Canavanine Resistance and the Mechanism of Arginine Uptake in the Fission Yeast *Schizosaccharomyces pombe*

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The mechanism of resistance to the arginine analogue L-canavanine, and of arginine uptake, were examined in the fission yeast *Schizosaccharomyces pombe*. Two mutants with increased resistance to canavanine were analysed genetically: both were double mutants, and in each case one mutation conferred resistance to canavanine, while the other enhanced this resistance. Evidence is presented that *can1.1* strains are defective in one system for arginine uptake, which presumably prevents entry of canavanine into the cell. This system operates in the wild-type whether the nitrogen source supplied is ammonium or glutamate. Double mutants carrying *can1.1* and an arginine requirement are unable to grow on ammonium medium even when supplied with exogenous arginine, while growth can occur on glutamate plus arginine. This suggested the existence of a second uptake system for arginine which is absent during growth on ammonium, and direct measurements of the rates of arginine uptake under various conditions confirmed this. Our observations closely parallel those made on the budding yeast *Saccharomyces cerevisiae*. The ability to select for or against function of the *can1* gene should facilitate certain types of genetical analysis in *S. pombe*.

INTRODUCTION

The fission yeast *Schizosaccharomyces pombe* is one of the best-characterized eukaryotic micro-organisms for genetic studies. In addition to the methods of classical genetics, recent advances (Beach & Nurse, 1981) have stimulated interest in the use of recombinant DNA procedures combined with transformation of *S. pombe*. In contrast, the biochemistry and physiology of *S. pombe* lags behind that of the budding yeast *Saccharomyces cerevisiae*. In particular, no mutants have been reported in which a specific uptake system is genetically defective, though more general uptake defects have been described (Johnston & Coddington, 1983). We have initiated an investigation into the genetic and physiological basis of resistance to the arginine analogue L-canavanine, for three main reasons. First, the genetics of canavanine resistance have been investigated (Kohli *et al.*, 1977) and resistant mutants are available. Second, a substantial body of information exists about canavanine resistance in *Sacch. cerevisiae* (for a review, see Cooper, 1982), and it is of interest to find whether the two distantly related yeasts are similar in this respect. Third, canavanine resistance may prove to be a useful genetic marker in *S. pombe*, since it may be selected or counter-selected, as in *Sacch. cerevisiae* (Whelan *et al.*, 1979).

METHODS

Strains and genetical procedures. All strains used were derived from the wild-types 972 *h*⁻ and 975 *h*⁺ (Gutz *et al.*, 1974). All the auxotrophs, and the canavanine resistant strains, were kindly provided by the Microbiology Institute of the University of Bern, Switzerland. Strain ED 428 was sent to us as '*can1.1 h*⁻', and strain ED 429 as '*can2.1 h*⁻'. Crosses were carried out according to Gutz *et al.* (1974); stable diploid strains were constructed using the *mat2.102* mating type mutation as described by Kohli *et al.* (1977).

Media and culture conditions. EMM-A was essentially EMM3 (Creanor & Mitchison, 1982), but with 2% (w/v) glucose, and supplemented with KCl (1 g l⁻¹). EMM-A contained ammonium chloride (5 g l⁻¹) as nitrogen source.

EMM-G was identical to EMM-A but the nitrogen source was monosodium glutamate (3.74 g l^{-1}) in place of ammonium chloride. Solid media contained 2% (w/v) Bacto-agar (Difco). Complex medium was YEA [yeast extract 0.5% (w/v), glucose 3% (w/v), Bacto-agar 2% (w/v)]. Growth supplements were included at 75 mg l^{-1} for the growth of auxotrophic strains. L-Arginine and L-canavanine were added as hydrochlorides: canavanine hydrochloride was added from a filter-sterilized stock to autoclaved medium. The growth and sampling of liquid cultures, and cell number estimation, were carried out according to Mitchison (1970). Strains were grown at 30°C .

Arginine uptake. This was determined by adding to a sample (0.5–2 ml) of growing cells the desired amount of L-[5(n)- ^3H]arginine monohydrochloride (Amersham; $703 \text{ GBq mmol}^{-1}$; 37 MBq ml^{-1}) plus unlabelled L-arginine monohydrochloride and/or other amino acids as required. Samples were removed, filtered onto 2.1 cm GF/C filters (Whatman), and washed four times with unlabelled arginine hydrochloride solution (1 mg ml^{-1}) and four times with water. The filters were dried and counted in 0.5% (w/v) butyl PBD in toluene, on a Packard model 2407 liquid scintillation spectrometer. Counting efficiency was 35%.

Chemicals. These were all of analytical grade.

RESULTS

The effect of various concentrations of canavanine on the growth of wild-type and canavanine resistant strains was investigated by measuring the diameter of colonies derived from single cells after 3 d growth. Two media were used, EMM-A containing ammonium chloride as nitrogen source and EMM-G containing monosodium glutamate. Table 1 shows the effect of different concentrations of canavanine on the wild-type 972, and the two canavanine resistant strains ED 428 (carrying *can1.1*) and ED 429 (carrying *can2.1*). Strain ED 460 is a derivative of ED 428 described below.

At all canavanine concentrations, the strains were more sensitive when grown with glutamate as nitrogen source than with ammonium. The use of glutamate has been reported (Strauss, 1979) to allow a higher rate of uptake of certain substrates, and this was presumably responsible for the increased sensitivity to canavanine. The wild-type 972 was completely inhibited at the lowest canavanine concentration tested during growth on glutamate. Under the same conditions both ED 428 and ED 429 showed substantial growth, though neither strain grew as well in the presence of canavanine at any concentration as in its absence (Table 1). During growth on ammonium medium, the wild-type and the mutants showed greater resistance to canavanine than during growth on glutamate. However the wild-type was substantially more sensitive than either ED 428 and ED 429 (Table 1). Despite the different levels of resistance shown by the three strains, the extent of inhibition was consistently greatest for the wild-type and least for ED 428 under both sets of conditions (Table 1).

The tests just described were based on the ability of single cells to proliferate and form colonies under the various conditions. For genetical analysis, a purely qualitative test was required so that strains could be easily and unambiguously classified as sensitive or resistant. For this, patches of cells growing actively under non-selective conditions were replicated onto agar medium containing glutamate as nitrogen source and canavanine at $10 \mu\text{g ml}^{-1}$. After 3–5 d incubation strains could be classified according to the degree of resistance.

The genetic basis of canavanine resistance in strains ED 428 and ED 429 was investigated by crossing each strain to the wild-type 975 *h*⁺ and analysing the meiotic progeny by tetrad dissection. In both crosses the segregation of canavanine resistance was irregular, in contrast to the regular 2:2 segregation of the other marker in the cross, mating type. In any single tetrad, either 0, 1 or 2 progeny were as resistant as the resistant parent. The remainder were either sensitive or showed some growth in the presence of canavanine after prolonged incubation. Varying the concentration of canavanine did not help to resolve this complex situation. The patterns of segregation led us to suspect that more than one gene with an effect on canavanine resistance was segregating, and prompted more detailed genetical analyses.

Genetic analysis of ED 428

Two progeny of the cross between ED 428 and wild-type were picked for further crosses. One of these, ED 428-1c, showed strong resistance similar to ED 428, while the other, ED 428-4d, showed weak resistance.

Table 1. Sensitivity of *S. pombe* strains to L-canavanine

Strains were streaked onto the surface of agar medium (EMM-A has ammonium as nitrogen source, and EMM-G has glutamate), and incubated at 30 °C. After 3 d incubation the mean diameter of isolated colonies was estimated.

Strain	Diameter (μm) on EMM-A				Diameter (μm) on EMM-G			
	Canavanine (μg ml ⁻¹):				Canavanine (μg ml ⁻¹):			
	0	3	10	30	0	3	10	30
972 <i>h</i> ⁻	1000	150	100	60	1000	s.c.	s.c.	s.c.
ED 428	1000	700	700	700	1000	350	200	175
ED 429	700	400	350	250	700	150–300*	100–250*	50–125*
ED 460	1000	900	700	600	1000	200	75	50

s.c., only single non-dividing cells present.

* Great heterogeneity in colony diameter.

Table 2. Progeny types in crosses between *can1.1* and wild-type strains

Strains ED 428-1c and ED 428-4d are progeny of the cross between ED 428 and 975 *h*⁺ (see text). ED 428-1c shows strong canavanine resistance; ED 428-4d shows weak resistance.

Tetrad types*:			No. of tetrads	
			ED 428-1c × 975 <i>h</i> ⁺	ED 428-4d × 972 <i>h</i> ⁻
R	(R)	S		
2	0	2	3	0
1	1	2	3	0
0	2	2	0	8

* R, fully resistant; (R), weakly resistant; S, sensitive.

The cross between ED 428-4d and 972 *h*⁻ yielded only sensitive and weakly resistant progeny, showing a 2:2 segregation in each of eight tetrads analysed (Table 2). Microscopic examination of the cells showed that sensitive strains consisted of swollen and dead cells, while the weakly resistant progeny showed nearly normal cellular morphology after growth on canavanine.

In contrast, the cross between ED 428-1c and 975 *h*⁺ gave an irregular segregation similar to that of the cross between ED 428 and 975 *h*⁺, with sensitive, weakly resistant and fully resistant progeny. The segregation pattern (Table 2) was consistent with the segregation of two genes controlling resistance to canavanine. One of these (*can1.1*) was presumably required for canavanine resistance, while the other (*canx*) interacted with the mutant *can1.1* allele to enhance the degree of resistance. On this basis ED 428-1c and the original resistant strain ED 428 were genotypically *can1.1 canx*, since they both generated three resistance classes when crossed to a wild-type. In contrast ED 428-4d was *can1.1 canx*⁺ since only sensitive and weakly resistant progeny resulted from a cross to wild-type. An alternative explanation, that the wild-type 975 *h*⁺ carried *canx* as a cryptic mutation, can be ruled out since a cross between an *h*⁻, weakly canavanine resistant strain derived from the cross between ED 428-4d and 972 *h*⁻ gave only sensitive and weakly resistant progeny when crossed to 975 *h*⁺. A twice-backcrossed derivative of ED 428 of genotype *can1.1 canx*⁺ *h*⁻ was designated ED 460 (see Table 1) and was used as our standard *can1.1* strain in further genetical and physiological experiments.

Canavanine is an arginine analogue, and one mechanism of resistance to it might be a defect in the uptake of the analogue and of arginine itself, by analogy with the better-defined situation in *Sacch. cerevisiae* (Grenson *et al.*, 1966). To test this genetically, crosses were carried out between ED 460 and two arginine auxotrophs, *arg1.230* and *arg6.238*. The former strain is unable to use ornithine in place of arginine, while the latter is able to do so (P. Munz, personal communication). Two different auxotrophs were used to rule out any possible gene-specific interactions with *can1.1*.

The two sets of crosses gave essentially similar results (Table 3). The arginine requirement segregated 2:2 in each tetrad. Of the Arg⁺ progeny, about half (type B) were resistant to

Table 3. *Progeny of crosses between ED 460 and arg⁻ strains*

Crosses were (a) ED 460 (*can 1.1 h⁻*) × *arg1.230 h⁺*; (b) ED 460 × *arg6.238 h⁺*. Eight tetrads were dissected for each cross, and pooled data are shown.

Progeny type	No. of progeny		Growth* on:					Genotype	
	Cross (a)	Cross (b)						<i>can1.1</i>	<i>arg</i>
			EMM-G	EMM-G + arg	EMM-A	EMM-A + arg	EMM-G + can		
A	10	9	+	+	+	+	-	+	+
B	6	7	+	+	+	+	+	-	+
C	6	7	-	+	-	+	ND	+	-
D	10	9	-	(+)	-	-	ND	-	-

* Scoring: -, no growth; (+), poor growth; +, strong growth; ND, not determined.

canavanine and about half (type A) sensitive (Table 3). The Arg⁻ progeny could be tested for canavanine resistance, since inclusion of arginine in the medium to permit growth abolished the inhibitory effect of canavanine. However, two types of Arg⁻ progeny could be distinguished. One type (type C in Table 3) grew on arginine supplemented medium as well as Arg⁺ strains, irrespective of the nitrogen source. The other type (D in Table 3) grew poorly on EMM-G supplemented with arginine, and not at all on EMM-A supplemented with arginine. In addition, type D strains grew poorly on complex medium. It is very likely that the type D strains are *arg can1.1*, since their phenotype resembles neither of the parents and is not wild-type. Detailed inspection of the segregation pattern within each tetrad, and backcrosses of putative double mutants (not shown) showed this supposition to be correct. Thus the double mutant strains (*arg can1.1*) demonstrate an interaction between the two mutations at the phenotypic level. This is strongly reminiscent of the situation in *Sacch. cerevisiae* where *can1 arg* double mutants were unable to grow even when provided with arginine under conditions of nitrogen repression (Whelan *et al.*, 1979; Grenson & Hou, 1972). The gene *CAN1* in *Sacch. cerevisiae* is known to code for a specific permease concerned with the uptake of arginine and other basic amino acids (Grenson *et al.*, 1966), and this prompted us to propose and test directly a model for arginine uptake and canavanine resistance in *S. pombe*. The model proposes the existence of two systems which are able to mediate the uptake of arginine. One of these (system I) shows some specificity for arginine, and is the major entry route for canavanine. The other system (system II) also allows the uptake of arginine. System II is repressed or inhibited by the presence of ammonium in the medium. This model explains all the observations described so far if the *can1⁺* gene codes for a component of system I. In *can1.1* strains canavanine is unable to be taken up and the cells are resistant to its inhibitory effects. Double mutants (*can1.1 arg*) need to assimilate arginine from the medium in order to grow. This is possible when the nitrogen source is glutamate, when arginine enters by system II, but impossible during growth on ammonium since system II is ammonium inhibited. Direct tests of this model are presented below.

Chromosome mapping of can1

During the construction of *can1* strains also bearing a *leu1.32* mutation, linkage between *can1* and *leu1* was fortuitously discovered. Of 20 tetrads analysed, 10 were parental ditype and 10 tetratype, suggesting a map distance of about 25 map units. This was confirmed by the analysis of 386 random spores, which gave a map distance of 25.4 map units. Three-point crosses including *can1*, *leu1* and either *tps13.24* or *cdc10.129* demonstrated that *can1* was linked to *tps13* but not to *cdc10*. The distance between *can1* and *tps13*, a centromere-linked marker (Gyax & Thuriaux, 1984) was 34 map units, based on 192 progeny. The *can1* gene may be a useful genetic marker since this region of chromosome II is otherwise rather sparsely covered.

Analysis of ED 429

Aberrant segregations for canavanine resistance were observed in the cross ED 429 × 975 *h⁺*. Both 2:2 and 3:1 segregations for sensitive:resistant progeny were observed initially. Closer

Fig. 1. Effect of arginine concentration on arginine uptake rates in *S. pombe* strains. Samples (500 μ l) of the exponential cultures indicated were added to 1 μ Ci [3 H]arginine and amounts of unlabelled arginine to bring the final concentration to those shown. Samples were incubated at 30 $^{\circ}$ C for 8 min, filtered, washed and counted as described in Methods. The counts were converted into pmol arginine taken up per min per 10^6 cells, taking the counting efficiency as 35%. (a) Cultures growing in EMM-A: ●, 972 h^- ; ▲, ED 428; ■, ED 429. (b) Cultures growing in EMM-G: ●, 972 h^- ; ▲, ED 428; ■, ED 429. Lineweaver-Burk plots of the uptake rates give the following values for K_m of the uptake system(s). For ammonium-grown cells the K_m values are 10.5 μ M, 14.3 μ M and 20.0 μ M for 972, ED 428 and ED 429 respectively. For glutamate-grown cells the corresponding values are 7.7 μ M, 7.8 μ M and 13.8 μ M.

scrutiny showed that of the three progeny initially classified as sensitive in each tetrad of the latter type, one showed a low level of canavanine resistance. This situation is analogous to that shown by ED 428, that is, it suggests the presence of an enhancer mutation in ED 429. In crosses between enhancer-free *can2.1* and *arg* strains, no interaction was observed in double mutants, which grew well when supplemented with arginine, irrespective of nitrogen source. This suggested that *can2.1* was not intimately involved in arginine or canavanine uptake, although experiments described below point to some kind of uptake defect in *can2.1*.

Arginine uptake by *can* strains

Arginine uptake was determined for cells of 972, ED 428 and ED 429 during growth in EMM-A (ammonium) or EMM-G (glutamate) media. Fig. 1 shows the rates of arginine uptake at various arginine concentrations. (Other experiments showed that the uptake was linear with time over the period used.) In all cases uptake approached saturation at 100 μ M-arginine; calculations based on Lineweaver-Burk plots (not shown) give K_m values between 7 and 20 μ M (see Fig. 1). The most striking observation was that strain ED 428 showed a much reduced uptake rate compared with the wild-type during growth in EMM-A. During growth in glutamate medium ED 428 showed a reduced uptake rate compared with 972, though the relative reduction was only about 50% in this case. However, the wild-type rate in glutamate medium was higher than that in ammonium medium; in fact, the absolute difference in rates between ammonium and glutamate medium was nearly the same for both ED 428 and 972. Expressed another way, the total rate of arginine uptake by 972 growing in EMM-G was additively composed of the rate of uptake in ammonium medium and an extra component. In ED 428 the uptake rate during growth in ammonium medium was very low, yet the 'extra' component present during growth in EMM-G was very similar to that for the wild-type. Assigning the uptake rate during growth in EMM-A to system I and the rate enhancement during growth in EMM-G to system II is entirely consistent with the model presented above.

Strain ED 429 showed a reduced level of uptake of arginine during growth in EMM-A or EMM-G. In both these cases the decrease relative to the wild-type was between 60 and 70%. Thus ED 429 shows no specific loss of either uptake system, but an overall reduction. Possible reasons for this are discussed below.

Table 4. *Uptake of arginine by strains 972, ED 428 and ED 460*

Cells of the strains indicated were grown in EMM-A or EMM-G. Samples (500 μ l) were added to 2 μ Ci [3 H]arginine and unlabelled arginine to a final concentration of 10 μ M. Samples (150 μ l) were taken at 10 min intervals, washed and counted. Uptake followed linear kinetics in each case. Samples for the determination of cell number were taken at the start of labelling.

Strain	Uptake rate ($10^{-3} \times$ c.p.m. min^{-1} per 10^6 cells) during growth on:	
	EMM-A	EMM-G
972	4.59	8.28
ED 428	0.34	5.43
ED 460	0.73	7.31

Table 5. *Effect of various amino acids on the uptake of arginine*

Cultures of strain 972 growing in EMM-A and strain ED 460 growing in EMM-G were assayed for arginine uptake rate. Labelling conditions were: 1 ml cells added to 1 μ Ci [3 H]arginine plus either 1 μ M- or 23 μ M-unlabelled arginine final concentration, plus 10 μ l of 10 mM-L-amino acid solution. Uptake rates were calculated from the linear portions of the uptake curves (generally between 10 and 30 min) and are expressed as percentages of the rates in the absence of any added amino acid. The absolute uptake rates for the control cultures (no additional amino acids included) of strain 972 in EMM-A were 1.3×10^3 c.p.m. min^{-1} per 10^6 cells for 1 μ M-arginine and 1.2×10^3 c.p.m. min^{-1} per 10^6 cells for 23 μ M. Corresponding values for ED 460 were 1.4×10^3 and 1.4×10^3 respectively.

Culture*	Amino acid	Relative uptake (%) in the presence of arginine at:	
		1 μ M	23 μ M
972 in EMM-A (system I)	None	100	100
	L-Ornithine.HCl	55	58
	L-Lysine.HCl	36	41
	L-Canavanine.HCl	41	42
	L-Tryptophan	129	86
	L-Leucine	112	63
ED 460 in EMM-G (system II)	None	100	100
	L-Ornithine.HCl	69	91
	L-Lysine.HCl	62	102
	L-Canavanine.HCl	81	114
	L-Tryptophan	92	127
	L-Leucine	82	141

* Presumptive arginine uptake system functional shown in parentheses.

Several other experiments were done in order to test further the hypothesis that two distinct uptake systems were capable of mediating the uptake of arginine. First, strains ED 428, ED 460 and the wild-type 972 were compared. Table 4 shows the relative rates of arginine uptake during growth in EMM-A or EMM-G. Under both sets of conditions the backcrossed derivative of ED 428, ED 460, showed a slightly higher level of uptake than ED 428, though still lower than the wild-type values. Other experiments (not shown) indicated that minor changes in the incubation conditions had no significant effect on the relative uptake rates of the strains. In all subsequent experiments the backcrossed derivative ED 460 was used.

To investigate further the difference between arginine uptake rate during growth on glutamate and on ammonium, shift experiments were done. Cells of 972 and ED 460 were grown in EMM-G; portions were added to [3 H]arginine or to [3 H]arginine plus ammonium chloride to bring the final concentrations to that in EMM-A. Samples taken at intervals to estimate arginine uptake showed that the rate of arginine uptake fell rapidly after addition of ammonium. In wild-type cells the reduction was by about 75%, while the *can1.1* strain showed a more drastic reduction by nearly 90%. The observed reductions in rate are presumably due to rapid inhibition

Table 6. *Properties of diploid strains heterozygous for can1.1*

Diploid strains heterozygous *can1.1/can1⁺* and the corresponding homozygotes were tested for growth on EMM-G in the presence of L-canavanine. HCl at 10 µg ml⁻¹. Colony diameter after 5 d growth was measured as described in Table 1. For arginine uptake determination, cells were grown in EMM-A at 30 °C. Samples (2 ml) were added to 2 µCi [³H]arginine with a final arginine concentration of 23 µM.

Strain	Colony diameter (µm)	Arginine uptake†
<i>can1⁺/can1⁺</i>	Single cells	6.01
* <i>can1.1/can1⁺</i>	100	2.34
* <i>can1.1/can1⁺</i>	100	3.54
<i>can1.1/can1.1</i>	200–300	1.75

* Results for two independent diploids heterozygous for *can1*.

† Expressed as 10⁻³ × c.p.m. min⁻¹ per 10⁶ cells.

of uptake system II by the presence of ammonium: in the *can1.1* strain the reduction is by almost 90% since system I operates at only a very low level. In the wild-type system I is still active and the reduction is less dramatic.

As stated above, rapid inhibition of system II is the most likely explanation for the rapid fall in uptake following the addition of ammonium. An alternative explanation might be that some component of system II whose synthesis is ammonium repressed is very labile and has a short turnover time *in vivo*.

Further evidence that systems I and II are physiologically distinct comes from experiments where the effect of various amino acids on arginine uptake was determined. To allow the maximum possible sensitivity in discriminating between the systems, wild-type cells growing in EMM-A (system I only) were compared with ED 460 cells growing in EMM-G (essentially only system II functional). The results (Table 5) show that presumptive system I is inhibited by basic amino acids such as lysine, ornithine or canavanine, but not significantly affected by tryptophan. Leucine showed some inhibition in one case. In contrast, system II showed little inhibition by any of the amino acids tested, and showed slight stimulation in the presence of tryptophan or leucine, at the higher arginine concentration tested (Table 5).

Dominance relationships for can1.1

It has been argued so far that the *can1⁺* gene codes for a component of an uptake system for arginine and canavanine. Both genetical and physiological data suggest that in the *can1.1* strain ED 460, one uptake system is defective. According to this hypothesis a diploid strain heterozygous for *can1.1* would be expected to show a rate of arginine uptake midway between those of the corresponding homozygotes, as in *Sacch. cerevisiae* (Grenson *et al.*, 1966), assuming the *can1* product to be limiting. Results for the two homozygotes and two independent heterozygotes are shown in Table 6. While the heterozygote values clearly fall between those for the homozygotes, the former are closer to the mutant homozygote than to the wild-type homozygote. Other experiments (not shown) indicated that minor changes in the incubation conditions had no significant effect on the relative uptake rates of the strains.

The diploid strains were examined for their sensitivity to canavanine by replicating actively growing cells onto the surface of EMM-G containing canavanine. The wild-type diploid showed no growth. The mutant homozygote grew, though not as strongly as the corresponding haploid mutant. The heterozygotes showed some growth, though this was weaker than that of the mutant homozygote. Within the patches of poorly growing heterozygous diploids faster growing colonies were observed: these presumably resulted from cells which had undergone mitotic crossing-over and become homozygous for the *can1.1* allele. To obtain a better estimate of the extent of inhibition by canavanine, the strains were streaked for single colonies onto EMM-G plus canavanine (10 µg ml⁻¹). After 5 d growth the homozygous *can1.1* diploid showed better growth than the heterozygotes (Table 6), while the wild-type diploid showed no growth.

In conclusion, diploids heterozygous *can1.1/can1⁺* show uptake and resistance phenotypes intermediate between those of the two homozygotes.

DISCUSSION

We describe in this paper an investigation into the mechanisms of canavanine resistance and arginine uptake in the fission yeast *S. pombe*. The strains we obtained from Bern as '*can1.1*' (ED 428) and '*can2.1*' (ED 429) both appear to be double mutant strains, each carrying a major canavanine resistance mutation and an additional modifier or enhancer mutation. It is not certain whether ED 428 and ED 429 share the same modifier mutation. In the case of ED 428 the modifier, provisionally designated *canx*, increases the level of resistance to canavanine during growth with glutamate as nitrogen source, but not with ammonium (Table 1, compare ED 428 and ED 460).

Two lines of evidence lead to the conclusion that the *can1*⁺ gene product is required for activity of one arginine uptake system, referred to as system I. First, the inability of *arg can1.1* double mutants to grow on EMM-A plus arginine (Table 3) is consistent with an uptake defect. Second, direct measurements in *can1.1* and *can1*⁺ strains (Table 4) show that the presence of *can1.1* reduces the rate of arginine uptake substantially. During growth on ammonium the reduction is about 90%, while a smaller reduction was found for cells growing on glutamate. It is likely that the *can1*⁺ gene itself codes for a component of arginine uptake system I, though the possibility that the gene has a regulatory rather than structural role cannot be excluded at present.

There is a substantial difference in the rates of arginine uptake between cells growing on ammonium and cells growing on glutamate (Fig. 1, Table 4). This difference, particularly marked in *can1.1* strains, points strongly to the existence of a second system which can mediate the uptake of arginine. This system, system II, appears to be strongly inhibited or repressed by ammonium (Table 4). System I is inhibited by basic amino acids while system II is not (Table 5), suggesting that system I may be a permease primarily concerned with basic amino acid uptake, similar to the *CAN1* system in *Sacch. cerevisiae* (Grenson *et al.*, 1966; Whelan *et al.*, 1979). The physiological role of system II is uncertain, though its inhibition or repression by ammonium shows clear parallels with the general amino acid uptake system in *Sacch. cerevisiae* (Grenson *et al.*, 1966, 1970). It may be significant that ED 460 (*can1.1*) shows the same level of canavanine resistance as ED 428 (*can1.1 canx*) during growth with ammonium as nitrogen source, but during growth on glutamate ED 428 is significantly more resistant (Table 1). It is possible that *canx* is defective in an element of uptake system II. The rather higher rate of arginine uptake by ED 460 compared with ED 428 (Table 4) is consistent with this proposal.

The nature of the defect in ED 429 is uncertain. This strain grows slightly more slowly than either wild-type or *can1* strains in the absence of canavanine (Table 1). Arginine uptake is reduced by about 60% compared with the wild-type, irrespective of the nitrogen source (Fig. 1). There is no genetic interaction between *can2.1* and *arg* mutations, which is expected since arginine uptake is only incompletely blocked. It is possible that *can2.1* is defective in uptake of many substrates in the same way as *cyh3* and *cyh4* in *S. pombe* (Johnston & Coddington, 1983), or the *aap* and *apf* mutants of *Sacch. cerevisiae* (Surdin *et al.*, 1965; Grenson & Hennaut, 1971).

In conclusion, there are strong similarities between the mechanisms of genetic resistance to canavanine in *S. pombe* and in *Sacch. cerevisiae*. A mutation in a single major gene, designated *can1* in both yeasts, drastically reduces the activity of an uptake system with specificity for arginine and other basic amino acids. A second system can mediate arginine uptake in both organisms, and this system is strongly inhibited during growth on ammonium. In *Sacch. cerevisiae* this system (coded by the *GAP* gene) also mediates the uptake of many amino acids of various types: in *S. pombe* the genetic basis of the system is uncertain, though it is possible that *can2.1* is defective in it. Further work will be required to elucidate this.

In *S. pombe* as in *Sacch. cerevisiae*, *arg can1* double mutants are unable to utilize exogenous arginine under conditions of nitrogen repression. This should permit selection in *S. pombe* both for *can1*⁺ and for *can1* genotypes. The latter can be selected by growth in the presence of canavanine, while the former should be favoured by selecting for growth of an arginine auxotroph in the presence of ammonium.

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