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Metabolite Compartmentation in *Saccharomyces cerevisiae*

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Uninduced cultures of *Saccharomyces cerevisiae* exhibit high basal levels of allantoinase, allantoicase, and ureidoglycolate hydrolase, the enzymes responsible for degrading allantoin to urea. As a result, these activities increase only 4- to 8-fold upon induction, whereas the urea-degrading enzymes, urea carboxylase and allophanate hydrolase, have very low basal levels and routinely increase 30-fold on induction. Differences in the inducibility of these five enzymes were somewhat surprising because they are all part of the same pathway and have the same inducer, allophanate. Our current studies reconcile these observations. *S. cerevisiae* normally contained up to 1 mM allantoin sequestered in a cellular organelle, most likely the vacuole. Separation of the large amounts of allantoin and the enzymes that degrade it provide the cell with an efficient nitrogen reserve. On starvation, sequestered allantoin likely becomes accessible to these degradative enzymes. Because they are already present at high levels, the fact that their inducer is considerably removed from the input allantoin is of little consequence. This suggests that at times metabolite compartmentation may play an equal role with enzyme induction in the regulation of allantoin metabolism. Metabolism of arginine, another sequestered metabolite, must be controlled both by induction of arginase and compartmentation because arginine serves both as a reserve nitrogen source and a precursor of protein synthesis. The latter function precludes the existence of high basal levels of arginase.

Recent work has shown that allantoin transport is only slightly reversible and that the rate of allantoin accumulation does not diminish with time as long as logarithmic growth is maintained (20). One way of explaining these observations is to suggest that allantoin is located in two cellular compartments. One of these compartments, the cytoplasm, would be expected to contain allantoin available to the plasma membrane allantoin transport system, whereas allantoin in the second compartment, the vacuole, would not likely be directly available to this transport system.

Davis and his collaborators have suggested just such an arrangement for intracellular arginine compartmentation in *Neurospora crassa* (3-5, 8, 18, 25-27). Their conclusions were derived from indirect kinetic experiments in which a pulse of high-specific-activity [^{14}C]arginine was provided to growing cultures of *Neurospora* and changes in the specific activities of arginine, its precursors, and metabolic products were followed as a function of time.

Similar conclusions have also been reached concerning intracellular arginine distribution in yeast. Here, however, a lower resolution, direct method of assay was used. Several early reports claimed that basic proteins made plant cell membranes permeable (12, 16, 35). Svihla et al. performed analogous experiments in yeast and found that basic proteins differentially disrupted the plasma membrane while leaving the tonoplast intact (23). Using this method, they sequentially extracted various sulfonium compounds, including *S*-adenosylmethionine, from the cytoplasm and internal, osmotically sensitive organelles (12-14, 17, 24). Visual studies confirmed the principal localization of *S*-adenosylmethionine within the cell vacuole (21, 22, 24). Recently, Schwencke and deRobichon-Szulmajster have partially characterized a transport activity for this metabolite in isolated vacuole preparations (15).

Wiemken and his collaborators applied similar techniques to studies of intracellular arginine and glutamate distribution in yeast (10, 32-34). They selected cytochrome *c* as the basic protein for disrupting the cell membrane and observed that most of the soluble arginine was found in the vacuolar compartment (i.e., it was released after hypotonic treatment of the tonoplast). Glutamate, on the other hand, was largely cytoplasmic (i.e., it was released after treatment of cells with cytochrome *c* only) (34). Durr et al. have since surveyed a number of polybasic substances in search of more effective agents for selectively

disrupting the yeast cell membrane. Polylysine appears to be one of the most useful compounds in this regard. It was rapidly and completely absorbed at 0°C, but did not cause significant injury to the vacuoles (6). Using these methods, Boller et al. reported the characteristics of a specific transport system for arginine in isolated yeast vacuoles (1).

Here, we have adapted and used these direct fractionation methods to study intracellular allantoin distribution in an attempt to determine whether or not it is compartmentalized. Throughout this report, we will operationally designate metabolite pools as cytoplasmic if they can be released by only polylysine treatment. Vacuolar pools will denote metabolites that require treatment of the cells with polylysine followed by hypotonic shock of the tonoplast for their release.

MATERIALS AND METHODS

Strains and media. All of the strains used in this work were prototrophic diploid organisms. Their genotypes and biochemically defined defects are listed in Table 1. These strains were constructed by using standard genetic techniques (9). The medium used throughout these experiments was that of Wickerham (31). Glucose (0.6%) and ammonia (0.1%) were added as sole carbon and nitrogen sources, respectively. Cell density measurements were made using a Klett-Summerson colorimeter (500- to 570-nm band-pass filter). One hundred Klett units is approximately equivalent to 3×10^7 cells per ml of culture.

Transfer of cells from one medium to another. In a number of experiments, it was necessary to transfer cell samples from one medium to another. This was done by filtering the culture through membrane filters and resuspending the harvested cells in fresh medium. All filtrations were performed with nitrocellulose filters (0.45-μm pore diameter; Millipore Corp.) and were completed in less than 15 to 20 s. The extent of cell loss during this procedure was determined by using radioactively labeled cells and found to be negligible (2).

Synthesis of radioactive allantoin. [^{14}C]allantoin was synthesized by condensing [^{14}C]urea and diethoxyacetic acid ethyl ester under acidic conditions by using standard procedures (C. Zellner and J. Stevens, U.S. Patent 2,158,098, May 1939). The product was recrystallized once from hot water and dried in vacuo at 4°C. The final product was obtained at an overall yield of 31%. The specific activity of allantoin was determined by carefully weighing a sample, dissolving it in a precisely known volume of water, and measuring the radioactivity content of the solution. Characterization of the product and verification of its identity with authentic allantoin have been reported elsewhere (19).

Isolation of cytoplasmic and vacuolar constituents. The procedures described here were patterned after those reported by Wiemken and Nurse (34). A 10-ml sample of the test culture was harvested by filtration and washed twice with 5 ml of cold, fresh minimal medium, twice with 5 ml of cold, 0.25 M sodium acetate buffer (pH 4.75), and twice with 3 ml of cold, 1 M sorbitol prepared in 0.05 M sodium acetate buffer (pH 5.0). Approximately 1.3 min were required to complete the wash procedures. Washed cells were suspended in 2 ml of the 1 M sorbitol solution described above. Polylysine (Sigma type VII-B, 68,000 molecular weight) was added to the sorbitol solution at a final concentration of 0.25 mg/ml just before it was used. Cells were incubated in the presence of polylysine for 2 min at 0°C unless otherwise indicated. After incubation, 1.5 ml of the suspension was harvested by filtration and, unless otherwise indicated, washed four times with 0.8 ml of cold, 0.25 M sodium acetate buffer (pH 4.75) containing 1 M sorbitol. The initial solution and the four wash solutions were all collected in one vessel and designated as the cytoplasmic fraction. Next, 1 ml of water was allowed to filter slowly through the cells. This was performed by decreasing the amount of suction exerted on the Millipore filter apparatus. This was repeated a second time with 1 ml of water and followed by washing the sample four times with 0.8 ml of cold, 0.25 M sodium acetate buffer (pH 4.75). The water and acetate buffer wash solutions were all collected in one vessel and designated as the vacuole fraction. A sample (80 μl) of each fraction was added to 15 ml of aqueous scintillation

TABLE 1. *Strains of S. cerevisiae*

Designation	Genotype	Enzyme defect	Reference
M25	$\frac{\mathbf{a}\ his6\ lys1\ ura1}{\alpha\ ade6\ leu1}$	None	28
M62	$\frac{\mathbf{a}\ his6\ ura1\ dur1\text{-}E145}{\alpha\ ade6\ leu1\ dur1\text{-}E145}$	Urea carboxylase	29
M64	$\frac{\mathbf{a}\ his6\ ura1\ dur2\text{-}E142}{\alpha\ ade6\ leu1\ dur2\text{-}E142}$	Allophanate hydrolase	29
M85	$\frac{\mathbf{a}\ his6\ ura1\ dal1\text{-}N16}{\alpha\ ade6\ leu1\ dal1\text{-}N16}$	Allantoinase	9
M104	$\frac{\mathbf{a}\ his6\ ura1\ dal2\text{-}N18}{\alpha\ ade6\ leu1\ dal2\text{-}N18}$	Allantoicase	9
M58	$\frac{\mathbf{a}\ his6\ ura1\ car2}{\alpha\ ade6\ leu1\ car2}$	Arginase	30

fluid, and the amount of radioactivity that it contained was determined immediately. The filter, which according to Wiemken and Nurse (34) contained unbroken vacuoles, was transferred to a vial, and the radioactivity that it contained was measured. The amount of radioactivity observed on the filter was added to that found in the vacuolar fraction. In most cases the filter contained 6 to 20% of the total radioactive material accumulated in the initial 10-ml cell sample. The total amount of radioactive material accumulated by a representative cell sample is indicated in the legend to each figure and table. The amounts of material accumulated have been expressed in terms of percentages and counts per minute, because we have no way of determining the concentrations of metabolites already present within the cell. Without these parameters, expression of the data in terms of molar concentrations is meaningless.

Isolation of intracellular allantoin. To demonstrate the presence of intracellular allantoin, a large culture of wild-type *Saccharomyces cerevisiae* was grown in yeast nitrogen base medium (Difco Laboratories). Ammonia was the sole nitrogen source. The cells (74 g) were harvested by centrifugation, frozen, thawed, and suspended in a mixture of methanol (70 ml), chloroform (10 ml), and water (20 ml). The suspension was clarified by centrifugation, and the pellet was resuspended in a similar amount of the above solution. After centrifugation, the two supernatant solutions were combined and evaporated to dryness in a rotary evaporator. The residue was extracted with a small amount of ethyl ether and then dissolved in 187 ml of 0.05 M tris(hydroxymethyl)aminomethane-acetate buffer, pH 7.0. The resulting intensely yellow solution possessed a pH of 5.7. One half of this solution was diluted (final volume, 350 ml) to decrease its ionic strength and passed over a column containing Dowex-1-acetate resin. The pale yellow eluant was collected and brought to 0.1 M HCl by addition of concentrated reagent. This solution was passed over a column containing Dowex-50 resin that had been previously equilibrated with 0.1 M HCl. The eluant from the Dowex-50 resin was evaporated to dryness in a rotary evaporator, and the resulting residue was dissolved in 3 ml of water. The sample was divided into two portions of 0.5 and 2.5 ml, respectively. The 0.5-ml sample was analyzed by paper chromatography as described earlier by Sumrada and Cooper (19). KOH (2.5 ml of a 3 M solution) was added to the remaining 2.5-ml sample. This mixture was heated to 75°C for 30 min, a treatment known to convert allantoin to potassium allantoate (19). This solution was diluted and passed over a column containing Dowex-1-acetate resin. The resin was eluted with a linear NaCl gradient (0 to 0.33 M), and the resulting fractions were assayed for ureido group-positive material. The preparation and use of these ion-exchange resins and the methods for assaying ureido group-containing compounds have been described previously (19).

RESULTS

Assay procedures. The assay procedures we have used were modified from those of Wiemken and Nurse, who used cytochrome *c* to make *Candida utilis* cells permeable (34). Unfortunately, we were unable to use this basic protein successfully for rupturing the plasma membrane in *S. cerevisiae*. However, polylysine was found to be an effective reagent for this purpose.

It appeared from earlier reports that the precise experimental parameters of these methods might be empirical and somewhat variable (7). Therefore, we performed several experiments to determine the best polylysine concentration to use and the appropriate time of treatment. For these experiments we measured the intracellular distribution of arginine, because Wiemken and his collaborators had previously shown that this amino acid was largely sequestered in cellular compartments other than the cytoplasm (34). All experiments involving radioactive amino acids were conducted in the presence of cycloheximide to prevent their incorporation into protein. As shown in Fig. 1, treatment of *S. cerevi-*

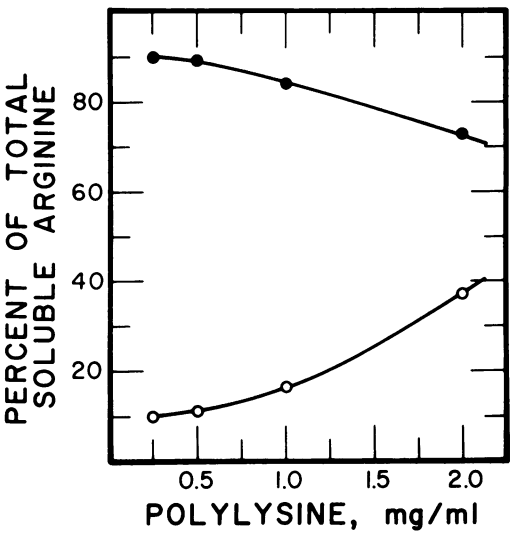

FIG. 1. *Effect of polylysine concentration on the observed intracellular distribution of arginine. Symbols: ●, percentages of total radioactive material observed in the vacuolar fraction; ○, those found in the cytoplasmic fraction. A 42-ml culture of an arginase-minus strain (M58) of S. cerevisiae was grown to a cell density of 50 Klett units in minimal ammonia medium. At that time cycloheximide was added to the culture (200 μg/ml, final concentration), and [^{14}C]arginine (0.1 μCi/ml; specific activity, 5 Ci/mol) was added 5 min later. Cells were allowed to accumulate radioactive arginine for 1 h. At the conclusion of incubation, four 10-ml samples were removed from the culture and processed as described in the text with two exceptions: (i) the concentration of polylysine used was that indicated in the figure and (ii) polylysine treatment was carried out for 5 min. The amount of [^{14}C]arginine accumulated in the 0.25-mg/ml sample was 700,800 cpm. Approximately the same amount of accumulation was observed with all of the other samples.*

siae with 0.25 mg of polylysine per ml resulted in release of only 10% of the total soluble arginine. However, treatment with increasing concentrations of polylysine resulted in a greater proportion of soluble arginine appearing in the cytoplasmic fraction. Because 0.25 and 0.5 mg of polylysine per ml gave approximately the same metabolite distribution pattern, we chose the smaller of the two concentrations. It must be emphasized that the amounts of polylysine used here are likely to be applicable only to the specific growth conditions and cell densities (ca. 1.5×10^8 cells per 2 ml) used in this work.

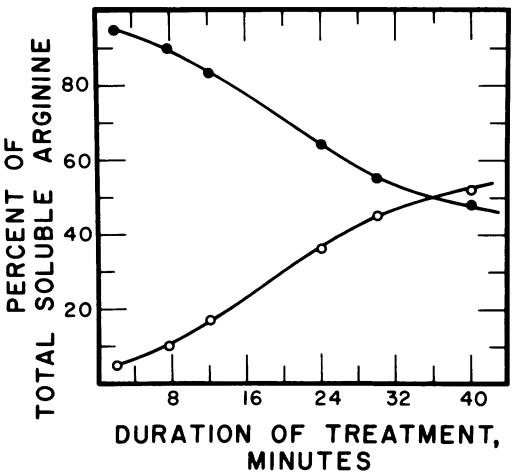

FIG. 2. *Effect of increasing times of polylysine treatment on the observed intracellular distribution of arginine. This experiment was performed in a manner similar to that shown in Fig. 1. Here, however, 0.25 mg of polylysine per ml was used for the time periods indicated in the figure. The amount of [^{14}C]arginine accumulated in the sample treated for 2 min was 470,500 cpm.*

As shown in Fig. 2, increasing the time of polylysine treatment also increased the proportion of total arginine found in the cytoplasm. After 2 min of treatment, the ratio of vacuolar to cytoplasmic arginine was about 20:1. After 40 min, however, the distribution was uniform. Therefore, we selected a 2-min incubation period in the presence of polylysine as most advantageous.

To identify optimum wash procedures, a culture of *S. cerevisiae* was allowed to accumulate arginine for 60 min, and the cytoplasmic and vacuolar metabolites were isolated as described above. As shown in Table 2, four 1-ml washes with 0.25 M sodium acetate buffer were sufficient to remove all of the radioactive material liberated by polylysine breakage of the cell membrane. Removal of this liberated material was not nearly so efficient if the ionic strength of the wash solution was lowered. The cells were then treated with water (2 ml) to burst the membrane-bounded organelles that remained inside of the punctured cells. As shown in Table 2, four 1-ml washes with sodium acetate buffer were sufficient to remove most of the remaining radioactive arginine. If the water treatment was omitted, only 16% of the remaining radioactivity was observed in subsequent washes.

The most rigorous test of our procedures was to compare the distributions of arginine and glutamate that we observed with those reported earlier (34). As shown in Table 3, arginine and its analog, homoarginine, appeared to be efficiently sequestered in compartments other than the cytoplasm, whereas glutamate appeared to be found predominantly in the cytoplasm. There was a gradual shift in the glutamate distribution with time; a uniform distribution of glutamate was observed after 10 min of accumulation.

TABLE 2. *Washing procedures for assay of intracellular distribution of metabolites*[a]

Metabolite pool assayed	Washing procedure[b]	Radioactive metabolite extracted (cpm)
Cytoplasmic	0.25 M sodium acetate + 1 M sorbitol	4,091
	0.25 M sodium acetate + 1 M sorbitol	1,165
	0.25 M sodium acetate + 1 M sorbitol	320
	0.25 M sodium acetate + 1 M sorbitol	273
Vacuolar	Water	7,321
	Water	2,517
	0.25 M sodium acetate	2,869
	0.25 M sodium acetate	737
	0.25 M sodium acetate	973
	0.25 M sodium acetate	617
	0.25 M sodium acetate	417

[a] A culture of strain M58 was grown to 50 Klett units and permitted to accumulate radioactive arginine as described in the legend to Fig. 1. Processing of the samples was performed as described in the text. However, in this experiment the wash solutions were collected in separate vessels.

[b] All washes were 1 ml.

TABLE 3. *Intracellular distribution of arginine, homoarginine, and glutamate*[a]

Amino acid and cellular compartment assayed	% of total amino acid extracted		
	2 min[b]	6 min	10 min
Arginine			
Cytoplasm	8	11	11
Vacuole	92	89	89
Homoarginine			
Cytoplasm	11	13	15
Vacuole	89	87	85
Glutamate			
Cytoplasm	73	63	51
Vacuole	27	37	49

[a] Cultures of strain M58 were grown and permitted to accumulate carrier-free [^{14}C]arginine (0.1 μCi/ml; specific activity, 5 Ci/mol), [^{3}H]glutamate (0.1 μCi/ml; specific activity, 2.8 Ci/mmol), or [^{14}C]homoarginine (0.03 μCi/ml; specific activity, 5 Ci/mol) for varying lengths of time as described in the legend to Fig. 1. At the times indicated in the table, samples were removed from the culture and processed as described in the text. The amounts of radioactive material observed in representative samples were 342,500 cpm for glutamate, 343,500 cpm for arginine, and 247,300 cpm for homoarginine.

[b] Times that cells were allowed to accumulate radioactive amino acids from the medium before being analyzed.

These data are in good agreement with those of Wiemken and Nurse (34).

Distribution of allantoin-related metabolites. To determine the intracellular distribution of urea, cultures of two mutant strains were allowed to accumulate [^{14}C]urea for increasing lengths of time and then processed as described above. A mutant strain defective in urea carboxylase activity was used to prevent any metabolism of accumulated urea. The strain that was defective in allophanate hydrolase was expected to accumulate some allophanate as well as urea (29). As shown in Table 4, most of the urea was accumulated in the cytoplasmic fraction of the urea carboxylase-minus strain (M62). In strain M64, somewhat more of the radioactive material was observed in the vacuolar fraction.

Allantoin distribution within the cell was determined in a manner similar to that used for urea. The period of accumulation, however, was considerably extended, because radioactive allantoin was available only at low specific radioactivity and entered the cells via a rather low-capacity uptake system (19). As shown in Table 5, most of the radioactive material was found in the vacuolar fraction whether the accumulated allantoin remained unmodified (strain M85) (19) or was metabolized in part to allantoic acid (strain M104).

Existence of allantoin as a normal cellular constituent. The presence and characteristics of allantoin-degradative enzymes in yeast are well documented. However, allantoin per se has never been shown to exist in yeast cells. To determine whether or not allantoin is a normal cellular constituent, we attempted to isolate al-

TABLE 4. *Intracellular distribution of urea in mutant strains of S. cerevisiae*[a]

Defective enzyme and cellular compartment assayed	% of total urea extracted			
	2 min[b]	8 min	30 min	60 min
Urea carboxylase (M62)[c]				
Cytoplasm	78	74	68	62
Vacuole	22	26	32	38
Allophanate hydrolase (M64)[c]				
Cytoplasm	57	63	64	52
Vacuole	43	37	36	48

[a] Cultures of strain M62 and M64 were grown and permitted to accumulate carrier-free [^{14}C]urea (1.2 μCi/ml; specific activity, 5 Ci/mol) for varying lengths of time as described in the legend to Fig. 1. Oxaluric acid (0.5 mM) was included in the culture medium of strain M62 to bring about induction of the urea permease. In strain M64 this uptake system is already present at fully induced levels. At the times indicated in the table, samples were removed from the culture and processed as described in the text. The amount of radioactive material observed in a representative sample was 1,157,200 cpm.

[b] Times that cells were allowed to accumulate radioactive urea from the medium before being analyzed.

[c] Strain number of the mutant organisms used in this experiment.

TABLE 5. *Intracellular distribution of allantoin in mutant strains of S. cerevisiae*[a]

Defective enzyme and cellular compartment assayed	% of total allantoin extracted		
	2 h[b]	6 h	10 h
Allantoinase (M85)[c]			
Cytoplasm	10	5	4
Vacuole	90	95	96
Allantoinase (M104)[c]			
Cytoplasm	11	6	7
Vacuole	89	94	93

[a] Cultures of strains M85 and M104 were grown and permitted to accumulate [^{14}C]allantoin (0.020 μCi/ml; specific activity, 0.19 Ci/mol) for varying lengths of time as described in the legend to Fig. 1. At the times indicated in the table, samples were removed from the culture and processed as described in the text. The amount of radioactive material observed in a representative sample was 384,000 cpm.

[b] Times that cells were allowed to accumulate radioactive allantoin from the medium before being analyzed.

[c] Strain number of the mutant organisms used in this experiment.

lantoin from a culture grown in vitamin-enriched glucose-ammonia medium (yeast nitrogen base medium [Difco]). Two methods were used to identify allantoin in the cell extract. Qualitatively, a ureido group-positive compound with an R_f value identical to that of authentic allantoin was observed when a portion of the cell extract was subjected to paper chromatography. The chromatographic system used for this analysis was capable of resolving all of the known allantoin-related metabolites. A more quantitative approach was predicated on the behavior of allantoin and its related metabolites on ion-exchange resins. Allantoin is unique among the ureido group-containing compounds because it is uncharged at both acid and mildly alkaline pH's. Allantoate, urate, ureidoglycolate, and allophanate are all anions at pH values above 5 and were, therefore, removed from the crude cell extract by passing it over a Dowex-1-acetate resin. The resulting eluant was then acidified and passed over a cation-exchange resin (Dowex-50). This treatment removed all of the remaining amino acids, urea, adenine, and its derivatives from the cell extract. Allantoin, however, would not be retarded by any of these chromatographic conditions. Finally, the extract was treated with base, a treatment known to convert allantoin to potassium allantoate. The base-treated eluant from the Dowex-50 resin contained ureido group-positive material and was, therefore, neutralized to about pH 7 and passed over a Dowex-1-acetate resin. All of the ureido group-positive material remained bound to this resin and was eluted with a linear NaCl gradient; elution occurred at the position normally occupied by allantoate. In view of these chromatographic characteristics, the ureido group-positive material was assumed to be allantoin. Using an extinction coefficient obtained with authentic allantoin, we approximated the test compound to be present at a concentration of 3.9 μmol/g (dry weight) of cells. Using the convention that we used previously to calculate the concentration of radioactive allantoin accumulated from culture medium (19), we calculated the intracellular allantoin concentration to be approximately 1 mM.

Urea, which may be eluted from the Dowex-50 resin and quantitated by the ureido group assay, was not observed to be present in the extract at significant concentrations.

DISCUSSION

Evidence presented in this report indicates that allantoin is a normal cellular constituent which appears to be located in two separate compartments. This conclusion rests on the selective extraction of radioactive allantoin from the "cytoplasmic" and "vacuolar" metabolite pools. In analogy to other published reports (6, 32–34) it is reasonable to suggest that the large sequestered pool of allantoin is located in the vacuole. However, an unequivocal case in support of this suggestion cannot be made. The procedures we used, like those of earlier workers, essentially divide the soluble metabolites into two fractions: those that are released by disrupting the cell membrane and those that are lost by bursting all of the remaining osmotically sensitive intracellular organelles. Included here are nuclei, mitochondria, microbodies, and vacuoles. Although a vacuolar location for allantoin seems most likely, a role for the other compartments cannot be completely ruled out. Direct microscopic observation of allantoin within the vacuole or isolation and purification of vacuoles containing allantoin would be required for the strongest validation of our conclusions. However, in the absence of such data it is important to point out that the distribution pattern of allantoin was the same as that observed for S-adenosylmethionine (12–14, 24), a compound visually located within the vacuole (21, 22, 24).

The likely vacuolar location of allantoin raises the possibility that it functions as a reserve source of intracellular nitrogen. Structurally, it is well suited to such a function, containing a higher ratio of nitrogen to carbon than any other molecule but urea. Urea does not function well in this respect due to its toxicity and that of isocyanate, its major spontaneous decomposition product. Allantoin, on the other hand, is neutral, nontoxic, and normally quite inert. Arginine, an alternative nitrogen storage product, has the disadvantage of being quite basic. Sumrada and Cooper have shown that very high concentrations of arginine, lysine, or ornithine can result in depletion of intracellular histidine (J. Gen. Microbiol., in press).

Allantoin is a purine degradation product derived from uric acid. Although Roush reported observing purine crystals in the vacuoles of *C. utilis* (11), there is presently no evidence concerning the intracellular formation of allantoin or the enzyme uricase in *S. cerevisiae*.

Compartmentation characteristics of allantoin and urea might well be correlated with control of the enzymes associated with their degradation. Lawther et al. demonstrated that uninduced cells contained a very high basal level of allantoinase (9). As a result, the enzyme was induced only 4- to 8-fold, whereas allophanate hydrolase activity routinely increased by 20- to 30-fold on induction (30). Contrast between these two levels of induction is emphasized by the fact that both enzyme activities have been shown to possess the same inducer, allophanic acid. If allantoin were present in the cytoplasm,

it would be easily subject to degradation by the high basal levels of allantoinase and allantoicase. The ensuing production of urea would have resulted in induction of urea carboxylase and allophanate hydrolase. However, this has not been observed experimentally. The compartmentation of allantoin that we observed would clearly solve this problem and raises the possibility that control of allantoin compartmentation may play an important role in the regulation of allantoin metabolism. Arguments similar to these have been made by Weiss and Davis for control of arginase in *Neurospora* (27).

In *S. cerevisiae* several laboratories (32–34), including our own, have shown that arginine is distributed into two cellular compartments. However, unlike the situation in *Neurospora* (27), yeast arginase is present only at very low basal levels and is highly inducible. Control of arginine metabolism both by compartmentation of arginine and induction of arginase seems reasonable because arginine is synthesized as a precursor of protein synthesis and also appears to serve as a reserve nitrogen source. High basal levels of arginase, if present, might result in needless competition between degradative and biosynthetic systems for a small pool of cytoplasmic arginine. Such a disadvantageous situation is avoided by regulating arginine metabolism at two levels.

Urea, on the other hand, appears to be a predominantly cytoplasmic component that does not accumulate to significant intracellular concentrations. Although only limited and indirect data are available, allophanic acid could be suggested to accumulate in the vacuole because the percentage of radioactive material accumulating in the vacuole increased when metabolism of [^{14}C]urea to [^{14}C]allophanate was permitted.

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