Rao, E. and A. G. DeBusk. A mutant of Neurorpora deficient in the general (Pm G) amino acid transport system.

Studier from a "umber of laboratories, including our own, have led to the view that three major, overlapping amino acid transport systems exist in Neurorpora. All three systems hove bee" established on the basis of kinetic competition analysis (Poll 1969 Biochim. Biophys. Acta 173: 113). However, only two systems, the neutral transport system (Pm N, mtr, system I, etc.) and the basic transport system (Pm B, bat, can^{R}, etc.) have bee" established on genetic grounds by the isolation of corresponding mutants. Representative mutants have bee" isolated in this laboratory and their transport characterized in conidia (Wolfinbarger and DeBusk 1971 Arch. Biochem. Biophys. 144: 503) and early stages of conidial development (Tisdale and DeBusk 1970 J. Bacteriol. 104: 689). Both the neutral (Pm N) and basic (Pm B) mutants still retain considerable residual transport in the early developmental stages. This residual transport in such mutants can be accounted for by an overlapping system referred to as the general

transport system (Pm G). Competition for Pm G transport occurs between all amino acids, (e.g., neutral by boric or neutral) rather than only between members of the neutral (e.g. , phe by leu) or basic (e.g., lys by arg) classes. However, in spite of an extensive effort, no mutants uniquely involving the general transport system were isolated.

It eventually became clear why a transport-deficient mutant such as pm-nb (neutral-boric double) was analog-resistant in spite of the presence of an intact general system. The general system is subject to regulation by ammonium ions, such as that provided from NH_4NO_3 of Vogel's medium N. If a modified KN03 Vogel's is employed, pm-nb again becomes sensitive to amino acid analogs. It is therefore simple to select for a second resistant locus. The mutant reported here was isolated from a pm-nb (neutral-basic deficient) parent after UV irradiation and plating on ammonium-deficient Vogel's medium. The growth responses to fluorophenylalanine of wild type (strain 74A), the pm-nb parent and the triple mutant, pm-nbg, are shown in Figure 1. This figure also reveals the basis for the selection of the general transport-deficient mutant discussed above.

One such isolate was chosen ($pm\text{-}nbg^{27}$), crossed to 74A and the various recombinants types recovered as single spore isolates. Screening of isolates can best be carried out with transport assay and the results of such a screening are shown in Table 1. The relative transport of a neutral (phe) and a basic (arg) amino acid are shown. The competition between phe and arg allows detection of the presence or absence of overlapping general activity in the various recombinants. It may be seen that very little transport activity remains in pm-nbg for either amino acid.

Figure 1.

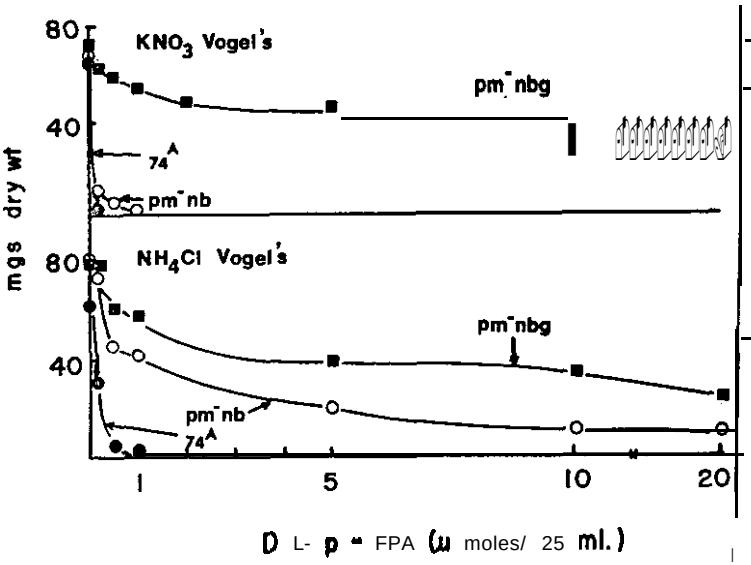

Table 1. Single point uptake assay for screening isolates from a cross between wild type strain 74A and pm-nbg27.

Strains	^{14}C-phe	^{14}C-arg	^{14}C-arg + ^{12}C-phe
74A (wild type)	1708	3375	2843
pm-n	783	3956	3041
pm-b	1783	1480	283
pm-nb		1251	
$pm\text{-}nbg^{27}$	664	112	326 89
pm-ng	79	2436	2878
pm-bg	1156	200	83
pm-g	1588	2533	2330

'0 5 mg cells were incubated for 90 minutes with 1% glucose /in 1X Vogel's medium containing either ^{14}C-phenylalanine or ^{14}C-arginine or ^{14}C-arginine with 10 X ^{12}C-phenylalanine (specific activity of ^{14}C-amino acids was 0.01 uci/0.1 μmoles/ml). The numbers represents counts/minute/mg. dry weight of conidia.

More extensive studier support the preliminary results presented and further verify the isolation of a general transport-deficient mutant (pm-g). It shows tight linkage to mating type (L.G. I) and is th erefore unlinked to the pm-n locus (L. G. IV) or pm-b (L. G. V). Further studies with the new transport mutant will be reported elsewhere. - - - Genetics Group, Deportment of Biological Sciences, Florida State University, Tallahassee, Florida 32306.