Control of Arginine Metabolism in Neurospora

INDUCTION OF ORNITHINE AMINOTRANSFERASE*

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In Neurospora, most of the intracellular arginine pool is sequestered in osmotically sensitive organelles, the "vesicles." In this paper, we describe the factors influencing the induction of the arginine degradative enzyme ornithine-oxo-acid aminotransferase (EC 2.6.1.13). Upon addition of arginine to the growth medium, increased activity for the enzyme appears after a 30- to 40-min lag. The initial rate of enzyme accumulation remains constant for approximately 80 min despite a continuously increasing intracellular arginine pool. Approximately 30 min following complete expansion of the arginine pool, the rate of enzyme accumulation increases abruptly until the steady state-induced level is achieved. Induction requires both protein and RNA synthesis. Synthesis of ornithine aminotransferase-forming capacity commences within minutes following addition of arginine to the growth medium. The initial rate of accumulation of this forming capacity is related to the rate of arginine uptake from the growth medium.

Induced levels of the enzyme begin to decline 30 min after the removal of arginine from the growth medium. The rate of accumulation of ornithine aminotransferase appears to revert to the basal level within minutes following arginine removal from the growth medium. Reversion to the basal rate of accumulation occurs while the intracellular arginine concentration is still higher than that required for enzyme induction. We suggest that compartmentation of arginine plays a significant role in controlling arginine metabolism in Neurospora.

Many prokaryotic organisms differ from typical eukaryotic cells in having much smaller pools of metabolites, especially amino acids. These levels are high enough to satisfy the concentration requirements of the enzymes which use the amino acids for protein synthesis. However, little catabolism occurs because the catabolic enzymes are neither induced nor very active at such low concentrations. One can hypothesize that small intracellular pools provide prokaryotes with great sensitivity to changes in the composition of the growth medium. For example, amino acid concentrations in Escherichia coli are typically on the order of 0.05 to 1 mM (1). If E. coli growing in minimal medium is given exogenous arginine, uptake of only a small amount of the amino acid from the medium should be sufficient to cause an increase in the concentration of the intracellular pool large enough to initiate a regulatory response at the gene level. Results consistent with this hypothesis were obtained by Krzyzek and Rogers (2) in their study of the repression of the arginine biosynthetic enzymes by arginine in E. coli. Their results indicated that the rate of messenger RNA synthesis for the arginine biosynthetic enzymes fell to the repressed level immediately after the addition of arginine to the cell culture. Thus, as was predicted, uptake of only a small amount of arginine was sufficient to begin the regulatory response.

In eukaryotes, large storage pools of metabolic intermediates are often present. In Neurospora, the intracellular concentration of arginine would be 8 mM in cell water if it were equally distributed throughout the cell (3). This concentration is large enough to maximally inhibit arginine biosynthesis (4), yet biosynthesis occurs. Furthermore, the amino acids coexist with large amounts of arginase (EC 3.5.3.1) and ornithine aminotransferase, the first two enzymes of arginine degradation, yet little catabolism occurs (3, 5). These apparently contradictory aspects of arginine metabolism in Neurospora have been investigated in depth in recent years. The study has produced detailed information on the interrelationship between subcellular structure and arginine metabolism. The major results are conveniently summarized in Fig. 1.

Most of the large arginine pool is located inside a membrane-bound organelle which has been termed "the vesicle" (6). The final step of arginine biosynthesis occurs in the cytosol (7). Most of the biosynthetically generated arginine is incorporated into protein, and the remainder is taken up by the vesicle. The biosynthetic enzyme subject to feedback inhibition by arginine, the arginine-catabolizing enzymes, and the arginine charging enzyme are cytosolic (7, 8). Protein synthesis occurs in the absence of significant catabolism because the concentration of cytosolic arginine is maintained at a level too low for binding to arginase but sufficient for binding to the charging enzyme (9, 10). Catabolism occurs only when arginine is present in the growth medium (3, 5). Under such conditions, the cytosolic arginine concentration is elevated about 75-fold (11), catabolic enzymes are induced.
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Materials and Methods

Strains and Chemicals—The wild type strain 74A was obtained from R. H. Davis, and the mutant strain bat (FGSC 1683) was obtained from the Fungal Genetics Stock Center (Arcata, Calif.). Bat is a strain lacking the basic amino acid transport system. Basic amino acids are taken up in this mutant only by the general amino acid transport system (15). The strains were maintained in liquid culture in Vogel’s minimal medium N (14) supplemented with 1% sucrose. Nutrient broth (Difco), containing 0.1% L-arginine, was also used.

Growth and Changing Growth Medium—Growth was carried out at 25°C in a flat-bottomed 2-liter boiling flask through which hydrated air was bubbled to aerate and mix the culture (16). Dry weights were determined by collecting the mycelia from 15 to 25 ml of the culture by suction filtration on Whatman No. 1 filter paper and weighing the resulting mycelial pad with acetone as aspiration continued. About 14 h after inoculation, the culture was in the exponential growth phase at a density of 0.3 to 0.4 mg dry weight of mycelia/ml of culture. Exponential growth continued up to a density of about 1.2 mg dry weight/ml. All experiments reported here used exponential cultures to ensure that all biochemical parameters had established steady state values.

Transfer of cells from one growth medium to another was accomplished as follows. Cells were collected by filtration using Whatman No. 1 filter paper, washed with 4 liters of sterile medium, and suspended in 2 liters of the new sterile and prewarmed medium. Care was taken to avoid drying the cells completely during the transfer procedure. Addition of arginine to the growth medium was accomplished by adding sufficient solid arginine to achieve the desired concentration.

Measurement of Ornithine Aminotransferase Activity—The mycelia from 30 to 200 ml of a culture were collected by suction filtration on Whatman No. 1 filter paper and were ground with sand in 3 to 4 ml of 0.02 M potassium phosphate, 0.01 M EDTA, pH 7.5. The resulting slurry was centrifuged at 20,000 × g for 30 min at 4°C. The supernatant was poured off and dialyzed against 0.02 M potassium phosphate, pH 7.5, at 4°C. Following dialysis, the protein content of the crude extract was determined by the method of Lowry et al. (16). Bovine serum albumin was used as a standard.

Ornithine aminotransferase was assayed by a modification of the method of Davis and Mora (17). The assay mixture contained 0.2 ml of 0.1 M α-ketoglutarate (pH 7.4), 0.2 ml of 0.1 M L-ornithine HCl (pH 7.4), 0.2 ml of 0.5 M pyridoxal phosphate, and 0.4 ml of 0.5 M potassium phosphate buffer (pH 7.4). The assay was carried out at 37°C. The enzyme reaction was started by the addition of 0.5 ml of diluted prewarmed protein extract containing 0.1 to 0.5 mg of protein and terminated after 30 min by placing the reaction tubes in a boiling water bath for 45 min. The reaction tubes were brought to 25°C and treated with 0.4 ml of a fresh solution of 0.2% o-aminobenzoic acid. The resulting color reaction proceeded to completion within 30 min at 25°C. At this time, 1.1 ml of 30% perchloric acid was added. The tubes were placed in an ice bath for 30 min to deposit the acid-insoluble material, then centrifuged for 10 min in a clinical centrifuge at top speed. Zero time blanks received the identical treatment, but were placed in the boiling water bath immediately after the addition of protein. The yellow color of the supernatant in the reaction tubes was measured in a Klett-Summerson colorimeter, using a No. 42 filter against a distilled water blank, and corrected for the amount of color in the zero time blanks. The intensity of the color was linear with time and enzyme concentration in the limits used in these experiments. The amount of product (3-pyruvyl-5-carboxylate) formed was computed using the conversion factor that 100 Klett units is equivalent to 0.195 μmol (17). Specific activities are reported as micromoles of product formed per min per mg of protein at 37°C.

Determination of Total Arginine Pool—The mycelia from 15 to 25 ml of an exponential culture were collected by suction filtration on a membrane filter (0.45 μm pore size), washed with ice cold water to remove any growth medium and stop uptake, and placed in 3 ml of ice cold 5% trichloroacetic acid for 1 h. The insoluble material was removed by centrifugation, re-extracted with 1 ml of 5% trichloroacetic acid, and centrifuged again. The supernatant was combined and extracted three times with approximately 4 ml of diethyl ether to remove the trichloroacetic acid. The resulting solution was brought to approximately pH 5.3 by the addition of 2 ml of 0.116 M Na2 citrate, pH 5.3. Purification of arginine was performed using Dowex AG 50W-X8-Na+ columns (200 to 400 mesh, 0.7 × 15 cm) previously equilibrated with the same buffer. Neutral, acidic compounds, and other basic amino acids, were eluted with 40 ml of the citrate buffer. Arginine was then eluted with 10 ml of 0.2 M sodium hydroxide. Arginine was determined colorimetrically by the method of Van Pilsum et al. (18).

Results

Arginine Uptake and Induction of Ornithine Aminotransferase—Fig. 2 shows the increase in activity of ornithine aminotransferase and the intracellular arginine pool at various times after the addition of arginine to the growth medium. Enzyme activity increased linearly for approximately 90 min following an initial lag of 30 to 40 min. Similar results have been observed for arginase (19). The rate of enzyme accumulation then increased abruptly. Enzyme activity reached a value characteristic of cells grown in arginine-supplemented medium after approximately 4 h. The intracellular arginine pool increased rapidly and reached a steady state value after approximately 90 min. Note that the increase in the rate of enzyme accumulation at 120 to 130 min occurs approximately 30 to 40 min following arginine pool expansion.

Fig. 1. Enzymes and spatial features of arginine metabolism in Neurospora. Dashed arrow indicates translocation. Solid arrows indicate enzymatic reactions: 1, argininosuccinate lyase (1-argininosuccinate lyase, EC 4.3.2.1); 2, arginyl-tRNA synthetase; 3, arginase (EC 3.5.3.1); 4, ornithine aminotransferase (L-ornithine:Z-oxoacid aminotransferase, EC 2.6.1.15).
for ornithine aminotransferase activity and for intracellular arginine pool. Wild type cells growing in minimal medium at a density of 0.3 mg dry weight/ml were supplemented with arginine to a final concentration of 5 mM at time zero. At various intervals thereafter, samples were withdrawn and analyzed for ornithine aminotransferase activity and for intracellular arginine.

The experiments below were designed to distinguish between these alternatives. The experiments below were designed to distinguish between these alternatives.

Kinetics of Induction of Ornithine Aminotransferase Anamoptosis In order to determine the time of induction initiation, an attempt was made to measure the level of ornithine aminotransferase-forming capacity at intervals following arginine supplementation. This required an assay for specific enzyme forming capacity. In the experiments reported here, enzyme-forming capacity was assayed by its ability to yield enzyme. Kepes (21) and Cybis and Wegleni (22) have successfully used this method. In order to use an assay of this type, it was necessary to determine the time required to get maximum expression of enzyme-forming capacity as its enzyme product. Cells growing in minimal medium were exposed to arginine for 80 min, washed, and transferred to arginine-free medium. The specific activity of ornithine aminotransferase was examined at various intervals after the transfer (Fig. 3). The specific activity rose, reached a peak approximately 90 min after the transfer, and subsequently fell. This behavior is independent of the time of exposure to arginine. These results indicate that full expression of enzyme-forming capacity occurs after 90 min of incubation in arginine-free medium.

Enzyme activity present 90 min after transfer was used as a measure of the amount of ornithine aminotransferase-specific enzyme-forming capacity produced during and after exposure of cells growing in minimal medium to arginine for various periods of time. The activity has been plotted versus the time of exposure to arginine (Fig. 4). The results are compared with the normal induction kinetics. Increased enzyme-forming capacity for ornithine aminotransferase appears to be present immediately after arginine addition. Thus, the induction must begin to uptake of only a small amount of arginine (see Fig 2) In addition, the accumulation of enzyme-forming capacity was constant for at least 60 min and was not affected by the continuous increase in the size of the arginine pool.

Effect of Inducer Removal on Ornithine Aminotransferase Activity—Following the study of the response to arginine addition, response to arginine removal was investigated. The goal of the investigation was to determine the relation between the size of the intracellular arginine pool and the level of ornithine aminotransferase. This information was obtained by following the enzyme activity and the fall of the arginine pool when wild type cells grown in arginine-supplemented medium were transferred to arginine-free medium. Fig. 5

To further characterize the induction, the effect of inhibitors of RNA and protein synthesis were investigated. Table I shows that 6-methylpurine, an inhibitor of RNA synthesis (19), and cycloheximide, an inhibitor of protein synthesis (20), prevented a significant rise in ornithine aminotransferase specific activity. Thus it appears that induction of the enzyme requires both RNA and protein synthesis.

These results demonstrate that the appearance of increased enzyme activity occurs only after a sizeable increase in the intracellular arginine pool. Induction of ornithine aminotransferase might not begin until the arginine pool has reached a sufficiently high intracellular concentration. Once the pool reached this level, processes involved in the increased rate of enzyme accumulation could proceed rapidly. However, it is also conceivable that ornithine aminotransferase induction might begin soon after arginine addition and long before major expansion of the intracellular arginine pool. The 30- to 40-min lag preceding the rise in enzyme specific activity could simply be a reflection of the time required for expression of increased enzyme-forming capacity as accumulated enzyme.

The experiments below were designed to distinguish between these alternatives.

### Table I

| Treatment                        | Specific activity (μmol/min/mg protein) |
|----------------------------------|----------------------------------------|
| None                             | 0.021                                  |
| Arginine, 5 mM                   | 0.096                                  |
| 6-Methylpurine (0.5 mM) + 5 mM arginine | 0.028                                  |
| Cycloheximide (10 μg/ml) + 5 mM arginine | 0.018                                  |

Fig. 2. Interrupted induction of ornithine aminotransferase. Wild type cells growing in minimal medium were supplemented with 2 mM arginine at -80 min. At time zero, the cells were collected, washed, and transferred to arginine-free medium. At various times throughout the experiment, samples were withdrawn and assayed for ornithine aminotransferase activity.
shows that the size of the arginine pool began to decrease immediately after the transfer and fell toward the basal level (20 nmol/mg dry weight). The activity of ornithine aminotransferase began to drop 30 min after the transfer and also decreased toward the basal level (0.2 pmol/min/mg of protein). The activity of the enzyme fell with a half-life of 3.4 h, which is longer than the 2.05-h doubling time of the cells. This longer half-life suggested that the decay in specific activity was not due to rapid enzyme turnover. To further substantiate this conclusion, the effect of cycloheximide on the decline in enzyme activity was investigated. Table II shows that cycloheximide prevented a significant drop in the specific activity of ornithine aminotransferase. Thus, it appears that the induced enzyme is quite stable. These results suggest that the decline in enzyme activity is not due to rapid degradation or enzyme turnover, but probably results from a reduction in the rate of active enzyme production. This would cause the specific activity of the enzyme to drop as a result of dilution by newly synthesized protein.

Evidence supporting this mode of decay comes from the close agreement of the experimental results with those predicted by the following model. The decline in ornithine aminotransferase activity is the result of a decrease in the rate of its production and resulting dilution by new protein; the rate of production decreases in the absence of inducer from the induced to the basal rate within minutes after the transfer to arginine-free medium; previously produced enzyme-forming capacity is expressed and then decays; ornithine aminotransferase is infinitely stable. It is possible to calculate theoretical curves based on these assumptions. If one assumes a negligible half-life for enzyme-forming capacity, enzyme specific activity at any time following inducer removal should conform to the following model. The decline in ornithine aminotransferase activity is the result of a decrease in the rate of its production and resulting dilution by new protein; the rate of production decreases in the absence of inducer from the induced to the basal rate within minutes after the transfer to arginine-free medium; previously produced enzyme-forming capacity is expressed and then decays; ornithine aminotransferase is infinitely stable. It is possible to calculate theoretical curves based on these assumptions. If one assumes a negligible half-life for enzyme-forming capacity, enzyme specific activity at any time following inducer removal should conform to the following model.

**Table II**

| Medium                                      | Specific activity (μmol/min/mg protein) |
|---------------------------------------------|----------------------------------------|
| Arginine-supplemented (2 mM)                | 0.084                                  |
| Minimal medium                              | 0.031                                  |
| Minimal medium + cycloheximide (10 μg/ml)  | 0.076                                  |

**Fig. 6.** Comparison of theoretical and observed decline in ornithine aminotransferase activities following transfer from arginine-supplemented to arginine-free medium. Calculated curve assuming immediate shift to basal rate of transcription (---) or assuming 30-min lag for expression of pre-existing mRNA molecules (- - -). See text for calculations.
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$6.025 I b 2 2 0.020 -- 0.005$

MOLE FRACTION ARGININE

FIG. 7 (left). Control of arginine uptake rate in bat strain. Mutant cells growing in minimal medium were exposed to 1.0 mm arginine and various concentrations of glycine at time zero. At various intervals (to 30 min), samples were withdrawn and assayed for intracellular arginine. The steady state rate of uptake was calculated to the following equation:

$$SA_i = \frac{SA_{(induced)} + SA_{(uninduced)} \times (e^{kt} - 1)}{e^{kt}}$$

where $SA$ is the specific enzyme activity, $k$ is the growth constant calculated for cells with a generation time of 123 min, and $t$ is the time after transfer. If one assumes that expression of pre-existing enzyme forming capacity requires 30 min (Fig. 2), the equation becomes:

$$SA_i = \frac{SA_{(induced)} \times e^{30t} + SA_{(uninduced)} \times (e^{30t} - e^{0t})}{e^{0t}}$$

The two curves differ only in the length of time following the transfer to arginine-free medium for the expression of pre-existing enzyme-forming capacity. In one curve, the assumption is made that this occurs immediately after the transfer and in the other, 30 min after the transfer. The theoretical curves and the experimental results are shown in Fig. 6. Since most of the experimental points fall on or between the two curves, it appears that the rate of ornithine aminotransferase production decreases to the basal level within minutes after the transfer to arginine-free medium. Fig. 5 shows that the size of the arginine pool 30 min after transfer to arginine-free medium is about 100 nmol/mg dry weight, more than 5 times the size of the pool in cells grown in minimal medium.

**Rate of Arginine Uptake and Enzyme Induction** - The results described above suggest that production of ornithine aminotransferase is not proportional to the size of the intracellular arginine pool. To explore this apparent contradiction, we have examined the initial rate of enzyme accumulation under conditions in which the rate of arginine uptake has been varied. In the bat mutant, arginine is taken up from the medium only by the general amino acid permease (13). The arginine uptake rate can be controlled by using other amino acids to compete with arginine for uptake by this transport system. Cells growing in minimal medium were simultaneously exposed to arginine and various concentrations of glycine for a short period of time. Fig. 7 shows that arginine uptake rate during the exposure period was proportional to the molar fraction of arginine in the medium.

In a similar experiment, the rate of ornithine aminotransferase accumulation was determined by assaying for the total enzyme-forming capacity produced in a given length of time. Cells growing in minimal medium were exposed to arginine and various concentrations of glycine for 60 min, then washed and transferred to minimal medium for 90 min to allow full expression of the enzyme-forming capacity. Fig. 8 shows that the rate of accumulation was determined by the molar fraction of arginine in the medium. The previous experiment indicated that the rate of ornithine uptake was also determined by the molar fraction of arginine. Therefore, it follows that the rate of ornithine aminotransferase accumulation is determined by the rate of arginine uptake into the cell.

**DISCUSSION**

The results in Fig 2 indicate that the rate of ornithine aminotransferase accumulation does not increase for 30 to 40 min following the addition of its inducer, arginine, to the growth medium. This is in contrast to the 5- to 10-min lag observed for kynureninase (EC 3.7.1.3) in Neurospora (23) and the 9- to 4-min lag for arginase and allophanate hydrolase in Saccharomyces cerevisiae (24-27). These lags may represent the time required for transcription, processing, and translation of specific mRNA molecules. This hypothesis remains to be investigated. In each case, however, the increased rate of enzyme accumulation appears to be constant for an extended period (when corrected for cell growth) after the lag phase. In the case of ornithine aminotransferase, this initial
constant induced rate of accumulation occurs despite a continually increasing intracellular arginine pool (Fig. 2). Approximately 30 min after maximal expansion of the arginine pool, the rate of enzyme accumulation increases abruptly and continues at this increased rate until the steady state level characteristic of cells growing in arginine-supplemented medium is achieved. These results suggest that the concentration of arginine (or its derivative) which controls the rate of enzyme production remains constant during the steady state induction phase but increases significantly when the intracellular pool reaches its maximal value. This is reflected following a 30-min lag by an increase in the rate of enzyme accumulation.

The long lag phase observed (Fig. 2) might reflect the establishment of an inducing level of intracellular arginine or the time required for expression of enzyme-forming capacity as active enzyme. Fig. 3 shows that 90 min are required for complete expression following the removal of the inducer, arginine. This compares to the 16 min observed for kynureninase (23) and 10 min for allophanate hydrolase (26). In all cases (22-24, 26; also see below) it appears that the increased rate of production of enzyme-forming capacity ceases abruptly following inducer removal. Using these results, it has been possible to examine the initiation of the increase in the rate of production of ornithine aminotransferase-forming capacity (Fig. 4). It appears that the increased rate of ornithine aminotransferase-forming capacity accumulation is initiated within minutes following arginine supplementation, remains constant, and parallels enzyme accumulation for at least 60 min. This occurs despite a steadily increasing intracellular arginine concentration. It appears unlikely that this response is mediated by the total intracellular arginine concentration.

When arginine is removed from arginine-supplemented medium, ornithine aminotransferase activity begins to decline following a lag of approximately 30 min (Fig. 5). This occurs at a time when the intracellular arginine pool is approximately 5 times that found in cells growing in minimal medium. Fig. 6 compares the observed decay of ornithine aminotransferase specific activity with models based upon an abrupt shift to the noninduced rate of enzyme production. In view of the long time required for the expression of ornithine aminotransferase-forming capacity (Fig. 3), it is likely that much of this lag period reflects expression of such capacity formed in the presence of arginine. It would appear that the rate of production of ornithine aminotransferase shifts to the noninduced rate shortly after arginine is removed from the growth medium and long before the intracellular pool level drops below that required for induction (Figs. 2 and 4).

These results suggest that ornithine aminotransferase production is independent of the total intracellular arginine concentration, but instead depends on the presence or absence of arginine uptake from the medium. It has previously been shown that much of the intracellular arginine pool of Neurospora is sequestered within a membrane-enclosed organelle (6). A schematic diagram of the structural and locational relationships between the relevant enzymes and arginine is shown in Fig. 9. During growth in minimal medium, the cytosolic pool remains small, catabolic enzymes are at a basal level, and catabolism is almost nonexistent (3, 5, 9). During growth in arginine-supplemented medium, the cytosolic arginine concentration increases much more than that of the total arginine pool, enzymes are induced to a small degree (4-fold), and catabolism proceeds rapidly (3, 11, 12). During transitions between growth in the presence and absence of arginine, the rate of catabolism responds fully within minutes (12). The model shown in Fig. 9 suggests that control of the level of gene expression might also respond to changes in the cytosolic arginine concentration.

During growth in minimal medium, the cytosolic arginine concentration would remain low and ornithine aminotransferase would be uninduced. Upon arginine addition to the growth medium, uptake would occur, the cytosolic concentration would rise until it reached a steady state value dependent on the rates of arginine entrance into and exit from the cytosol. This steady state value is likely to largely depend on the rate of uptake from the medium. The immediate response to arginine addition shown by ornithine aminotransferase-forming capacity and the constant increase during pool expansion are consistent with this model (Fig. 4). Once the "vesicular pool" becomes fully occupied, the cytosolic arginine concentration would rise until it reached a steady state value further since one means of its exit is now eliminated. This is reflected in an increased rate of production of enzyme-forming capacity. The latter results in new enzyme following the 30-min lag required for its expression (Fig. 2). The increased cytosolic arginine concentration will control the rate of uptake by transinhibition until the steady state level characteristic of long term growth in arginine-supplemented medium is achieved. If arginine is removed from the growth medium, the cytosolic concentration will drop as uptake ceases. This results in rapid cessation of catabolism (12) and return to the basal rate of ornithine aminotransferase production (Fig. 6).

A basic tenet of this model is that responses governed by the cytosolic arginine concentration will be affected by the rate of arginine uptake from the medium. The rate of arginine uptake can be controlled by competitive inhibitors of its
transport system. This is best accomplished using a mutant which used a nonspecific transport system for arginine. Such a mutant is the bat strain which can transport arginine only via the general amino acid permease (13). The feasibility of this approach has been demonstrated (Fig. 7). The relationship between the initial rate of accumulation of enzyme-forming capacity and the arginine uptake rate clearly suggests that the rate of uptake affects the cytosolic arginine concentration and the induction process which responds to this concentration (Fig. 8).

It remains to be examined why the response of arginase (12) and ornithine aminotransferase is significantly slower than that of kynureninase in Neurospora and arginase and allophanate hydrolase in yeast. Mechanistic possibilities include: slower rates of mRNA processing, activation or assembly of completed enzyme molecules, or slow insertion of required cofactors. These possibilities remain to be investigated. From a functional point of view, two features distinguish the kynureninase catabolic system from the arginine degradative pathway. First, the level of the enzyme in cells growing in minimal medium is low, and the activity increases 120-fold in the presence of inducers (23). Second, the intracellular tryptophan and kynurenine pools are very small during growth in minimal medium (23). These observations suggest that addition of tryptophan to the growth medium will quickly affect the total intracellular tryptophan concentration, but that efficient metabolism will require significant enzyme induction. In contrast, arginase, ornithine aminotransferase, and arginine levels are high in cells growing in minimal medium. The enzymes are only induced 4-fold and the total arginine pool expands only 7-fold (3, 11). Compartmentation (Fig. 9) coupled with pre-existing enzyme allows Neurospora to respond quickly to changes in the availability of arginine (12). Although there is some evidence for the compartmentation of tryptophan in Neurospora, its significance is not clear (28).

In yeast, arginine is compartmentalized in the vacuole, an organelle similar to the vesicle of Neurospora (29). The difference in response would appear to be a consequence of the necessity for enzyme induction for maximal catabolism since enzyme levels are low during growth in minimal medium (30). Compartmentation would appear to add an additional means of controlling amino acid metabolism. In arginine metabolism in Neurospora, it would appear to play a decisive role, whereas in yeast it may contribute significantly although enzyme induction appears to play a major role. The rationale for such differences may reside in the life-styles of the organisms. Neurospora's arginine metabolism appears to be ideally suited to fluctuating environments in which arginine alternately becomes available or is depleted. The overall significance of compartmental processes in higher eukaryotes remains to be investigated.

REFERENCES

1. Tempest, D. W., Meers, J. L., and Brown, C. H. (1970) J. Gen. Microbiol. 64, 171–185
2. Krzyzek, R., and Rogers, P. (1972) J. Bacteriol. 110, 945–954
3. Davis, R. H., Lawless, M. B., and Port, L. A. (1970) J. Bacteriol. 102, 299–305
4. Cybis, J. J., and Davis, R. H. (1974) Biochim. Biophys. Acta 36, 625–634
5. Davis, R. H. (1970) Biochim. Biophys. Acta 215, 412–414
6. Weiss, R. I. (1970) J. Biol. Chem. 245, 5404–5413
7. Weiss, R. L., and Davis, R. H. (1973) J. Biol. Chem. 248, 5403–5408
8. Cybis, J., and Davis, R. H. (1975) J. Bacteriol. 123, 196–200
9. Subramanian, K. N., Weiss, R. L., and Davis, R. H. (1973) J. Bacteriol. 115, 284–290
10. Nazario, M. (1967) Biochim. Biophys. Acta 145, 146–152
11. Weiss, R. L. (1970) J. Bacteriol. 126, 1173–1179
12. Weiss, R. L., and Davis, R. H. (1977) J. Bacteriol. 129, 866–873
13. Thwaites, W. M., and Pandyala, L. (1969) Biochim. Biophys. Acta 192, 455–461
14. Vogel, H. J. (1964) Am. Nat. 98, 435–446
15. Davis, R. H., and deSerres, F. J. (1970) Methods Enzymol. 17A, 79–143
16. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275
17. Davis, R. H., and Mora, J. (1968) J. Bacteriol. 96, 383–388
18. Van Pilaum, J. F., Martin, R. P., Kito, E., and Hess, J. (1956) J. Biol. Chem. 222, 225–236
19. Key, J. L. (1966) Plant Physiol. 41, 1257–1264
20. McKeenan, W., and Hardesty, B. (1969) Biochim. Biophys. Acta 92, 655–660
21. Kepes, A. (1963) Biochim. Biophys. Acta 76, 293–309
22. Cybis, J., and Weglenski, P. (1972) Eur. J. Biochem. 20, 262–268
23. Turku, J. R., Sorko, W. A., and Matchett, W. H. (1970) J. Bacteriol. 103, 364–369
24. Whitney, P. A., and Magasanik, B. (1973) J. Biol. Chem. 248, 2137–2142
25. Whitney, P. A., Cooper, T. G., and Magasanik, B. (1973) J. Biol. Chem. 248, 6203–6209
26. Lawther, R. P., and Cooper, T. G. (1973) Biochem. Biophys. Res. Commun. 55, 1100–1104
27. Lawther, R. P., and Cooper, T. G. (1975) J. Bacteriol. 121, 1064–1073
28. Matchett, W. H., and DeMoss, J. A. (1964) Biochim. Biophys. Acta 86, 91–99
29. Wiemann, A., and Nurse, P. (1973) Planta (Berl) 109, 293–306
30. Wiame, J. M. (1971) Curr. Top. Cell. Regul. 4, 1–38
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