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ISOLATION AND CHARACTERIZATION OF A MUTANT OF *NEUROSPORA CRASSA* DEFICIENT IN GENERAL AMINO ACID PERMEASE ACTIVITY

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SUMMARY

A mutant of *Neurospora crassa* (*pm-nbg*^{27}) was isolated on the basis of its resistance to *p*-fluoro-phenylalanine on ammonium-deficient Vogel's medium. This mutant was found to be devoid of both conidial and post-conidial (after 180 min of preincubation) transport activity of all amino acids.

Genetic analysis of *pm-nbg*^{27} by crossing it to wild-type (74^{A}) resulted in the predicted segregants exhibiting transport characteristics of *pm-n*, *pm-b*, *pm-g*, *pm-nb*, *pm-ng*, *pm-bg* and parental types

The above observations confirm the postulated general amino acid permease system as well as a single genetic locus control of that activity

INTRODUCTION

For the last decade this laboratory has had the characterization of amino acid transport in *Neurospora crassa* as one of its major avenues of interest. During these years we, along with other laboratories, have been successful in isolating mutant strains deficient in the neutral amino acid transport system and in the basic amino acid transport system [1–7]. Mutants of the third major amino acid transport system, the general, have resisted numerous attempts at isolation by a variety of techniques.

Recently there has developed considerable interest in the regulatory and kinetic aspects of transport by the general amino acid transport system. In earlier studies, the presence of specific neutral (PmN) and basic (PmB) amino acid transport systems hindered analysis of this system. The availability of a double mutant strain (*pm-n*; *pm-b*) which is deficient in the two specific systems, has allowed study of the general system unhindered by other complicating transport systems [8]. We wish to report here the isolation and preliminary characterization of a mutant deficient in this, the general amino acid transport system, which has specificity for both neutral and basic amino acids.

Maintenance of high general (PmG) transport system activity by ammonia starvation was employed to isolate a *p*-fluoro-phenylalanine-resistant strain of the

already resistant *pm-n*; *pm-b*, a strain which, under these conditions, was sensitive to the analog. The isolated triple mutant *pm-n*; *pm-b*; *pm-g* was outcrossed to a wild-type and the single mutant *pm-g* segregant was isolated and characterized.

MATERIALS AND METHODS

Strains. The wild-type 74 OR23-1A was obtained from Fungal Genetic Stock Center, Arcata, California. The *pm-nb* double mutant was isolated as previously described by Wolfinbarger and DeBusk [8]. All stocks were maintained on Vogel's minimal medium (N) [9] with 2 % sucrose as carbon source and 2 % agar (Difco-Bactoagar) at 25 °C 7-day-old conidia were used in all experiments

Mutant isolation. Observations in this laboratory have indicated that PmG activity can be enhanced by ammonium starvation (Fig. 1). The enhanced transport activity restored a degree of sensitivity of *pm-n*, *pm-b* to the phenylalanine analog *p*-fluoro-phenylalanine (compared to routine growth on ammonia-supplemented media), thus making it possible for the isolation of mutants deficient for *pm-g* activity 7-day-old conidia from *pm-nb* (a double mutant lacking neutral and basic amino acid transport systems [8]) were irradiated with ultraviolet light and plated on KNO_3 Vogel's medium (equimolar concentrations of KNO_3 for NH_4NO_3 in Vogel's medium) containing 0.4 mM *p*-fluoro-phenylalanine. *pm-nbg*^{27} was isolated on the basis of its resistance to the phenylalanine analog

Transport studies Transport studies were performed as described by Wolfinbarger and DeBusk [8]. Radioactive amino acid was added to give a final concentration of 0.01 μCi/0 1 μmol per ml. Incorporation of the labeled compound was measured using a Beckman low β counter.

All radioactive amino acids (^{14}C uniformly labeled) used were of the L-form and were purchased from Schwartz, New York or International Chemical and Nuclear Corp., Calif. The non-radioactive amino acids, utilized as 'carrier' for the labeled amino acids or for competition studies, were also of the L-form and were obtained from Sigma Chemicals, St Louis, Mo

RESULTS

Observations in this laboratory have indicated that the presence of ammonium results in the disappearance of PmG system activity. This is illustrated by a growth experiment represented in Fig. 1. Wild-type (74^{A}) was found to be highly sensitive to the phenylalanine analog *p*-fluoro-phenylalanine both in NH_4Cl and KNO_3 Vogel's medium. The *pm-nb* double mutant still possessing residual transport activity for all amino acids through the general transport system, was resistant to *p*-fluoro-phenylalanine in NH_4Cl Vogel's medium. However, following derepression of the general transport system by ammonium starvation (KNO_3 Vogel's) *pm-nb* was found to be highly sensitive to *p*-fluoro-phenylalanine in KNO_3 medium. The *pm-n*; *pm-b*; *pm-g* triple mutant can therefore be characterized as resistant to *p*-fluoro-phenylalanine under both conditions (KNO_3 and NH_4Cl) for growth.

Amino acid transport experiments were performed to ascertain that the above-observed resistance of *pm-n*; *pm-b*; *pm-g* was due to deficiency in the transport of that particular amino acid and its analogs. Transport activity of neutral amino acids was

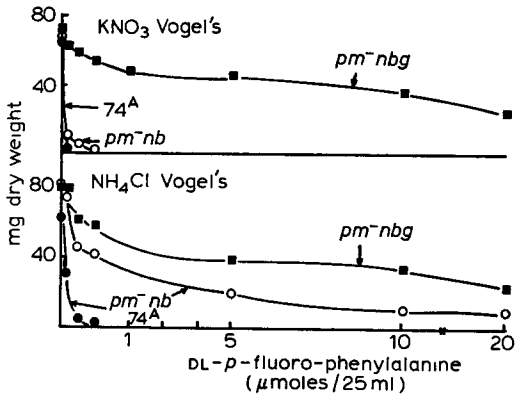

Fig. 1. Resistance to *p*-fluoro-phenylalanine in KNO_3 and NH_4Cl medium. Growth was measured in terms of dry weight in mg (A) after 3 days of incubation in KNO_3 Vogel's medium with 2% sucrose and specified concentrations of *p*-fluoro-phenylalanine, and (B) in a 3-days growth test in NH_4Cl Vogel's medium with 2% sucrose and specified concentrations of *p*-fluoro-phenylalanine.

measured using labeled phenylalanine (Fig. 2A). The triple mutant *pm-nbg*^{27} exhibits an almost negligible amount of transport of phenylalanine when compared to the parental strain *pm-nb*. Similarly labeled arginine was used to compare transport activity of basic amino acids, between *pm-nb* and *pm-nbg*^{27} (Fig. 2B). Again *pm-nbg*^{27} shows negligible transport of arginine compared to its *pm-nb* parent.

Neurospora crassa conidia were reported to possess [10] a post-conidial trans-

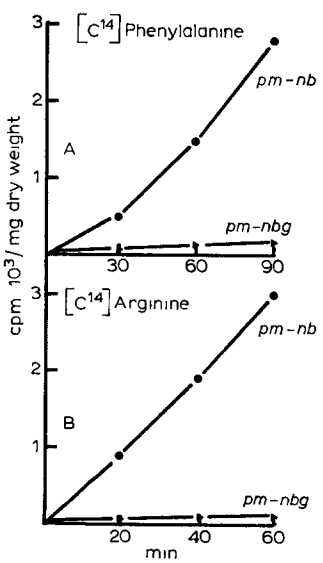

Fig. 2 (A) Transport of [^{14}C]phenylalanine Conidial transport of L-[^{14}C]phenylalanine was measured in *pm-nbg*^{27} and compared to transport in *pm-nb*. Uptake was initiated by adding labeled amino acid to the incubation medium (1 × Vogel's) with 0.1 mg/ml concentration of conidia (B) Transport of [^{14}C]arginine. Conidial transport of L-[^{14}C]arginine in *pm-nb* and *pm-nbg*^{27} was compared.

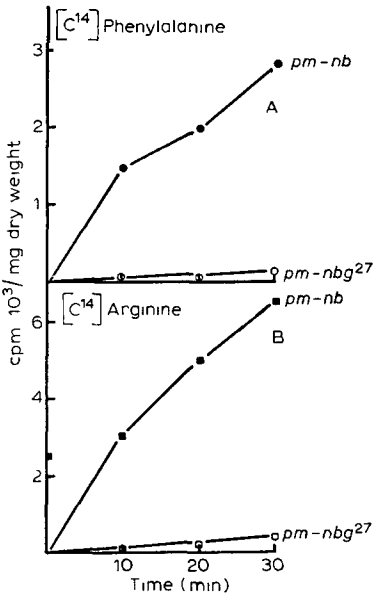

Fig 3. (A) Post-conidial transport of phenylalanine. *pm-nb* and *pm-nbg*^{27} conidia were incubated in 1 × Vogel's medium for 180 min with 1 % glucose Uptake of L-[^{14}C]phenylalanine was initiated by the addition of labeled amino acid (B) Post-conidial transport of arginine Post-conidial transport of arginine was measured and compared for *pm-nb* and *pm-nbg*^{27} by the addition of labeled arginine to conidia that were preincubated in 1 × Vogel's medium with 1 % glucose

port activity following incubation for 180 min in Vogel's minimal medium (N) plus glucose as carbon source. A 10-fold amplification of transport activity was observed, due mainly to the activity of the general transport system. Transport experiments with

TABLE I

SINGLE POINT UPTAKE ASSAY FOR SCREENING ISOLATES FROM A CROSS, 74^{A} AND *pm-nbg*^{27}

0 5 mg cells were incubated for 90 min with 1 % glucose in Vogel's medium containing either [^{14}C]-phenylalanine or [^{14}C]arginine or [^{14}C]arginine with 10 times unlabeled phenylalanine. The numbers represent cpm/mg dry weight of conidia

Strain	[^{14}C]Phenylalanine	[^{14}C]Arginine	[^{14}C]Arginine plus unlabeled phenylalanine	
74^{A}	1708	3375	2843	(16 %)*
pm-n	783	3946	3041	(33 %)
pm-b	1783	1480	283	(81 %)
pm-nb	664	1251	326	(74 %)
pm-nbg^{27}	71	112	89	(20 %)
pm-ng	79	2436	2878	(+19 %)
pm-bg	1156	200	83	(59 %)
pm-g	1588	2533	2330	(8 %)

* Values in parenthesis represent the degree of reduction, as a percentage value, of arginine transport by excess phenylalanine

both neutral and basic amino acids (Fig. 3) were performed to determine whether or not the deficiency in general amino acid transport activity was limited to the conidial stage. The triple mutant shows little transport of phenylalanine (Fig. 3A) and arginine (Fig. 3B), compared to the parental strain *pm-nb*. In order to confirm a genetic control of general amino acid permease activity (PmG), a genetic analysis of the mutant *pm-nbg*^{27} was made by crossing it to the wild-type strain (74^{A}). From this cross, single spore colonies were isolated and tested by a 5-ml end-point assay for labeled amino acid transport (phenylalanine and arginine representing neutral and basic amino acids, respectively). Activity of the general system was measured by determining the degree of competition for transport between neutral and basic amino acids (e.g. labeled arginine and 10 times the concentration of unlabeled phenylalanine). By comparing these competition patterns, eight different types of isolates were identified from the above-mentioned cross (Table I). They represent wild type, *pm-nbg*, *pm-n*, *pm-b*, *pm-g*, *pm-nb* and *pm-bg*. These observations confirmed that the activity of the general transport system was under the control of a single locus. One of the isolates obtained from the above cross (*pm-g*^{23}) was examined further for its transport of neutral and basic amino acids. Fig. 4B illustrates transport activity of neutral and basic amino acids and their competition patterns in *pm-g*^{23} as compared to wild-type (74^{A}) (Fig. 4A). In the mutant, 10-fold greater concentration of unlabeled phenylalanine and unlabeled arginine fail to compete for respective transport of labeled arginine and phenylalanine. The failure to obtain competition for transport indicates that the *pm-g* lacks the PmG system of transport activity for neutral and basic amino acids by the general transport system.

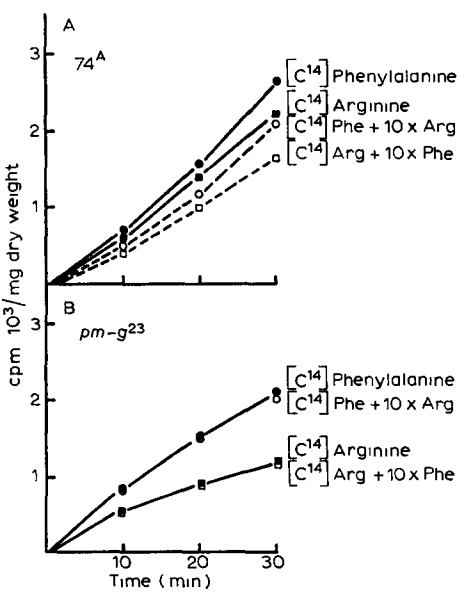

Fig 4. Transport and competition patterns for neutral and basic amino acids in 74^{A} (wild type) and *pm-g*^{23}. Conidial transport of neutral and basic amino acids was assayed using [^{14}C]phenylalanine and [^{14}C]arginine, respectively, in 74^{A} (wild type) (4A) and *pm-g*^{23} (4B). Competition patterns for neutral and basic amino acids were established by using a 10-fold concentration of competing amino acid (unlabeled arginine and unlabeled phenylalanine, respectively).

DISCUSSION

Genetic control of amino acid transport has been clearly established in yeast [11–16] and Neurospora [2, 5, 7, 8] by isolating mutants lacking particular transport activities. The deficiency is further related to the absence of a single gene product. Wolfinbarger and DeBusk [8] have isolated and characterized mutants lacking neutral amino acid permease activity (*pm-n*) and basic amino acid permease activity (*pm-b*). Stuart and DeBusk [17] have examined amino acid-binding glycoproteins using CNBr affinity column chromatography. The permease mutants were shown to lack certain fractions of these binding molecules. Thus the binding affinities of these complexes are shown to be under genetic control. However, efforts in isolating mutants in general amino acid permease activity have not so far been successful Thus it was not possible to show the control of general amino acid permease activity under the influence of a single gene product

Grenson et al. [11–14] have made extensive study of several amino acid transport systems in *Saccharomyces cerevisiae* They have reported that the physiological function of the general amino acid permease in yeast is probably to provide the cells with amino acids as nitrogen source [14, 15]. They were of the opinion that this system should be considered with general regulation of nitrogen metabolism. This was supported by the observation that ammonia inhibits the activity of the general amino acid permease

N crassa and *S. cerevisiae* exhibit similar amino acid transport and metabolic activities Preliminary studies in this laboratory using *N crassa* have confirmed the observations made in yeast Activation of the general amino acid transport system by ammonium starvation made it possible to select for *pm-g* mutants. The mutant *pm-nbg*^{27} obtained by its resistance to *p*-fluoro-phenylalanine on KNO_3 medium was found to meet all the criteria for a general transport-deficient lesion

The triple mutant *pm-nbg*^{27} shows negligible transport of several neutral and basic amino acids These observations were consistent with the observed resistance to *p*-fluoro-phenylalanine.

The lesion in the general amino acid permease system was not limited to conidia Even after 180 min of preincubation, the cells were still devoid of any transport activity (post-conidial transport [10]) for both neutral and basic amino acids

Genetic control of the general amino acid permease system was clearly established by the isolation of *pm-g* mutant from *pm-nbg*^{27} The *pm-g*^{23} mutant exhibits transport of phenylalanine and arginine by systems specific for neutral and basic amino acids, respectively. Lack of competition by 10-fold greater concentrations of competing amino acids clearly indicate the loss of general amino acid permease activity The genetic analysis also confirms a single genetic locus control for the general amino acid permease system

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REFERENCES

1 DeBusk, B. G and DeBusk, A. G (1965) Biochim Biophys. Acta 104, 139–159
2 Lester, G. (1966) J. Bacteriol. 91, 677–684
3 Luc, Nang-Ly (1967) Master's Thesis, Florida State University
4 Roess, W. B (1966) Ph. D. Dissertation, Florida State University
5 Roess, W B and DeBusk, A. G. (1968) J. Gen Microbiol. 52, 421–432
6 Thwaites, W. M and Pendyala, L. (1969) Biochim. Biophys. Acta 192, 455–461
7 Pall, M L (1970) Biochim Biophys Acta 211, 513–520
8 Wolfinbarger, L. and DeBusk, A G (1971) Arch Biochem. Biophys 144, 503–511
9 Vogel, H J (1956) Microbiol Genet. Bull 13, 42
10 Tisdale, J. H. and DeBusk, A G (1970) J. Bacteriol 104, 689–697
11 Gitts, J. J. and Grenson, M. (1967) Biochim Biophys Acta 135, 507–516
12 Grenson, M , Wiame, J M. and Becket, J (1966) Biochim Biophys Acta 127, 325–338
13 Grenson, M (1966) Biochim Biophys Acta 127, 339–346
14 Grenson, M. and Hou, C (1972) Biochem. Biophys Res. Commun. 48, 749–756
15 Surdin, Y. W , Sly, J., Sire, J , Bordes, A. M and De Robichon-Szulmajster, H. (1965) Biochim. Biophys Acta 107, 546–556
16 Stuart, D W and DeBusk, A G. (1971) Arch Biochem Biophys. 144, 512–518