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An inducible amino acid transport system in *Neurospora crassa*★

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SUMMARY

The general amino acid transport system in *Neurospora crassa* conidia is shown to be stimulated to a greater rate of transport activity by the basic amino acids L-arginine and L-lysine. The neutral amino acid L-phenylalanine causes a reduction in the rate of transport activity by this system. The possible mechanisms of regulation by these amino acids are discussed.

The amino acid and sugar transport systems in *Neurospora crassa* have been shown, in general, to be repressed by growth (or incubation) of cells in the presence of the substrate transported[1–3]. The repression appears to be specific in that neutral amino acids "transinhibit"[4] (reduce) the transport activity of the neutral, but not the basic amino acid transport system. Further, the basic amino acids "transinhibit" the basic, but not the neutral amino acid transport system[5]. Repression of sugar transport systems, however, is reported[6] to be non-specific in that glucose represses the glucose, galactose, lactose, *etc.*, transport systems in Neurospora.

This report presents evidence for substrate induced stimulation and repression of a general amino acid transport system in *N. crassa* conidia.

Strains employed. The strains of *N. crassa* used in these studies were 74-OR23-1A (the wild type) and the mutant *Pm⁻ AB # 10*. The geneology and transport characteristics of *Pm⁻ AB # 10* have been previously described[7]. Briefly, it is a double transport deficient mutant altered (reduced) in transport of neutral amino acids and basic amino acids. The transport deficiencies are the result of lesions in the neutral and basic amino acid transport systems. *Pm⁻ AB # 10* is analogous to the described *Pm⁻ NB* mutant, and is presumed to retain only the general amino acid transport system(s) for accumulation of neutral and basic amino acids. The *Pm B* locus has been tentatively mapped on linkage group V.

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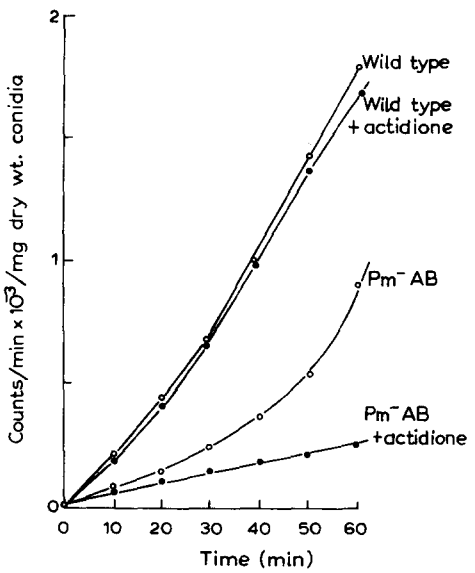
Techniques employed. The methods and materials employed in culture maintenance, transport experiments, and extraction and chromatography of label have been previously described[7].

This study was undertaken to examine the apparent increase in the velocity of L-arginine transport, in *Pm⁻ AB #10 (Pm⁻ AB)*, as a function of time of incubation in the presence of L-arginine[7]. As shown in Fig. 1, the increase in rate of L-arginine transport can be blocked by the addition of cycloheximide. This antibiotic has been shown to be a potent inhibitor of protein synthesis in Neurospora.

In order to more closely examine the velocity of L-arginine transport, cells were preincubated with or without 0.1 mM L-arginine in 1X Vogel's salts[8]. At appropriate intervals, aliquots were removed, filtered, washed and resuspended in 1X Vogel's salts *plus* L-[^{14}C] arginine (0.1 mM). Thereafter, short-term uptakes (< 16 min) provided an estimate of the velocity (in μmoles/min per mg dry wt. cells) of L-arginine transport. As shown in Fig. 2, the velocity of L-arginine transport in *Pm⁻AB* is greater in those cells preincubated 60–90 min in L-arginine than in those cells preincubated the same length of time in Vogel's salts. Further, calculation of the change in velocity of transport as a function of time of preincubation indicates that not only is the velocity of L-arginine transport greater in arginine incubated cells, but the rate of change in velocity is increasing. The increase in velocity of L-arginine transport in cells incubated in 1X Vogel's salts, as a function of time



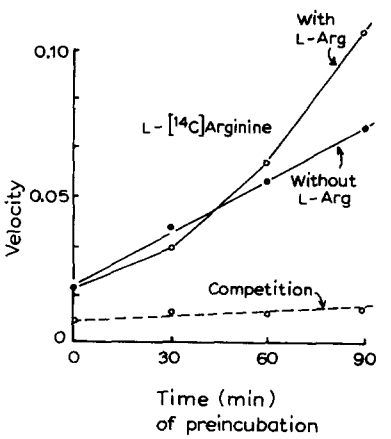


Fig. 1. Transport of L-[^{14}C] arginine in wild type and *Pm⁻AB* in the presence and absence of cycloheximide (actidione) (100 μg/ml). L-Arginine is at an external concentration of 0.1 mM and a specific activity of 0.01 μC/0.1 μmole.

Fig. 2. Velocity (μmoles x 10^{-2}/min per mg dry wt. conidia) of L-[^{14}C] arginine (0.1 mM) transport by cells preincubated for the designated period of time with or without 0.1 mM unlabeled L-arginine. See text for methods. The points designated "competition" represent the average velocity of L-[^{14}] arginine transport in the presence of 1.0 mM concentrations of unlabeled L-arginine, L-lysine, or L-phenylalanine Transport of L-arginine (by cells preincubated with or without unlabeled L-arginine) in the presence of competing amino acids did not exceed a velocity of 0.015 μmole x 10^{-2}/min per mg cells.

of preincubation, occurs at a constant rate. To insure that preloading of cells with unlabeled L-arginine does not restrict later incorporation of L-[^{14}C] arginine into trichloroacetic acid-insoluble material, *i.e.* apparent larger pool of L-[^{14}C] arginine, the trichloroacetic acid-insoluble incorporation of label into cells preincubated in Vogel's salts is subtracted from both sets of the "total" L-[^{14}C] arginine transport rates to obtain the "pool" values plotted

There are several plausible explanations for the observed rate changes: (1) A *de novo* synthesis of more "permease" molecules of the general (or a "new") transport system such that a greater transport rate is observed. (2) Increased degradation of L-arginine by arginase[9], *i.e.* increase in rate could be due to metabolic drag. (3) A decrease in rate of efflux of accumulated L-arginine. (4) A ligand induced allosteric modification of some component of the permease. Because the observed increase in velocity is measured as a trichloroacetic acid-soluble pool (in all figures), an increase in the rate of protein synthesis need not to be considered as a possible explanation. The pool values are obtained by subtraction of the ^{14}C-labeled amino acid incorporated into a trichloroacetic acid-insoluble fraction from the "total" ^{14}C label accumulated by the cells.

An increase in the rate of degradation has been ruled out by the finding that greater than 95% of the L-arginine accumulated, by both the wild type and the mutant, (after 60 min) is chromatographically similar to arginine[7]. These results were consistent with other studies involving transport studies with L-arginine[10]. Further, the accumulation of a large "free" pool of unlabeled arginine, during the preincubation, would tend to reduce, rather than increase, the apparent rate of degradation of the labeled L-arginine utilized in determination of transport velocities.

Because the presence of a large pool of unlabeled L-arginine would tend to reduce efflux of the ^{14}C-labeled L-arginine (during the velocity determinations), *i.e.* an apparent increase in influx, studies were undertaken to see if accumulated L-arginine would efflux. Table I shows that only 8% of accumulated arginine effluxes from the wild type after

TABLE I

EFFLUX OF L-[^{14}C] ARGININE FROM WILD TYPE AND *Pm⁻AB* CELLS

The cells were allowed to accumulate L-[^{14}C] arginine for 60 min. Following this incubation, they were filtered onto a membrane filter, washed, resuspended in 1X Vogel's salts to the original 0.1 mg/ml and aliquots removed with time, filtered, washed, and counted as before[7].

Time of efflux (min)	*Counts/min per mg dry wt. conidia*			
	Wild type		*Pm⁻AB*	
	Total	*Protein*★	*Total*	*Protein*★
0	1692	696	832	416
2	1702		794	
4	1714		796	
6	1664		776	
8	1648		772	
10	1596		762	
20	1562	864	712	446

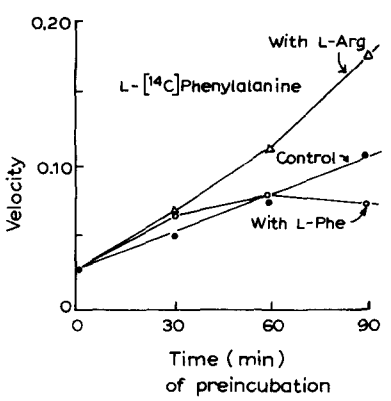
★The counts designated as occurring in protein represent the label incorporated into a trichloroacetic acid-insoluble fraction.

20 min and only 14% from the *Pm⁻AB* mutant. The finding of an insignificant efflux rate in these strains is consistent with the results of Roess and DeBusk[10] who reported an absence of arginine efflux in Neurospora conidia.

The sensitivity to cycloheximide earlier suggested a possible *de novo* synthesis of "permease" molecules. The major question is whether the new permease molecules are those described by the general amino acid transport activity or a separate basic amino acid transport activity. If L-arginine is indeed stimulating transport activity of the general amino acid transport system, an increase in L-phenylalanine transport should occur following a preincubation in L-arginine. Fig. 3 shows that this is exactly what happens. *Pm⁻AB* cells preincubated in 0.1 mM L-arginine transport L-phenylalanine (0.1 mM) at a velocity greater than cells preincubated in 1X Vogel's salts. Preincubation of cells in 0.1 mM L-phenylalanine, however, decreases the subsequent velocity of L-phenylalanine (and L-lysine, Fig. 4) transport, as compared to control cells preincubated in 1X Vogel's salts.

Further, if it is an increase in transport activity of the general transport system, the increased velocity of L-arginine transport should be subject to reduction by a neutral L-amino acid. Fig. 2 shows that competition by L-phenylalanine (and L-arginine or L-lysine) for L-[^{14}C] arginine transport drastically reduces the transport velocity in both the stimulated and nonstimulated cells. Because the velocity of L-arginine transport in the presence of different competing amino acids was similar in each preincubation stage, an average of the velocities was plotted. These competition studies and the arginine stimulated velocity increase of L-phenylalanine transport in *Pm⁻AB* are consistent with the hypothesis that the increased transport activity is due to the general amino acid transport system.

That the increase in general transport activity is not due specifically to incubation in L-arginine is shown by the observation that preincubation with 0.1 mM L-lysine also stimulates an increase in velocity of L-lysine transport. As with the L-phenylalanine transport velocity studies in Fig. 3, preincubation of cells with L-phenylalanine reduces the subsequent transport velocity of L-lysine.



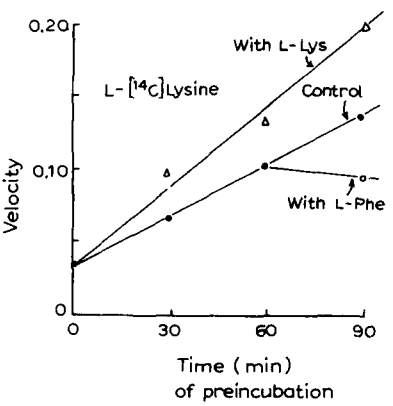


Fig. 3. Velocity (μmoles x 10^{-2}/min per mg cells) of L-phenylalanine (0.1 mM) transport by cells preincubated with 0.1 mM L-arginine, 0.1 mM L-phenylalanine, or in 1X Vogel's salts (control) prior to determination of transport velocity of L-phenylalanine.

Fig. 4. Velocity (μmoles x 10^{-2}/min per mg cells) of L-lysine (0.1 mM) transport by cells preincubated with 0.1 mM L-lysine, 0.1 mM L-phenylalanine or in 1X Vogel's salts (control) prior to determination of transport velocity of L-lysine.

These studies suggest that the general amino acid transport system is subject to regulation by its substrate molecules. Basic amino acids stimulate the system providing for an increase in transport activity, while L-phenylalanine (and perhaps other neutral amino acids) decreases its activity.

Regulation of amino acid transport has been proposed to occur by a variety of mechanisms. Each mechanism, however, has one common element, the transported molecule. Whether regulation occurs by turn-over of some labile component of the transport system, allosteric inhibition or binding to some active site by intracellular substrate, or total amino acid pool is not known at this time. All three (and probably other) mechanisms may be involved to some degree in regulating the transport process. The sensitivity of the inducible system, studied here, to cycloheximide could be due to a required *de novo* synthesis of some transport component or to a build up of amino acids which "transinhibit" the system.

Thwaites and Pendyala[11] have reported that conditions which affect the rate of arginine biosynthesis also regulate development of the general transport system. Low endogenous pools of arginine block development of the general transport system. Thus, stimulation, by exogenously supplied arginine, of the general system might seem reasonable.

That transport of L-arginine in the wild type remains linear, suggests that the presence of a functional basic amino acid transport system may modify the reaction(s) of the regulatory apparatus, *i.e.* either transport activity of the basic system decreases in proportion to increase of the general or stimulation of the general system does not occur in the wild type. J.R. Ferguson (personal communication) has observed an arginine stimulated increase in L-phenylalanine transport in the Lindegrin 1-A (FGSC No. 354) wild type of Neurospora. This observation (and others) indicates that strain differences, physiological state of cells, pH[12], *etc.*, are important in delineation of transport activity present in a dynamic cell population.

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