Mutant isolation and characterization. Cultures of SEY2102(pCBZ1) (pCBZ1 is a *URA3* 2 μ plasmid containing an *ATP2-lacZ* gene fusion which is irrelevant to this study; Emr *et al.*, 1986) were grown in MA medium overnight, transferred to phosphate buffer and treated with ethyl methanesulphonate (EMS, 1.5% final concn) for 30 min. The cells were then treated with sodium thiosulphate (5% w/v) and dilutions were plated on minimal-glycerol-ammonia plates. This treatment resulted in 50–60% killing. Two of the resulting mutants were found to be unable to grow on YPD plates; this phenotype was not altered by curing the strain of the fusion plasmid, pCBZ1. Standard crosses were performed as described by Sherman *et al.* (1986).

Amino acid transport assays. Uptake of ¹⁴C-labelled amino acids was assayed by the method of Woodward & Cirello (1977) in cells growing exponentially (OD₆₀₀ 0.2–0.5) in the media indicated. In each case radioactive amino acid was added to a final concentration of 0.1 mM with a specific activity of 0.5 μ Ci μ mol⁻¹ (18.5 kBq μ mol⁻¹) in the assay mixture. In all experiments uptake was linear over a 15 min assay period and the results given are the mean of at least two separate experiments. L-[¹⁴C]Arginine hydrochloride (150 mCi mmol⁻¹), L-[¹⁴C]glutamic acid (125 mCi mmol⁻¹), L-[¹⁴C]leucine (75 mCi mmol⁻¹) and L-[¹⁴C]valine (125 mCi mmol⁻¹) were obtained from Amersham. L-[ureido-¹⁴C]Citulline (47 mCi mmol⁻¹) and L-[¹⁴C]leucine (337 mCi mmol⁻¹) were obtained from New England Nuclear. Total protein was assayed using the method of Bradford (1976).

RESULTS

Two yeast mutants unable to grow on rich medium (YPD) were fortuitously isolated during a mutagenesis experiment. The original mutagenesis was designed to discover mitochondrial protein targeting mutants and EMS-mutagenized cells were selected on minimal-glycerol-ammonia medium. During subsequent stages of the isolation two mutants were identified which

Fig. 1

Fig. 2

Fig. 1. Growth of *aat1* yeast in minimal and rich media. Cultures of SEY2102 (*MAT α ura3-52 leu2-3,112 his4- Δ 519 gal2*) (open symbols) and JGY1001 (as SEY2102, *aat1-1*) (filled symbols) were grown in MA medium supplemented with histidine, leucine and uracil (as described in Methods) to OD₆₀₀ 0.5. The cells were collected and resuspended in either the same MA (○, ●), YP-glucose (□, ■) or YP-glycerol (△, ▲) at OD₆₀₀ 0.1. The cultures were incubated at 30 °C with aeration and the OD₆₀₀ followed for 12 h.

Fig. 2. Inhibition of growth of *aat1* yeast by hydrophobic amino acids. Cultures of SEY2102 (open symbols) and JGY1001 (filled symbols) were grown as described in Fig. 1. Exponentially growing cells were diluted into fresh medium (○, ●), or into medium to which either isoleucine (△, ▲) or phenylalanine (□, ■) was added to a final concentration of 0.05%. The cultures were incubated at 30 °C with aeration and the growth rate followed for 24 h. Methionine, tyrosine and valine had effects on the growth of *aat1* similar to those shown here.

failed to grow on YPD plates. When these mutants were backcrossed to the parental strain (SEY2102), tetrad analysis showed that the YPD⁻ and mitochondrial targeting phenotypes did not co-segregate. Haploid YPD⁻ segregants were backcrossed again and the resulting YPD⁻ strains (JGY1001, 1002) used in all subsequent experiments. The YPD⁻ phenotype is completely recessive and both mutants were found to be in the same complementation group, which has been called *aat1*.

The phenotype of these mutants is quite dramatic. As shown in Fig. 1, *aat1* strains grew normally at 30 °C (and 37 °C – data not shown) in minimal medium containing ammonia as principal nitrogen source but barely completed one doubling in rich media. Fig. 1 also illustrates that the phenotype is not growth rate dependent. The parental strain grew at approximately the same rate in MA medium as in YP-glycerol; the mutants could grow at this rate in MA medium but were unable to grow in YP-glycerol. In testing various components of rich media for growth inhibition, it was found that the growth of *aat1* in MA medium was inhibited by the addition of certain amino acids. Fig. 2 shows the effect of the addition of 0.05% isoleucine and phenylalanine on *AAT1* and *aat1* cells growing exponentially on ammonia. The same growth inhibition was observed on addition of the other hydrophobic amino acids methionine, tyrosine and valine, either individually, or in any combination (data not shown). Other amino acids had no effect on the growth of *aat1* strains, except serine and threonine which, at higher concentrations (0.2%), caused partial growth inhibition (data not shown). *aat1* cells that had stopped growing in these supplemented media could not be diluted and grown further in these media. This phenotype can also be clearly observed on plates; *aat1* strains form microcolonies on YPD plates or on minimal plates supplemented with any of the inhibitory amino acids.

Table 1. *Measurement of balanced growth in AAT1 and aat1 yeast*

All strains were *ura3-52 leu2-3,112 his4-Δ519 gal2*. The *LEU2* strain contained the 2μ plasmid pZUC13, which carries the *LEU2* gene. The growth rate was determined by repeatedly diluting exponentially growing cells into fresh media until a balanced growth rate was obtained for > 2 d. The media contained 1 × Bacto yeast nitrogen base (without NH₃ and amino acids), 2% (w/v) glucose, 0.2% of the nitrogen source indicated, histidine (20 mg l⁻¹), leucine (30 mg l⁻¹) and uracil (30 mg l⁻¹). The doubling time of all three strains on media with no nitrogen source added was > 10 h. NG, No growth above background. No growth above background was observed in any of the strains on the following nitrogen sources: cysteine, glycine, histidine, isoleucine, lysine, methionine, phenylalanine, tyrosine and uracil. Growth of *aat1 leu2* was within 10% of the parental strain on the following nitrogen sources: allantoin, arginine, asparagine, citrulline, glutamate, glutamine, leucine, ornithine, proline, tryptophan and valine.

Nitrogen source	Doubling time (min)		
	<i>AAT1 leu2</i>	<i>aat1 LEU2</i>	<i>aat1 leu2</i>
Ammonia	170	180	180
Adenine	230	220	320
Alanine	225	225	320
Aspartate	190	200	350
Serine	250	270	390
Threonine	260	350	360
Urea	170	175	270
YPD	140	140	NG

The aat1 phenotype is dependent on the presence of the leu2 allele

The two alleles of *aat1* were isolated in a *leu2-3,112 ura3-52 his4-Δ519 gal2* background. Repeated attempts to make prototrophic strains with the *aat1* phenotype for use in single nitrogen source growth rate experiments failed to give any YPD⁻ LEU⁺ strains. As an alternative method of constructing a LEU⁺ strain, JGY1001 was transformed with a *LEU2*-containing plasmid, pZUC13; the resulting JGY1001(pZUC13) transformants were all YPD⁺ and showed none of the amino acid sensitivities of the auxotrophic mutant strain.

The ability of *aat1* yeast to use different compounds as principal nitrogen source was investigated using *AAT1* and *aat1* strains (both *leu2-3,112 ura3-52 his4-Δ519 gal2*) and *aat1* transformed with the multicopy *LEU2* plasmid pZUC13 (Table 1). While growth of the *aat1 leu2* strain was similar to wild-type on many nitrogen sources, including leucine, this strain grew more slowly than the parent *AAT1 leu2* strain on adenine, alanine, aspartate, serine, threonine and urea; these differences were not observed in the *aat1 LEU2* strain.

Amino acid transport assays

The linkage of the *aat1* growth phenotypes with the presence of the *leu2* allele suggests that growth inhibition is associated with the ability of the cell to import and utilize leucine to supplement its leucine auxotrophy. This hypothesis is also suggested by the extreme sensitivity of *aat1* strains to leucine analogues: the growth of *aat1 leu2* strains on MA plates is completely inhibited by the addition of cycloleucine (500 μg ml⁻¹), norleucine (200 μg ml⁻¹) or trifluoroleucine (100 μg ml⁻¹). These leucine analogues have no effect on the growth of either wild-type or *aat1 LEU2* yeast in these conditions (data not shown).

S. cerevisiae has been shown to contain at least two mechanisms for leucine uptake, one specific ($K_T = 0.05$ mM) and one non-specific ($K_T = 1.0$ mM, GAP) (Horak, 1986). [¹⁴C]Leucine uptake was assayed in the parental strain at a range of substrate concentrations (Woodward & Cirello, 1977), and the presence of both transport systems, with kinetics similar to those published previously, was demonstrated (data not shown). The rate of [¹⁴C]leucine uptake by the specific transporter was assayed using 100 μM-leucine and was found to be similar in wild-type and mutant cells growing in MP medium [SEY2102, 6.65 nmol min⁻¹ (mg protein)⁻¹; JGY1001, 5.06 nmol min⁻¹ (mg protein)⁻¹]. The rate of leucine uptake in *aat1* yeast was

Table 2. Rate of [14 C]leucine uptake in yeast: effect of preincubation with other amino acids

Strains were *ura3-52 leu2-3,112 his4-Δ519 gal2*. One hour prior to assay, the indicated amino acid was added to cells growing exponentially in MA medium, to a final concentration of 0.05% in the medium. After 1 h incubation, the cells were harvested, washed, and incubated in 10 mM-potassium phthalate buffer containing 2% (w/v) sucrose for 15 min at 30 °C. Then 100 μ M-[14 C]leucine (0.5 μ Ci μ mol $^{-1}$) was added, and, at certain time intervals, samples were removed, filtered and washed with 10 ml ice-cold water. Radioactivity on the filters was measured by liquid scintillation counting. In all cases uptake was linear for >15 min.

Addition	[14 C]Leucine uptake [nmol min $^{-1}$ (mg protein) $^{-1}$]*	
	<i>AAT1</i>	<i>aat1</i>
None	4.80 (100)	2.20 (46)
Alanine	4.26 (89)	2.14 (45)
Arginine	3.36 (70)	2.26 (47)
Glutamate	3.74 (78)	2.54 (53)
Glutamine	4.70 (98)	2.02 (42)
Histidine	5.16 (108)	3.28 (68)
Isoleucine	4.89 (102)	1.40 (29)
Methionine	4.12 (86)	1.46 (30)
Phenylalanine	5.60 (117)	0.99 (21)
Serine	4.27 (89)	1.50 (31)
Threonine	3.75 (78)	1.48 (31)
Valine	4.17 (87)	1.61 (34)
YPD†	2.44 (51)	0.89 (19)

* The percentage of the *AAT1* rate in MA medium is shown in parentheses.

† Cultures were grown in MA medium and transferred to YPD for 1 h prior to the assay.

Table 3. GAP activity in *aat1* yeast

Strains and method were as described in Table 2 except that the cells were grown in either MA or MP medium and assayed for the uptake of [14 C]citrulline. A *gap1* strain gave no activity [<0.1 nmol min $^{-1}$ (mg protein) $^{-1}$] in these conditions.

Nitrogen source	[14 C]Citrulline uptake [nmol min $^{-1}$ (mg protein) $^{-1}$]	
	<i>AAT1</i>	<i>aat1</i>
Ammonia	0.17	5.38
Proline	5.32	6.93

approximately 50% of that of the wild-type in cells grown in MA medium. Preincubation of the cells in YPD medium led to a further significant inhibition of leucine uptake in the *aat1* strain (Table 2). In order to determine which amino acids were affecting leucine transport, this experiment was repeated with preincubation of MA-grown cells in the presence of individual amino acids for 1 h prior to assay. The data in Table 2 show that the amino acids which inhibit growth in *aat1* strains in MA medium are those which inhibit leucine uptake to less than 35% of the wild-type level.

The activity of the GAP system was assayed using [14 C]citrulline, which enters the cell solely by this pathway, as substrate (Grenson *et al.*, 1970). The results in Table 3 show that GAP activity in *AAT1* is regulated as expected: it is fully active on a poor nitrogen source (proline) and is repressed on ammonia. In contrast, the GAP activity of *aat1* is not repressed by ammonia but is inhibited completely by preincubation of the cells in YPD. This effect was investigated further; the rates of [14 C]citrulline uptake by both strains growing exponentially in MP medium after preincubation with individual amino acids are given in Table 4. Many of the amino acids completely inhibited GAP activity in both strains; however, certain amino acids (arginine,

Table 4. *Inhibition of GAP activity by preincubation with other amino acids*

Strains and methods were as for Table 2 except that the cells were grown in MP medium prior to the 1 h incubation and assayed for the uptake of [^{14}C]citrulline.

Addition	[^{14}C]Citrulline uptake [nmol min $^{-1}$ (mg protein) $^{-1}$]	
	<i>AAT1</i>	<i>aat1</i>
None	5.32	6.93
Alanine	<0.1	<0.1
Arginine	<0.1	2.10
Aspartate	0.3	2.39
Glutamate	<0.1	<0.1
Histidine	<0.1	<0.1
Isoleucine	<0.1	<0.1
Leucine	5.64	5.89
Methionine	<0.1	3.02
Phenylalanine	1.61	3.58
Serine	0.15	1.26
Threonine	<0.1	0.34
Valine	<0.1	<0.1
YPD	0.67	<0.1

Table 5. *Uptake of other ^{14}C -labelled amino acids by *aat1* yeast*

Cells were grown in either MA or MP medium and assayed using 100 μM - ^{14}C -labelled amino acid substrate as described in Table 2.

Amino acid	Amino acid uptake [nmol min $^{-1}$ (mg protein) $^{-1}$]					
	Ammonia		Proline		YPD*	
	<i>AAT1</i>	<i>aat1</i>	<i>AAT1</i>	<i>aat1</i>	<i>AAT1</i>	<i>aat1</i>
Arginine	2.86	5.75	4.86	10.57	1.60	2.52
Glutamate	2.84	3.13	2.41	1.66	1.63	1.13

* Cell* Cells grown in MA were transferred to YPD medium for 1 h prior to assay.

aspartate, methionine, phenylalanine, serine) that inhibited GAP in *AAT1* had little effect on citrulline uptake in *aat1* yeast.

The inhibition of both leucine-specific transport and GAP activity in *aat1* strains transferred to YPD or other non-permissive media could potentially be the result of a non-specific loss of membrane integrity. In order to test this, the rate of uptake of arginine and glutamate in *AAT1* and *aat1* yeast was also assayed (Table 5). No significant decrease in transport of these amino acids was observed on preincubation of *aat1* in YPD medium.

AAT1 is not allelic to GAP

JGY1001 (*MAT α ura3-52 leu2-3,112 his4- Δ 519 gal2 *aat1-1**) was crossed with BC14 (*MAT α adel *gap1-1**) and the resulting diploid (JGY1001/G) was tested for *aat1* and *gap* phenotypes. JGY1001/G grew normally on both YPD and MP + D-histidine (test for *gap* – Rytka, 1975), showing that *aat1* is non-allelic to *gap1*.

DISCUSSION

A novel group of yeast mutants (*aat1*) has been identified that are unable to grow on rich media or on minimal medium with certain amino acid supplements (Figs 1 and 2). This growth phenotype has been shown to be directly linked to the presence of the *leu2* allele; *aat1 LEU2* yeast grow normally in all conditions investigated (Table 1). Similar types of growth inhibition

have been observed in other studies on amino acid metabolism and transport. Meuris *et al.* (1967) isolated yeast mutants unable to grow on minimal media with individual amino acid additions. They found that this growth inhibition could be reversed by the addition of structurally related metabolites, e.g. inhibition by tyrosine of a *ty-i* mutant could be reversed by the addition of phenylalanine and tryptophan. This phenotype can be explained by a mutation in a step of the biosynthetic pathway of aromatic amino acids that is in common to all three; the presence of excess tyrosine inhibits synthesis of phenylalanine and tryptophan by feedback inhibition. Similar mutants have been studied extensively in bacteria (Smith *et al.*, 1962; Dalal *et al.*, 1966) and have contributed significantly to our knowledge of biosynthetic pathways. However, *aat1* is clearly a different type of mutant; addition of all the inhibitory amino acids together does not reverse the effect (data not shown) and the mechanisms discussed above are clearly not appropriate to a YPD⁻ phenotype.

One known example of a YPD⁻ phenotype in yeast is found in proline auxotrophs (*pro1*, *pro2*, *pro3*) that appear to be unable to take up proline for protein synthesis in rich medium because all the proline transport systems are known to be inhibited by components of YPD (Brandriss, 1979). The realization that the *aat1* growth defects are directly linked to the presence of the *leu2* allele came from the reversion of *aat1* to YPD⁺ when transformed with a *LEU2* plasmid. This suggests that the mutant phenotype is a result of defective leucine uptake in rich or supplemented minimal media. The strain is effectively starving for leucine, even on YPD, because it is unable to take up the amino acid from the surrounding medium. This explanation is consistent with the amino acid sensitivities of *aat1*; previous results have shown that isoleucine and valine are competitive inhibitors of leucine uptake (Bussey & Umbarger, 1970) or alternatively, possible substrates of the system (Ramos *et al.*, 1980). However, there have been no previous reports of the other amino acids that confer sensitivity to *aat1* (phenylalanine, methionine, etc.) affecting leucine transport. This explanation is also consistent with the sensitivity of *aat1 leu2* yeast to the leucine analogues cycloleucine, norleucine and trifluoroleucine.

Leucine has been shown previously to be taken up by two different transport systems in *S. cerevisiae* (Horak, 1986), and our parental strain (SEY2102) showed uptake kinetics consistent with this hypothesis (data not shown). Leucine transport was assayed at low (100 μ M) substrate concentrations to determine the activity of the leucine-specific system only. In these assays, *aat1* cells growing on proline had nearly wild-type (83%) leucine transport; however, leucine transport was inhibited to only 46% of wild-type in *aat1* yeast growing on ammonia. Preincubation of the cells with certain amino acids further inhibited leucine uptake in *aat1* cells, and it was found that the amino acids that inhibited leucine transport to <1.7 nmol leucine min^{-1} (mg protein^{-1}) were the same amino acids that inhibited growth (Table 2). The effect was particularly severe when *aat1* cells were preincubated in YPD for 1 h prior to the assay (Table 2). The preincubation of *AAT1* yeast with these amino acids or YPD had significantly less effect on leucine transport. This effect on leucine transport must be mediated through transinhibition of one, or both, of the leucine transport systems by the amino acids loaded into the cell during the preincubation period. One possible explanation is that the regulation of the leucine-specific transporter is extremely sensitive to intracellular amino acid pool size and this, in turn, affects GAP activity. Alternatively the leucine transporter could be completely defective and the observed leucine uptake in *aat1* could be solely through the GAP.

In order to distinguish between these hypotheses, the activity of GAP was determined in cells grown in various media. The results in Table 3 show that GAP is not subject to nitrogen catabolite repression in *aat1* yeast. This was surprising, as other enzymes known to be subject to nitrogen repression (NAD-linked glutamate dehydrogenase, arginase; Cooper, 1982a) were regulated normally in *aat1* strains (data not shown). This further suggests that the leucine uptake observed in *aat1* could be due to GAP activity in both MA and MP media. However, [¹⁴C]citrulline uptake was inhibited in both parental and mutant MP-grown yeast by preincubation with many alternative nitrogen sources (Table 4). This shows that leucine uptake in *aat1* cannot be explained by GAP activity; preincubation of MP-grown *aat1* cells with many amino acids (e.g. alanine, histidine, glutamate) completely inhibits GAP activity (Table 4) and

yet significant leucine uptake is observed after incubation with these amino acids (Table 2). It is significant that GAP activity in *aat1* strains responds differently from the wild-type on preincubation with certain nitrogen sources (e.g. arginine and urea). This is not understood at present but could be caused by altered internal pools of certain nitrogenous compounds in the mutant strain.

The conclusion from the transport studies is that both transport systems are not regulated normally in *aat1*. This is not simply a result of loss of membrane integrity and function in non-permissive media, since the rates of arginine and glutamate uptake in *aat1* strains were not altered in these conditions (Table 5). The phenotype does, however, appear to be linked to the size of internal amino acid pools: this relationship is being investigated further.

Mutants (*aap*, *apf*) that pleiotropically affect amino acid transport have been isolated previously by their resistance to multiple amino acid analogues in yeast (Surdin *et al.*, 1965; Grenson & Hennaut, 1971). Other characteristics of *aap1* mutants include: the inability to grow on proline as nitrogen source; repressed GAP activity in all media; and greatly decreased levels of specific amino acid transporters (2–25% of wild-type). These phenotypes contrast with those of the mutants described here, which grow normally on proline, have full GAP activity and in which only the leucine transport system has been found to be inhibited. The existence of both types of mutant is interesting because it suggests that amino acid transport systems are regulated both individually in response to specific nutrient availability and collectively to provide general nitrogen sources for growth. It has also been suggested that ammonium transport may be co-regulated with the amino acid transport systems (Larimore & Roon, 1978).

The mechanisms of control of uptake of nitrogenous compounds in yeast in response to changing environmental conditions appear to be very complex and are not yet understood. It is hoped that this novel class of mutants will provide a new approach to the investigation of these pathways. At present, spontaneous temperature-sensitive revertants of *aat1* and three other *aat* (YPD⁻) groups are under investigation. A preliminary analysis has shown that those mutants include strains with very limited nitrogen source range and a *cdc* terminal phenotype (unpublished observations).

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