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Physiological Adaptation to the Loss of Amino Acid Transport Ability

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A strain of *Neurospora crassa* devoid of constitutive amino acid transport ability can utilize arginine as the sole nitrogen source. Nitrogen starvation, presence of arginine, and mutational inactivation of the general permease are key factors in signaling production of an extracellular enzyme which removes the alpha-amino group from the amino acid.

The fungus *Neurospora crassa* has three constitutive amino acid transport systems: a neutral amino acid-specific (N) system, a basic amino acid-specific (B) system, and a general (G) system which transports all classes of amino acids (1, 2, 7, 10, 11, 15, 19, 20). As a result of mutations for each of the constitutive permeases, the triple mutant strain *pmn;pmb;pmg* has no constitutive amino acid permease activity (6, 12, 15–17). In the course of experiments designed to examine the fate of basic amino acids as a function of the transport system of entry, we found that arginine can serve as a nitrogen source for the *pmn;pmb;pmg* strain despite the fact that this basic amino acid cannot be transported. We describe here the key factors of an adaptation phenomenon which allows the transport-defective strain to utilize arginine.

Strains were maintained on solidified Vogel medium N containing NH_4NO_3 as the nitrogen source (2, 18). To examine the ability of an amino acid to serve as a nitrogen source, the NH_4NO_3 was removed (no-nitrogen Vogel medium), and an alternative nitrogen source was added (15 mM final concentration). Standard 72-h liquid growth assays were performed at 35°C (3). The ability of the wild-type (FGSC 987) and *pmn;pmb;pmg* (FGSC 2606) strains to utilize the basic amino acid L-lysine or L-arginine as the sole nitrogen source is shown in Fig. 1a. We expected the wild-type strain to grow by virtue of its functional B and G systems and the *pmn;pmb;pmg* strain not to grow because of its transport defects. Neither strain can grow on L-lysine, but both can utilize L-arginine.

Since no transport of L-arginine could be detected in the *pmn;pmb;pmg* strain at any stage of development (data not shown), we suspected that this strain had an alternate mechanism for procuring the desired nitrogen. Liquid cultures of both the wild-type and *pmn;pmb;pmg* strains grown with L-arginine as the sole nitrogen source were filtered (Whatman no. 1) to remove mycelial growth. The culture filtrates were dialyzed and incubated with L-[^{14}C]arginine by the methods of DeBusk et al. (3). The reaction products were separated by thin-layer chromatography, using precoated cellulose sheets with 1-butanol–acetic acid–water (80:20:20) as the solvent, and visualized with ninhydrin (amino acids), ferric chloride (keto acids), and autoradiography (3, 9). Media from the *pmn;pmb;pmg* strain, but not from the wild-type strain, converted the amino acid to a radioactive product that reacted with ferric chloride (Fig. 1b). Boiling of the *pmn;pmb;pmg* media for 5s eliminated conversion, suggesting that this activity was enzyme mediated. Since several keto acids are not commercially available, including the keto acid derivative of L-arginine, we verified that the enzymatic activity we detected involved the conversion of amino acid to keto acid by comparing the reaction product with that produced by L-amino acid oxidase (EC 1.4.3.2) obtained from *Crotalus adamanteus* (Sigma Chemical Co., St. Louis, Mo.), which is known to convert alpha-amino acids to alpha-keto acids. For each amino acid tested, the reaction products generated by the two enzymes were identical and were ferric chloride positive.

The arginine-induced enzyme is not specific for L-arginine but can convert a variety of amino acids to the respective keto acids. The ability to convert the neutral amino acid phenylalanine to the keto acid phenylpyruvate allowed us to detect conversion activity in test media, using a microassay that monitors the conversion of radiolabeled phenylalanine to radiolabeled phenylpyruvate (3). This assay cannot be used with charged amino acids such as arginine. Presumably, the enzymatic conversion of amino acids to keto acids is accompanied by the release of alpha-amino nitrogen as ammonium ions which then serve as the nitrogen source.

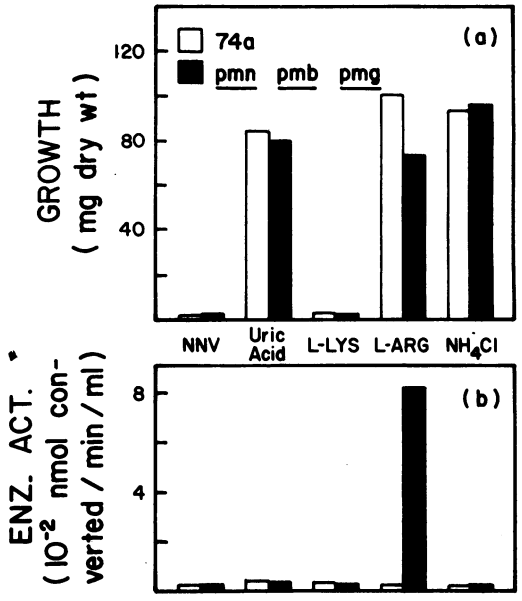

FIG. 1. Growth with various nitrogen sources (a) and production of extracellular deaminase (b) by the wild-type (74a) and *pmn;pmb;pmg* strains of *N. crassa*. All media consisted of no-nitrogen Vogel medium (NNV) plus a 15 mM nitrogen source as indicated. Growth assays were performed at 35°C for 72 h. Deaminase assays were performed at 35°C for 2 h.

We previously reported (3) the elaboration by the *pmn;pmb;pmg* strain of an extracellular deaminase in response to the presence of the amino acid analog *p*-fluorophenylalanine (FPA). This FPA-induced deaminase is also able to convert phenylalanine to phenylpyruvate. We are interested in determining whether the arginine-induced enzyme and the FPA-induced enzyme are the same protein. Although we cannot yet answer this question by definitive biochemical analysis of the isolated proteins, we can offer evidence that the production of the two activities is distinctly regulated. Whereas the FPA-induced enzyme is produced under nitrogen-sufficient conditions (NH_4Cl), the arginine-utilizing enzyme is not. Also, the addition of a new mutation, *lox*, which prevents the *pmn;pmb;pmg* strain from producing enzyme activity in response to FPA, does not affect enzyme production in response to arginine (Fig. 2). The *pmn;pmb;pmg*; *lox* strain was generated by UV mutagenesis and selected for its vigorous growth in the presence of KNO_3 plus 0.4 mM FPA.

Since the production of the enzyme responsible for arginine utilization appears to be an adaptive response, we are interested in the signal(s) which communicates the need for the response. We examined whether the presence of an amino acid in the growth medium, nitrogen starvation, or defective amino acid transport was involved in signaling enzyme production. The *pmn;pmb;pmg* strain produces the arginine-induced enzyme under conditions of nitrogen starvation plus the presence of arginine. Addition of NH_4Cl to the arginine medium prevents enzyme production, suggesting that nitrogen starvation is a key factor in triggering the adaptive response (Fig. 2). Nitrogen starvation alone is not sufficient, however, since the *pmn;pmb;pmg* strain does not produce enzyme in the presence of the purine uric acid which invokes nitrogen starvation conditions (Fig. 1). These data suggest that both nitrogen starvation and the presence of arginine are required for production of the enzyme. The fact that the *pmn;pmb;pmg* strain produces the enzyme in the presence of these two factors but the wild-type strain does not suggests that defective amino acid transport is additionally essential to the signaling process.

To determine whether the adaptive response

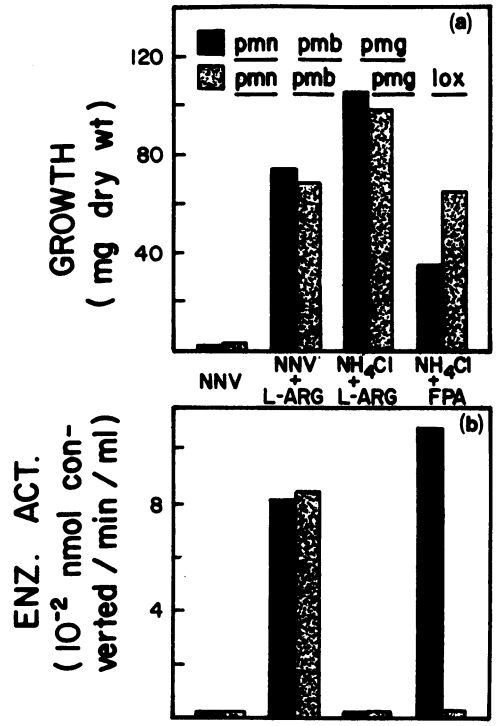

FIG. 2. Effect of the *lox* mutation on growth (a) and the production of extracellular deaminase (b) by the *pmn;pmb;pmg* strain in response to arginine or FPA. All media consisted of no-nitrogen Vogel medium (NNV) with additions as indicated: 15 mM L-arginine, 15 mM L-arginine plus 15 mM NH_4Cl, or 15 mM NH_4Cl plus 0.1 mM FPA. Growth and enzyme assays were conducted as for Fig. 1.

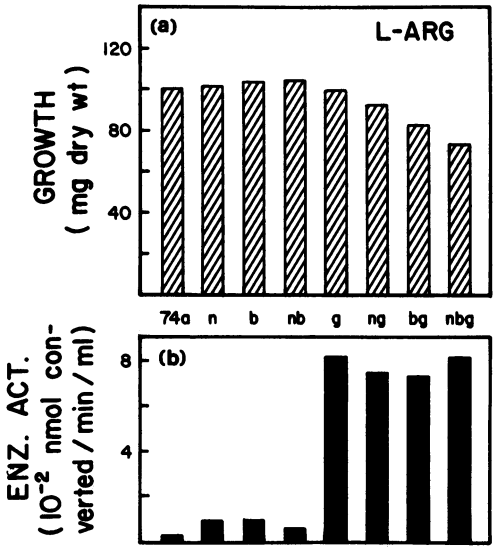

FIG. 3. Growth (a) and production of deaminase (b) by amino acid transport-defective strains in response to arginine. The strains are defective for amino acid transport activity as follows: wild type (74a), no defects; *pmn* (n), N system defective; *pmb* (b), B system defective; *pmn*;*pmb* (nb), N system defective and B system defective; *pmg* (g), G system defective; *pmn*;*pmg* (ng), N system defective and G system defective; *pmb*;*pmg* (bg), B system defective and G system defective; *pmn*;*pmb*;*pmg* (nbg), N system defective, B system defective, and G system defective. Growth and enzyme assays were conducted as for Fig. 1.

could be correlated with loss of a particular amino acid transport activity, the various transport mutant combinations were grown on arginine as the nitrogen source. All strains containing the *pmg* mutation produce the enzyme in response to arginine (Fig. 3). We conclude that all three factors are required for the adaptive response: nitrogen starvation (or the absence of ammonium ions), the presence of arginine, and the loss of general permease activity (or the presence of the *pmg* mutation).

We are currently examining the range of amino acids that can elicit enzyme production, the role of nitrogen starvation in the signaling process, and the contribution of the *pmg* mutation. The fact that the enzyme produced in response to arginine can deaminate phenylalanine suggests that the adaptive phenomenon is not arginine specific. Failure of lysine to elicit enzyme production might suggest that the phenomenon has a limited amino acid range, but we suspect that since lysine cannot support growth, possibly for secondary metabolic reasons, insufficient enzyme is produced for detection in our assay. The role of nitrogen starvation is most likely that of freeing the cell from nitrogen catabolite repression (8). Therefore, we are investigating whether the adaptive response is under control of the *nit-2* locus, which, in the absence of nitrogen catabolite repression, produces a positive effector that coordinately induces several nitrogen-procuring activities (14). To determine the contribution of the *pmg* mutation, we are isolating additional G system-defective strains. If an altered gene product is the key factor, the ability to elicit enzyme production should be locus specific. If inactivation of the G system per se is the critical factor, any mutation resulting in an inactivation of the G system should trigger enzyme production.

The signal transduction-adaptation response that we have observed with *N. crassa* is in principle similar to that described for several widely diverse systems: the interaction of hormones with specific cell surface receptors, neurotransmitters with synaptic receptors, antigens with immunoglobulin cell surface receptors, light with photoreceptors, and attractants and repellants with chemotactic receptors. A major limitation in studying such complex processes in higher organisms is the inability to dissect the process genetically. Microbial model systems such as the one we are developing with *N. crassa* have been very successfully applied to this area of biology because of their unique advantage of genetic manipulation (4, 5, 13).

LITERATURE CITED

1. **DeBusk, B. G., and A. G. DeBusk.** 1965. Molecular transport in *Neurospora crassa*. I. Biochemical properties of a phenylalanine permease. Biochim. Biophys. Acta **104**:139–150.
2. **DeBusk, R. M., and A. G. DeBusk.** 1980. Physiological and regulatory properties of the general amino transport system of *Neurospora crassa*. J. Bacteriol. **143**:188–197.
3. **DeBusk, R. M., D. T. Brown, A. G. DeBusk, and R. D. Penderghast.** 1981. Alternate mechanism for amino acid entry into *Neurospora crassa*: extracellular deamination and subsequent keto acid transport. J. Bacteriol. **146**:163–169.
4. **Foster, K. W., and R. D. Smyth.** 1980. Light antennas in phototactic algae. Microbiol. Rev. **44**:572–630.
5. **Koshland, D. E., Jr.** Biochemistry of sensing and adaptation in a simple bacterial system. Annu. Rev. Biochem. **50**:765–782.
6. **Lester, G.** 1966. Genetic control of amino acid permeability in *Neurospora crassa*. J. Bacteriol. **91**:677–684.
7. **Magill, C. W., S. O. Nelson, S. M. D'Ambrosio, and G. I. Glover.** 1973. Histidine uptake in mutant strains of *Neurospora crassa* via the general transport system for amino acids. J. Bacteriol. **113**:1320–1325.
8. **Marzluf, G. A.** 1981. Regulation of nitrogen metabolism and gene expression in fungi. Microbiol. Rev. **45**:437–461.
9. **Ogilvie-Villa, S., R. M. DeBusk, and A. G. DeBusk.** 1981. Characterization of 2-aminoisobutyric acid transport in *Neurospora crassa*: a general amino acid permease-specific substrate. J. Bacteriol. **147**:944–948.
10. **Pall, M. L.** 1969. Amino acid transport in *Neurospora crassa*. I. Properties of two amino acid transport systems. Biochim. Biophys. Acta **173**:113–129.
11. **Pall, M. L.** 1970. Amino acid transport in *Neurospora*

crassa. II. Properties of a basic amino acid transport system. Biochim. Biophys. Acta **203**:139–149.

12. **Rao, E. Y. T., T. K. Rao, and A. G. DeBusk.** 1975. Isolation and characterization of a mutant of *Neurospora crassa* deficient in general amino acid transport activity. Biochim. Biophys. Acta **413**:45–51.
13. **Ray, J., and R. A. Lerner.** 1982. A biologically active receptor for the carbohydrate-binding protein(s) of *Dictyostelium discoideum*. Cell **28**:91–98.
14. **Reinert, W. R., and G. A. Marzluf.** 1975. Genetic and metabolic control of the purine catabolic enzymes of *Neurospora crassa*. Mol. Gen. Genet. **139**:39–55.
15. **Roess, W. B., and A. G. DeBusk.** 1968. Properties of a basic amino acid permease in *Neurospora crassa*. J. Gen. Microbiol. **52**:421–432.
16. **Stadler, D. R.** 1966. Genetic control of the uptake of amino acids in *Neurospora*. Genetics **54**:677–685.
17. **Thwaites, W. M., and L. Pendyala.** 1969. Regulation of amino acid assimilation in a strain of *Neurospora crassa* lacking basic amino acid transport activity. Biochim. Biophys. Acta **192**:435–461.
18. **Vogel, H. J.** 1964. Distribution of lysine pathways among fungi: evolutionary implications. Am. Nat. **98**:435–446.
19. **Wolfinbarger, L., Jr., and A. G. DeBusk.** 1971. Molecular transport. I. *In vivo* studies of transport mutants of *Neurospora crassa* with altered amino acid competition patterns. Arch. Biochem. Biophys. **114**:503–511.
20. **Wolfinbarger, L., Jr., H. H. Jervis, and A. G. DeBusk.** 1971. Active transport of L-aspartic acid in *Neurospora crassa*. Biochim. Biophys. Acta **249**:63–68.