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Regulation of Allantoate Transport in Wild-Type and Mutant Strains of *Saccharomyces cerevisiae*

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Accumulation of intracellular allantoin and allantoate is mediated by two distinct active transport systems in *Saccharomyces cerevisiae*. Allantoin transport (*DAL4* gene) is inducible, while allantoate uptake is constitutive (it occurs at full levels in the absence of any allantoate-related compounds from the culture medium). Both systems appear to be sensitive to nitrogen catabolite repression, feedback inhibition, and trans-inhibition. Mutants (*dal5*) that lack allantoate transport have been isolated. These strains also exhibit a 60% loss of allantoin transport capability. Conversely, *dal4* mutants previously described are unable to transport allantoin and exhibit a 50% loss of allantoate transport. We interpret the pleiotropic behavior of the *dal4* and *dal5* mutations as deriving from a functional interaction between elements of the two transport systems.

Allantoate is an intermediate of adenine and guanine degradation that can serve as the sole nitrogen source for *Saccharomyces cerevisiae*. This compound enters the cell by way of an energy-dependent active transport system with a Michaelis constant of approximately 50 μM (23). Although the transport process has been characterized in detail, little is known about its physiology and regulation. This dearth of information prompted our study. One objective of this work was to identify the principal physiological influences that impact on the operation of allantoate uptake, thereby ascertaining its role in the overall control of allantoin metabolism in *S. cerevisiae*. Another was to determine whether the allantoate transport system shares functionally common elements with the closely related allantoin transport system.

S. cerevisiae possesses two allantoin pathway-specific active transport systems in addition to the one for allantoate. The first is a low-K_m urea transport system whose production is induced by allophanate or oxalurate in a manner similar to that observed for the pathway enzymes (9). Urea transport also appears subject to nitrogen repression; i.e., minimal transport activity is observed when cells are grown in the presence of a good nitrogen source as asparagine (9). The second is a low-K_m allantoin transport system whose production is induced by allantoin or its nonmetabolized analog hydantoin acetate (6, 19, 22). This system, like urea transport, appears to be subject to nitrogen repression. In contrast to urea uptake, however, allantoin accumulation also appears to be regulated by feedback and trans-inhibition (5, 6, 19, 22). The most convincing demonstration of control being exerted at the level of transport activity is the <3-min half-life observed for the loss of ability to accumulate allantoin following addition of asparagine to the culture medium (4).

Genetic studies have been performed on the urea and allantoin transport systems. Mutations in the *dur3* locus result in the loss of urea active transport (21). Allantoin transport may be similarly lost by mutation of the *dal4* locus, which is situated in the *DAL* gene cluster on the right arm of chromosome IX (6). *dal4* mutants fail to grow when provided with allantoin as the sole nitrogen source but behave like wild-type cells when grown in minimal allantoate, urea, or arginine medium.

In this report, we show that allantoate transport activity is produced at a high, constitutive (defined as transport activity being present at high levels in the absence of allantoate-related compounds in the culture medium) level. We were unable to demonstrate the presence of an induction system. However, allantoate transport activity was observed when cells were provided with a poor nitrogen source such as proline but not when they were provided with good ones such as asparagine or glutamine. This is a strong indication that production of the transport system is subject to nitrogen catabolite repression. Uptake activity was also feedback inhibited by intracellular allantoate and transinhibited by a variety of L- and D-amino acids. Finally, we demonstrated that the allantoin and allantoate transport systems potentially have elements in common, since mutation of the *dal4* gene decreased allantoate transport and, conversely, mutation of *dal5* diminished allantoin transport.

(A preliminary report of this work has been presented [V. Turoscy and T. G. Cooper, Abstr. Annu. Meet. Am. Soc. Microbiol. 1980, K109, p. 144]).

MATERIALS AND METHODS

Strains. The strains used in this work are listed in Table 1 along with their genotypes and biochemical phenotypes. *dal2* or *dal1 dal2* mutants were used throughout this work to dissociate the transport of allantoate from its degradation. These strains are operationally referred to as wild-type in this publication, because they contain no defects in transport or its regulation.

Culture conditions. Wickerham medium (27), buffered to pH 6.0 by the addition of 1% sodium citrate (the pH of the final medium was adjusted with HCl), was used throughout these experiments. Glucose (0.6%) and ammonium sulfate (0.1%) were used as the sole sources of carbon and nitrogen, respectively, unless otherwise noted. Cell density measurements were made with a Klett-Summerson colorimeter (500- to 570-nm band-pass filter). One hundred Klett units are equivalent to approximately 3×10^7 cells per ml of culture. Resting-cell cultures were prepared as described by Turoscy and Cooper (23). Procedures for transferring cells from one

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TABLE 1. Strains used in this work

Strain	Genotype
M25	*MATa his6 ura1 lys1* *MATα ade6 leu1*
M104	*MATa his6 ura1 dal2-N18* *MATα ade6 leu1 dal2-N18*
M926	*MATa his6 dal5-N1262* *MATα ino1-13 met8 dal5-N1262*
M927	*MATa his6 ura1 dal1-N16 dal2-N18* *MATα ade6 leu1 dal1-N16 dal2-N18*
M934	*MATa ura1 met8 dal1-N16 dal2-N1229 dal4-N1175* *MATα his6 ino1-13 dal1-N16 dal2-N1229 dal4-N1175*
M938	*MATa ino1-13 met8 dal1-N16 dal2-N18 dal5-N1262* *MATα his6 ura1 dal1-N16 dal2-N18 dal5-N1262*
M1613	*MATa his7 tyr1 cdc11 dal5* *MATα gal1 hom6 trp1 met14*
M1614	*MATa hom6 ade6 his4 ura1 gal2* *MATα his7 tyr1 cdc11 dal5*

medium to another, synthesis of radioactive allantoate, and the allantoate transport assay conditions were also described in that publication (23).

Enzyme assay conditions. All enzyme assays were conducted in a manner similar to that described previously. Cell extracts were routinely prepared by a method similar to that of Cooper et al. (7). Permeabilized cells were prepared by the methods of Whitney and Cooper (26) with minor modifications as described by Bossinger and Cooper (2). The assay procedures for determination of allantoinase, allantoicase, and ureidoglycolate hydrolase activities were described previously (3, 13, 14, 24). Allophanate hydrolase and urea-amidolyase activities were assayed in cell extracts or nystatin-permeabilized cells by the methods of Whitney and Cooper (25).

Mutagenesis and isolation of mutants. Mutants were generated by the mutagenic techniques of Fink (10). Two methods were used to enrich cultures for strains with specific mutant phenotypes. The use of nystatin, as described by Snow (18), allowed us to enrich for strains that are unable to use a given nitrogen source. The second enrichment method was that of Henry et al. (11), which is based on the observation that actively growing inositol auxotrophs die when deprived of inositol. Growth-arrested cells, however, survive this treatment. When this method was used, we chose a strain containing the *ino1-13* mutant allele.

Purification of mutant strains, sporulation, micromanipulation, and tetrad analysis were performed by standard genetic methods (15, 16). Complementation among various mutant alleles was tested by assessing the growth of heterozygous diploid strains on an appropriately supplemented solid medium or by assessing quantitative growth rates in liquid cultures.

RESULTS

Allantoate transport is a constitutively produced system. The urea and allantoin transport systems have both been shown to be induced by allantoin pathway intermediates (9, 22). Our first question about allantoate transport control was whether it responded to these inducers. Cultures (strains M104 and M927) were grown overnight in buffered glucose-ammonia medium containing one of several test compounds. At a cell density of 50 Klett units, a sample from each culture was harvested by centrifugation, suspended in medium devoid of the test compound, equilibrated at 30°C for 5 min, given 0.1 mM (final concentration) [^{14}C] allantoate, and assayed for allantoate uptake. None of the allantoin pathway intermediates or gratuitous inducers resulted in increased production of allantoate transport system activity (Table 2). On the contrary, all of these compounds lowered the rates of uptake. Decreases observed after pregrowth of cells with allantoin or allantoate probably derived from feedback inhibition of allantoate uptake by intracellular allantoate. This conclusion was strengthened by the observation that pregrowth of cells in minimal allantoate medium decreased uptake 50% in a *dal2* mutant (M104) compared with 85% in a *dal1 dal2* double mutant (M927). The difference between the two strains is the absence of allantoinase in the latter. We have previously shown this enzyme catalyzes the reversible hydrolysis of allantoin to allantoate (23). Therefore, functional allantoinase would decrease the intracellular concentrations of allantoate by establishing an equilibrium mixture of allantoin and allantoate. In the *dal1 dal2* mutant, all of the preaccumulated allantoate would remain as allantoate, whereas in the *dal2* mutant, approximately half of the accumulated allantoate would be converted to allantoin.

An intriguing observation derived from this experiment was the inhibition of allantoate transport in cells grown in medium containing oxalurate, the nonmetabolized inducer of genes encoding the allantoin pathway enzymes. Inhibition was independent of allantoin metabolism, as indicated by similar degrees of inhibition in both the *dal2* and *dal1 dal2* mutants.

Nitrogen catabolite repression and transinhibition of allantoate transport. To determine whether allantoate transport was sensitive to nitrogen catabolite repression, M104 cells were grown in buffered minimal medium containing one of several nitrogen sources. At 50 Klett units, a sample of each culture was transferred to a prewarmed flask containing [^{14}C]allantoate (final concentration, 0.12 mM). Negligible levels of transport were observed in cells provided with asparagine or glutamine, two repressive nitrogen sources (Table 3). On the other hand, uptake was highest in proline-

TABLE 2. Effect of various compounds on the levels of allantoate transport

Expt	Genotype (strain)	Test compound (concn)	Allantoate accumulated[a]
1	*dal2* (M104)	None	8.7
		Allantoin (0.1%)	4.0
		Allantoate (0.1%)	4.2
		Hydantoin acetate (10 mM)	5.1
2	*dal2* (M104)	None	6.3
		Oxalurate (0.5 mM)	0.8
		Urea (10 mM)	3.0
3	*dal1 dal2* (M927)	None	4.6
		Allantoin (0.1%)	2.5
		Oxalurate (0.5 mM)	0.7
4	*dal1 dal2* (M927)	None	4.7
		Allantoate (0.1%)	0.9

[a] Data are expressed as nanomoles of allantoate accumulate per 40 min per milliliter of the original culture.

TABLE 3. Effect of nitrogen source on allantoate transport activity in strain M104 (*dal2*)

Expt	Nitrogen source (final concn, 0.1%)	Allantoate accumulated[a]
1	Ammonia	8.1
	Asparagine	ND[b]
	Proline	10.7
2	Ammonia	5.5
	Glutamine	ND
3	Ammonia	6.8
	Aspartic acid	2.1

[a] Data are expressed as nanomoles accumulated in 40 min per milliliter of culture at a cell density of 50 Klett units.
[b] ND, Not detectable (at background level for assay).

grown cells and intermediate when ammonia or aspartate were provided. These are the results expected of a system that is sensitive to nitrogen catabolite repression. The only apparently exceptional result is the lack of repression by ammonia. Strains used in this experiment were derived from a genetic background (analogous to strain M25) in which ammonia is not a repressive nitrogen source (1).

The above experiment leads to the simple conclusion that allantoate transport, like that of allantoin and urea, is sensitive to nitrogen catabolite repression. The physiology of the system appears, however, to be more complex. This was demonstrated by monitoring the onset of asparagine inhibition of allantoate uptake. Strain M927 was allowed to accumulate allantoate for 30 min. It was then divided into two aliquots; one received no further additions, while asparagine was added to the other (final concentration, 0.1%). Accumulation slowed immediately in the portion receiving asparagine and reached a plateau in 30 min (Fig. 1). Similar inhibition was observed with serine (data not shown). We showed earlier that intracellular allantoate does not effuse from the cell (23). Therefore, the plateau in accumulation

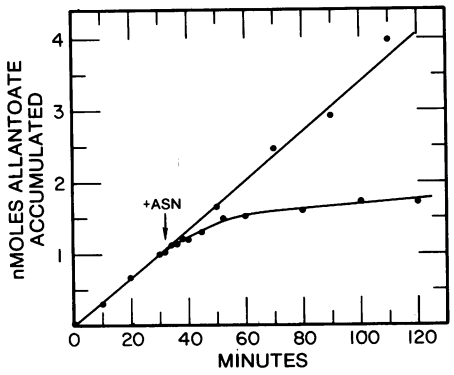

FIG. 1. Inhibition of allantoate transport by asparagine. A culture of strain M927 was grown overnight in buffered glucose-ammonia medium to a cell density of 30 Klett units. At this time, a portion was transferred to a prewarmed flask containing [^{14}C]allantoate at a final concentration of 0.1 mM. Samples were removed at the indicated times, and the amount of accumulated allantoate they contained was determined. At 31 min, the culture was split into two portions. Asparagine (ASN; final concentration, 0.1%) was added to one portion (lower curve subsequent to 30 min); no further addition was made to the other. Sampling and assay of accumulated allantoate were continued as before at the times indicated. Data are expressed in nonamoles of [^{14}C]allantoate accumulated per milliliter of the culture.

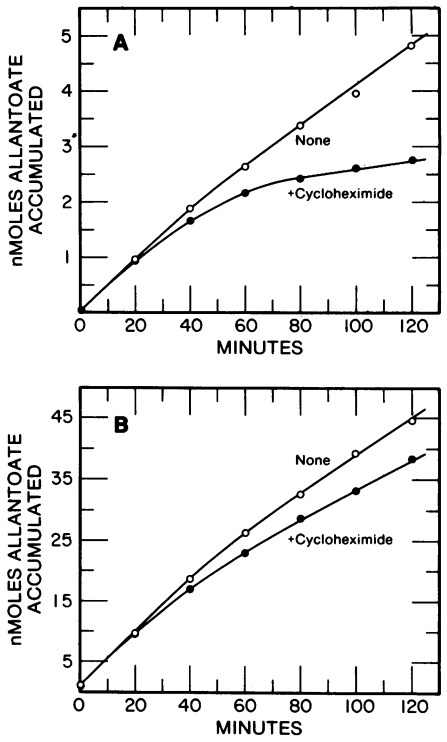

FIG. 2. Effect of cycloheximide on allantoate accumulation in growing and resting cells. (A) A culture of strain M927 was grown to a cell density of 52 Klett units in buffered glucose-ammonia medium. The cells were divided into two portions, transferred to small prewarmed flasks, and allowed to accumulate [^{14}C]-allantoate (final concentration, 0.1 mM). Cycloheximide (final concentration, 100 μg/ml of culture) was added to one portion at time zero; no addition was made to the other. Samples (1 ml) were removed at the indicated times, and the amount of radioactive allantoate they contained was determined. Data are expressed as nanomoles of allantoate accumulated per milliliter of culture. (B) This experiment was performed in a similar manner to that in panel A. However, a resting-cell culture (cell density, 290 Klett units) was used in place of growing cells. A resting-cell culture was produced by starving the cells for nitrogen as described in Materials and Methods. As before, one portion received cyclohexmide at a final concentration of 100 μg/ml.

indicates that transport of extracellular allantoate ceased. In other words, transport machinery, present before the addition of asparagine, no longer appeared to function. These are the characteristics of trans-inhibition, i.e., inhibition of the accumulation of a metabolite by another compound that is structurally unrelated to it (5). This phenomenon has been similarly documented for allantoin transport (5, 19, 22).

Another way of eliciting trans-inhibition is by inhibiting protein synthesis (22). Therefore, growing cells were allowed to accumulate allantoate in the presence or absence of cycloheximide (Fig. 2A). With cycloheximide present, allantoate accumulation decreased to a plateau level in 120 min. Inhibition of protein synthesis would be expected to result in accumulation of intracellular amino acids, thereby causing transinhibition of transport, as was shown for allantoin transport. Starvation of the cells for nitrogen prior to inhibition of protein synthesis would be expected to deplete any intracellular amino acid reserves, and so subsequent treatment with cycloheximide would not be expected to result in the accumulation of amino acids. Allantoate accumulation was much less sensitive to cycloheximide when the

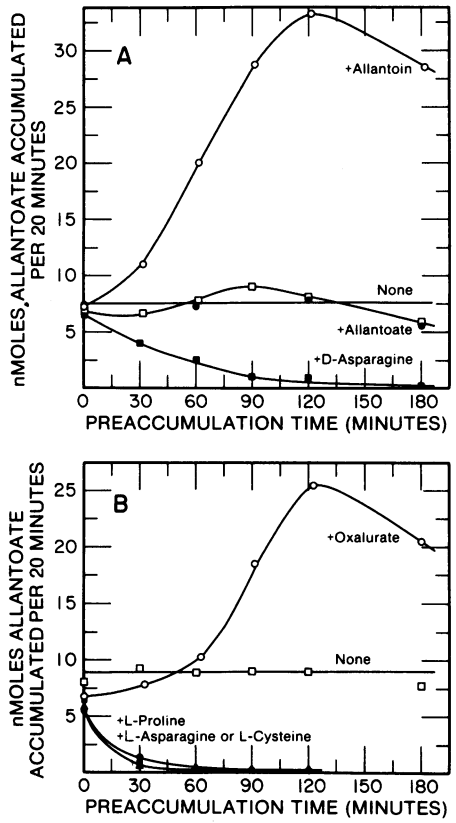

FIG. 3. Time course effect of accumulation of various compounds on the initial rate of allantoate transport in resting cells. A resting-cell culture of strain M927 (final cell density, 181 Klett units for panel A and 196 Klett units for panel B) was prepared as described in Materials and Methods. The experiment was initiated by harvesting portions of this culture by filtration, washing them with prewarmed buffered glucose medium, and transferring them to small flasks containing an equal volume of buffered glucose medium (no ammonia) or buffered glucose medium supplemented with allantoin, D-asparagine, allantoate (panel A), L-proline, L-asparagine, L-cysteine (all at 0.1% final concentrations) or oxalurate (2.0 mM) (panel B). These samples were allowed to preaccumulate a test metabolite for the times indicated. At each designated time, portions were removed from the flasks, harvested by filtration, washed with buffered medium and suspended in an equal volume of buffered medium containing 0.1 mM [^{14}C]allantoate. The cells were then allowed to accumulate the radioactive allantoate for 20 min, after which a 1.0-ml sample was removed and the amount of radioactivity it contained was determined. Each point on the graph indicates the amount of allantoate (in nanomoles) accumulated in 20 min after a designated time of preaccumulation.

cultures were first starved for nitrogen (Fig. 2B). The results of this experiment supported the suggestion that intracellular amino acids are responsible for the inhibition of allantoate transport.

As demonstrated above, a variety of compounds inhibit allantoate transport. To determine the time course over which inhibition was exerted, we performed the following experiments. Resting cultures (see Materials and Methods) were permitted to preaccumulate test compounds for the time indicated on the abscissa of Fig. 3. The cells were then removed from the medium, suspended in fresh medium devoid of the test compound, and assayed for allantoate uptake. Each point in the figure represents the amount of allantoate accumulated in 20 min (these are uptake rate

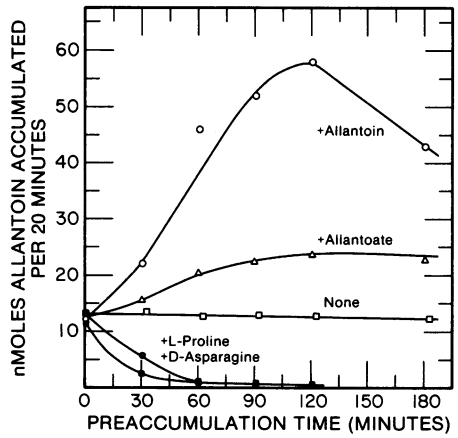

FIG. 4. Effect of preaccumlation of various compounds on the initial rate of allantoin transport in resting cells. Resting-cell cultures of strain M927 (final cell density, 185 Klett units) were assayed as described in the legend to Fig. 3. In this case, cells were allowed to preaccumulate allantoin, allantoate, L-proline, and D-asparagine (all provided at final concentrations of 0.1%). Allantoin transport was then measured as described in Materials and Methods.

measurements). Addition of allantoin (Fig. 3A) or oxalurate (Fig. 3B) to the resting cells increased allantoate uptake fivefold. After 120 min, however, the rate of uptake began decreasing at a moderate rate. Allantoate addition had little effect on uptake, but D-asparagine, L-proline, and L-cysteine were completely inhibitory within 2 h. The same behavoir was observed when the experiment was repeated and the cells were assayed for allantoin transport instead (Fig. 4).

Isolation of allantoate transport-deficient mutants. The similar enhancement of allantoin and allantoate transport by allantoin and oxalurate suggested that the two systems might possess common elements. However, this suggestion could be tested only with mutants each lacking one of the systems. Several early attempts to isolate allantoate transport-deficient strains proved unsuccessful. To enhance our chances of success, we incorporated several changes into the isolation strategy. The structural similarity of allantoin and allantoate raised the possibility that allantoate enters the cell by way of the *DAL4*-associated allantoin active transport system. This prompted us to begin with strains harboring a mutation at the *DAL4* locus. A second change involved alteration of the growth medium. Previous unsuccessful attempts at isolation of mutants were carried out with Wickerham medium which has a pH of 3.3 during the logarithmic phase of cell growth. Since allantoate decomposes to urea at low pH, we used citrate-buffered (pH 6.0) medium throughout the enrichment and screening procedures.

Strain M822-27b was mutagenized as described in Materials and Methods. After outgrowth, the mutagenized culture was subjected to a tandem enrichment: the first procedure

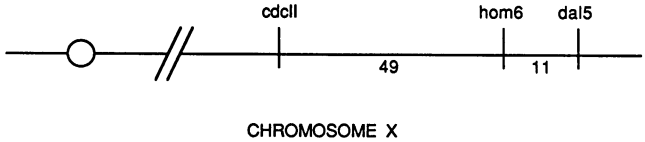

FIG. 5. Positions of *cdc11*, *hom6*, and *dal5* loci on chromosome X.

TABLE 4. Linkage relationships of the *cdc11*, *hom6*, and *dal5* loci on chromosome X

Cross (no. of tetrads analyzed)	Gene pair	PD	NPD	TT	Map distance[a]
M1613 (66)	*cdc11-hom6*	17	4	45	52.3
	hom6-dal5	58	0	8	6.1
	cdc11-dal5	15	5	46	57.6
M1614 (219)	*cdc11-hom6*	54	10	155	49.1
	hom6-dal5	177	1	41	10.7
	cdc11-dal5	45	11	163	52.3

[a] Calculated by using the formula of Perkins (17).

TABLE 6. Levels of allantoin-degradating enzymes in wild-type and *dal5* strains of *S. cerevisiae*

Enzyme assayed	Enzyme level (nmol/min per mg of protein) in:			
	Wild-type (M25)		*dal5* (M926)	
	Uninduced	With urea	Uninduced	With urea
Allantoinase	3.5	15.6	2.4	10.4
Allantoicase	3.4	6.6	3.8	5.7
Ureidoglycollate hydrolase	11.1	15.1	11.8	15.2
Urea-amidolyase	0.01	0.6	0.01	0.5
Allophanate hydrolase	5.3	55.7	6.0	56.1

involved growth of cultures in buffered minimal allantoate medium containing nystatin (18), while the second involved growth in the same medium devoid of both nystatin and inositol (11). Fifty-five strains, which failed to grow on allantoate, were identified and isolated. Preliminary complementation analysis revealed that 13 of these strains carried mutations in previously identified loci: *dal2*, *dur1*, and *dur2*. Twenty-eight of the remaining strains fell into a single new complementation group as assayed by their loss of ability to grow in medium containing allantoate as the sole nitrogen source. Allele N1262 was chosen as the representative for further analysis.

Genetic analysis of the strain containing this allele showed that the inability to use allantoate as a nitrogen source segregated in typical Mendelian fashion (2+:2−) when the strain was crossed to a wild-type strain of the opposite mating type. The mutation also segregated independently of the *dal4* mutation present in the mutagenized parent, as well as all other previously identified *dal* and *dur* loci. The locus containing the N1262 allele was then designated *dal5*. All of the mutant alleles in this class were recessive to the wild type. Using the method of Klapholz and Esposito (12), we localized the *dal5* locus to chromosome X. Recombination frequencies observed between the *dal5*, *cdc11*, and *hom6* markers suggested that *dal5* was situated 10 to 11 map units proximal to the *hom6* locus and distal to *cdc11* (Fig. 5; Table 4). Some caution is appropriate, given the distance between *cdcll* and the other markers available in the cross. The proposed gene order was also consistent with the marker patterns observed in recombinant tetrads. This means of analysis probably provides a more reliable indication of gene order for such widely separated loci than does comparison of recombination frequencies.

TABLE 5. Doubling times of wild-type and the *dal5* mutant strains of *S. cerevisiae*

Nitrogen source	Doubling time (min) of:	
	Wild-type (M25)	*dal5* (M926)
Ammonia	140	150
Allantoin	200	200
Urea	170	170
Allantoate[a]	320	NG[b]
Arginine	150	160
Asparagine	140	140
Proline	270	260
Citrulline	330	315
Ammonia[a]	125	120

[a] Medium was buffered with 1.0% citrate (pH 6.0).

[b] NG, No growth. On transfer to buffered allantoate medium, *dal5* cells doubled once and then stopped growing, while M25 cells grew to saturation.

Biochemical analysis of *dal5* mutants. A strain containing the N1262 allele grew normally on all nitrogen sources except allantoate (Table 5). Normal growth in buffered minimal ammonia medium eliminated the possibility that inability to use allantoate was due simply to inefficient growth in buffered medium. Similar results were observed with all nitrogen sources except allantoate (Table 5). When allantoate was provided as the sole nitrogen source, the absorbance of the *dal5* mutant culture doubled once and then ceased to increase. Wild-type cells grew slowly to saturation. These growth characteristics suggested that *dal5* mutants were deficient in the utilization of externally supplied allantoate. Allantoate generated internally, however, was metabolized normally, as indicated by normal doubling times when mutant cells were grown in minimal allantoin medium. To eliminate possible enzyme dysfunctions from further consideration, we assayed all five allantoin pathway enzymes. Cultures of strains M25 and M926 were grown to a cell density of 75 Klett units in the presence or absence of 10 mM urea as inducer. Cell extracts were prepared, and the enzyme activities were assayed as described in Materials and Methods. Both uninduced and induced levels of the enzymes were comparable in the wild-type strain and in *dal5* mutants (Table 6). Next we assayed the ability of a *dal5* mutant to accumulate allantoate from the medium. Allantoate transport and accumulation were drastically decreased in strain M938, which carried a *dal5* mutation (Fig. 6B). This experiment identified the loss of allantoate transport as the most likely defect in *dal5* mutants.

Functional interaction of the *DAL4* and *DAL5* gene products. The similar response of allantoin and allantoate transport to the addition of allantoin and oxalurate (Fig. 3 and 4) led us to suspect the existence of some interaction between components of the two transport systems. This was confirmed by the following experiments. We observed that a *dal4* mutation in a *dal1 dal2* mutant strain (M934) decreased allantoate uptake by approximately 25% (Fig. 6B). In the converse experiment, allantoin transport decreased to 60% of the wild-type level when a *dal5* mutation was included in a *dal1 dal2* mutant strain (M938) (Fig. 6A). Strains carrying both *dal4* and *dal5* mutations were unable to transport either compound (data not shown). Moreover, the apparent Michaelis constants of allantoin and allantoate transport exhibited by *dal4* and *dal5* mutants were both decreased by 30% compared with values found in corresponding wild-type strains grown under identical conditions (portions of resting-cell cultures of strains M927, M934, and M938 were allowed to accumulate [^{14}C]allantoate or [^{14}C]allantoin for 20 min at 30°C and were assayed at pH 6.0) (Table 7).

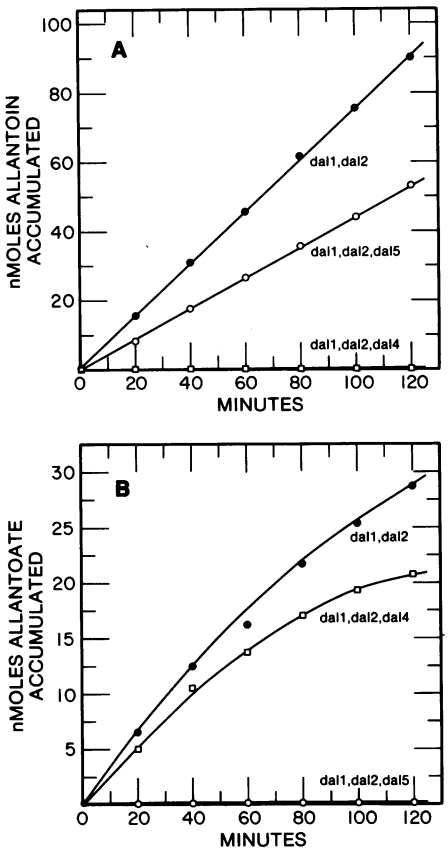

FIG. 6. Effect of mutations in the *dal4* and *dal5* loci on allantoin and allantoate transport. (A) Resting-cell cultures of strains M927 (*dal1 dal2*) (●) M934 (*dal1, dal2, dal4*) (□), and M938 (*dal1, dal2, dal5*) (○) were prepared and assayed for their ability to transport and accumulate [^{14}C]allantoin as described in Materials and Methods. Data are presented as nanomoles of allantoin accumulated per milliliter of culture, adjusted in each case to the cell density of the control culture (M927) as determined with a Coulter Counter (Coulter Electronics, Inc.). [^{14}C]allantoin was added to a final concentration of 0.1 mM. The wash medium used in these assays was buffered glucose medium containing 0.5 M NaCl. (B) Resting-cell cultures of strains M927 (●), M934 (□), and M938 (○) were prepared in buffered glucose medium (pH 6.0) and assayed for their ability to transport [^{14}C]allantoate as described in Materials and Methods. [^{14}C]allantoate was added to the cultures at a final concentration of 0.1 mM. Data were normalized to cell densities as described above.

We have previously shown that allantoin transport is induced by allantoin or its nonmetabolized analog hydantoin acetate. To assess whether the *DAL5* gene product plays a role in this process, we measured allantoin transport in wild-type and *dal5* mutant strains grown with and without 0.1% allantoin. Pregrowth of a *dal5* mutant (M938) in allantoin-containing medium resulted in the same degree of increase in allantoin transport as seen in the wild-type (M927). (Fig. 7).

DISCUSSION

We have shown that allantoate transport is subject to multiple forms of regulation. In contrast to allantoin and urea transport, which are inducible, allantoate transport does not appear to be controlled by induction. This conclusion was based on our inability to find compounds capable of increasing allantoate transport and on the substantial levels of activity present in cells grown under derepressive conditions. Allantoate transport was found to be sensitive to feedback inhibition by intracellular allantoate (Table 2) and highly sensitive to trans-inhibition by L- and D-amino acids in a manner similar to that observed for allantoin transport (5, 19, 22). The biochemical mechanisms associated with these modes of control remain to be elucidated for allantoate transport as they do for the other transport systems of yeasts and fungi.

TABLE 7. Apparent K_m values of the allantoin and allantoate transport systems for allantoin and allantoate in strains harboring mutations at the *dal4* or *dal5* locus

Strain	Genotype	K_m (mM)	
		Allantoin	Allantoate
M927	*dal1 dal2*	13.3	47.6
M934	*dal1 dal2 dal4*	ND[a]	33.3
M938	*dal1 dal2 dal5*	9.1	ND

[a] ND, Not determined.

Allantoate transport is also probably sensitive to nitrogen catabolite repression. Our conclusion here, however, must remain tentative for the moment. The uncertainty derives from superimposition of the control mechanisms that probably regulate allantoate transport activity. Nitrogen catabolite repression, which we have shown to be exerted at the level of gene expression (20, 28), acts only over long periods (4). Trans-inhibition, which can be elicited by some of the same molecules as nitrogen repression, is a rapidly acting mechanism of transport system inactivation. The dilemma that arises is that of rigorously demonstrating the slow process in the presence of the rapid one. Additional experiments assaying transport activity could be done to determine whether nitrogen catabolite repression operates on this

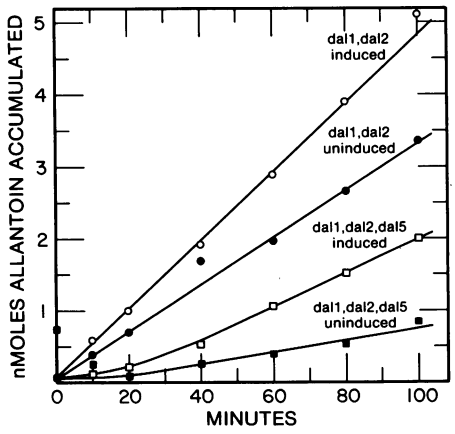

FIG. 7. Effect of mutating the *dal5* locus on the induction of allantoin transport. Cultures of strains M927 (*dal1 dal2*) and M938 (*dal1 dal2 dal5*) were grown in glucose-ammonia medium in the presence (open symbols) or absence (solid symbols) of 0.1% allantoin to a cell density of 45 Klett units. At that time, a portion of each culture was harvested by centrifugation, washed once with prewarmed, preaerated medium, and suspended in an equivalent volume of buffered glucose-ammonia medium. The cells were allowed to equilibrate at 30°C for 5 min, and then [^{14}C]allantoin was added to a final concentration of 0.1 mM. Samples (1 ml) were taken at the times indicated and processed as described in Materials and Methods.

system. The more direct approach, however, is not to answer the question until the gene has been cloned and its expression can be analyzed.

The responses of allantoate transport to allantoin pathway metabolites point to several paradoxes about its control. Enhanced transport was observed when oxalurate was added shortly before assay. However, the long-term presence of this gratuitous inducer inhibited allantoate transport (compare Table 2 with Fig. 3). These data suggest that oxalurate effects allantoate transport in two different ways. The inhibitory effects of oxalurate may be explained by suggesting that the allantoate transport system is one of the systems mediating oxalurate uptake. Consistent with this suggestion was the observation that allantoate is a severe competitive inhibitor of oxalurate uptake (8). Oxalurate-mediated enhancement of allantoate transport is a characteristic shared with allantoin transport. Although we do not understand the details of this phenomenon, we suggest that it may derive from the induction of a transport system component. We are in search of the component(s) at present.

Finally, we have shown that allantoin and allantoate transport have components in common. This was demonstrated by the fact that mutation of either transport system resulted in its complete inability to transport one of the compounds and its diminished ability to transport the other. While the two systems may have common components, they retain their independence. If this were not true, it would never have been possible to isolate the *dal4* and *dal5* mutants. The nature of the interaction awaits identification of the remaining components of the system. However, in this regard, it may be relevant that allantoin, which enhanced allantoate transport, has previously been identified as an inducer of the allantoin transport system. This reasoning supports the hypothesis that oxalurate-mediated enhancement of allantoate transport occurs via the induction of a transport system component. Continued visualization of the enhanced rate of transport was then probably masked by the inhibitory effects of the inducer as it accumulated in the cell (Fig. 3).

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