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MUTATIONS IN *NEUROSPORA CRASSA* WHICH AFFECT MULTIPLE AMINO ACID TRANSPORT SYSTEMS

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SUMMARY

Characterization of a double mutant, *his-6: hgu-4*, which is unable to utilize L-histidyl-glycine as a source of histidine has revealed a new locus on linkage group V. The *hgu-4* genotype results in a generalized reduced transport activity for amino acids, with a concomitant increased resistance to amino acid analogs. Transport rates and analog resistance for amino acids by this mutant are compared to the previously reported transport deficient mutants *fpr-1*, *nap* and *un-3*.

Transport of L-aspartate as a function of temperature is examined in a variety of transport deficient strains in an attempt to explain the mode of action of mutation which pleiotropically affect several genetically and biochemically distinct amino acid transport systems.

INTRODUCTION

In recent years a great many studies of amino acid transport in *Neurospora crassa* have resulted in isolation of mutations affecting specific amino acid transport systems, e.g. *mtr* and *pm-n* (defective in neutral amino acid transport [1, 2], *bat* and *pm-b* (basic amino acid transport) [3, 2] and recently *pm-g* (general amino acid transport system) [4]. These mutations have been valuable in that they have permitted the elucidation of individual amino acid transport systems.

A second class of transport-defective mutations has also been described, *fpr-1* [5], *nap* [6] and *un*-3 [7], which do not appear to affect specific systems, but instead reduce the overall rate of transport of a variety of metabolites; their importance for understanding amino acid transport activity by the individual systems has not been fully appreciated. The question must be asked, what component, or components, might transport systems have in common, and how might a mutational (or physiological) alteration of this component affect the individual transport systems?

We recently reported [8] the isolation (in a *his-6* auxotrophic background) of a mutant of *Neurospora*, designated *hgu-4*, which cannot utilize several histidine-containing dipeptides, such as L-histidyl-glycine, as sources of histidine. Further characterization of this mutant is reported here and *hgu-4* is shown to be phenotypi-

cally similar to both *fpr-1* and *nap*, located on the same linkage group (L.G. V), and yet genetically distinct. The *hgu-4* mutant is shown to be reduced approximately 50 % in transport of all amino acids tested and resistant to most amino acid analogs.

The transport deficiency is attributable to V_{max} differences for neutral and basic amino acid transport and K_m changes (or lack of change) for acidic amino acid transport. The *hgu-4* lesion does not directly alter a specific transport system but rather may affect the activity and development of several amino acid transport systems. The effects of temperature on acidic amino acid transport and the possibility of interaction(s) between the neutral and general amino acid transport systems are also examined and discussed.

MATERIALS AND METHODS

Strains and growth conditions. The mutant strains (*fpr-1*, *nap* and *un-3*) were obtained from the Fungal Genetics Stock Center, Arcata, California.

The wild-type (Tatum, $SY4f_8a$) *pm-n* and *hgu-4* were from stocks maintained at Ohio State University. A stock of the *hgu-4* mutant has been deposited with the Fungal Genetics Stock Center (FGSC # 2734). Isolation of a mutant deficient in the general amino acid transport system (*pm-g*) was recently reported [4]. The mutant strain *pm-g* was generously supplied by Edith and T. K. Rao.

The media employed for growth assays was Vogel's minimal medium N [9] plus 2 % (w/v) sucrose and supplemented with growth requirements or antimetabolites as indicated in the text.

Growth assays. To determine resistance to antimetabolites, growth tests were carried out in 125 ml Erlenmeyer flasks containing 31 ml of liquid minimal medium plus various concentrations of amino acid analogs. The flasks were inoculated with 1 to 2 drops of a nonturbid (approximately 2000 to 3000 conidia/ml) conidial suspension and incubated stationary and in the light at 30 °C for 3 days. After this growth period, each mycelial mass was harvested, pressed dry of excess moisture, and dried to a constant dry weight prior to weighing. The data were recorded as milligrams (dry weight) per mycelial pad.

Genetic data. The genetic data was obtained by standard procedures (see ref. 10).

Transport assays. Conidia for transport assays and germination (for cells employed in mycelial transport assays) were obtained as previously described [2]. Conidia were germinated in Vogel's minimal medium N supplemented with 20 mM potassium acetate (as sole carbon source) for either 6 h or 15 h prior to filtering and washing [11]. Transport assays were conducted as previously described [2] and measured initial transport velocities.

Amino acid pools. The analysis of amino acid pools was performed using standard techniques and applying the sample on a Beckman 121 amino analyzer in a buffer (Beckman) containing a nor-leucine standard. Chemicals, the radioactive amino acids were obtained from either Schwartz/Mann or New England Nuclear and were uniformly labeled with ^{14}C. The [^{14}C]acetate was labeled in the carboxyl carbon and was obtained from New England Nuclear, Boston, Mass. The specific activities of radioactive substrates employed in the transport assays were 0.1 Ci/1.0 mol.

The amino acid analogs were obtained from Sigma or Calbiochem Chemical Companies and were of the highest purity available.

RESULTS

Mapping data

It was previously reported that *hgu-4* and *his-6* are both located on linkage group V [8]. The discovery that the *hgu-4* mutation confers resistance to D-glutamate provided a convenient means of scoring for the locus in the absence of a histidine requirement. To test whether the *hgu-4* mutation was responsible for both resistance to D-glutamate and the inability to grow on L-histidyl-glycine, the *his-6* : *hgu-4* double mutant was crossed to wild type and the prototrophic progeny scored for the expected phenotypes. To determine growth on L-histidyl-glycine (or L-histidine), 3-amino-1,2,4-triazole (0.5 mg/ml) was added to induce a requirement for histidine in the prototrophs. Of 99 prototrophic progeny examined, 35 were sensitive to D-glutamate (3.3 mM) and grew on L-histidyl-glycine (0.33 mM) and 64 were resistant to D-glutamate and unable to grow on the dipeptide. No progeny sensitive to D-glutamate and unable to utilize histidyl-glycine were observed, suggesting that both phenotypes are a consequence of the *hgu-4* mutation.

Mapping of the *hgu-4* locus to *fpr-1* and *nap* was performed by standard genetic methods; their arrangement on chromosome V as well as approximate map distances are given in Fig. 1. We do not know whether the presence of the three loci on the same linkage group is significant, but the finding of three separate mutants

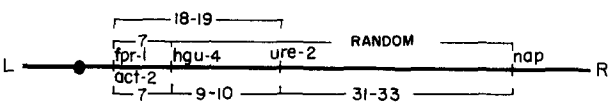

Fig. 1. Map of linkage group V showing map distances as percentage recombination between the respective loci. The map is not drawn to scale.

TABLE I

GROWTH ASSAYS ON VARIOUS AMINO ACID ANALOGS

Growth data as mg dry weight mycelial pad following 3-days growth at 30 °C as described in the text. All growth values were corrected to 100 mg dry wt. on minimal medium to facilitate comparison.

Analog*	Wild type	*fpr-1*	*hgu-4*	*nap*
None	100	100	100	100
D-Glutamate	4	79	82	78
PFPA	7	41	22	41
L-Ethionine	0	31	8	44
4-MTR	2	12	55	12
L-Canavanine	0	90	0	30
Thiosine	24	72	25	52

* Analogs at concentrations indicated: D-glutamate, 16.5 mM; *p*-fluorophenylalanine (PFPA), 0.016 mM; L-ethionine, 1.65 mM; D- L-4-methyl-tryptophan (MTR), 0.16 mM; L-canavanine sulfate, 0.0066 mM; thiosine (S-2-(amino-ethylcysteine), 3.3 mM.

with such similar phenotypes, each isolated by different selection techniques, is of considerable interest.

Analog resistance

Referal to Table I reveals that *fpr-1*, *hgu-4* and *nap* are resistant to the growth inhibitory properties of the amino acid analogs D-glutamate, D, L-parafluorophenylalanine, L-ethionine and 4-methyltryptophan. The sensitivity of *hgu-4* to L-canavanine sulfate and thiosine correlates with its capacity to transport arginine in conidia (discussed later), and the resistance of *nap* and *fpr-1* to these analogs is consistent with published reports [12].

Transport of amino acids

The resistance of *hgu-4* to D-glutamate, ethionine, 4-methyltryptophan and parafluorophenylalanine is correlated with an almost 50% reduction in L-aspartic acid, D-glutamic acid and L-phenylalanine transport in this mutant when compared to the wild type (Table II). This reduction in transport was found in conidia, 6 h old mycelia, and 15 h old mycelia. Older *hgu-4* mycelia (26 h growth in acetate minimal medium) displayed an even more reduced (approximately 80–90%) transport rate for L-aspartate and D-glutamate than found in younger mycelia (data not given).

It should be noted (Table II) that *hgu-4* conidia were not reduced in transport of L-arginine whereas mycelia grown 6 h in acetate minimal medium were reduced approximately 50%. The ability of conidia of *hgu-4* to transport arginine correlates well with the observed sensitivity of this mutant to the arginine analog, L-canavanine sulfate, and thiosine (Table I).

TABLE II

TRANSPORT ASSAYS OF WILD TYPE AND *hgu*-4 WITH VARIOUS AMINO ACIDS VERSUS GROWTH STAGE

Transport assays as described in Materials and Methods section of text. The amino acids L-arginine and L-phenylalanine and acetic acid were assayed at pH 5.8 whereas L-aspartate and D-glutamate were assayed at pH 3.8. All labeled substrates were at 0.1 mM concentrations.

Amino acid	Conidial	6 h mycelia*	15 h mycelia*
Wild type			
L-Arginine	0.45** ±0.03	3.45 ±0.21	–
L-Aspartic acid	2.90 ±0.17	5.40 ±0.32	–
L-Phenylalanine	0.80 ±0.05	8.80 ±0.53	–
D-Glutamic acid	–	2.15	4.10
Acetic acid	–	3.20 ±0.19	–
hgu-4			
L-Arginine	0.55 ±0.04	1.65 ±0.12	–
L-Aspartic acid	1.50 ±0.10	1.90 ±0.13	–
L-Phenylalanine	0.40 ±0.03	3.45 ±0.24	–
D-Glutamic acid	–	0.90	2.15
Acetic acid	–	2.30 ±0.16	–

* Stage of development as described in the text.

** Transport values as nmol/min/1.0 mg dry wt. cells ±S.E.

The *hgu-4* mutant is reduced approximately 30 % in transport of acetate. However, this reduction in initial transport velocity does not significantly alter this strains ability to grow on acetate as its sole carbon source, it's growth is comparable to that of wild type.

The analog resistance and transport data suggests that the *hgu-4* genotype results in a pleiotropic reduction in amino acid transport activity. Similar phenotypes have been reported for *fpr-1* (ref. 5 and Sadler, R. and DeBusk, A. G., personal communication), *un-3* [7, 13], and *nap* [6, 12] and thus the *hgu-4* phenotype is not new or unique. The reason for the transport deficiency of *nap* is unknown, but in addition to amino acids, *nap* is reduced in transport of other metabolites, e.g. glucose and uridine [12].

The *un-3* mutant (variously designated *un-t* [7] or 5570 lt [7, 13]) was originally isolated as a temperature-conditional irreparable mutant [14] which was subsequently reported to be a generalized membrane-defective mutant [7] and later a developmental mutant [13]. Kappy and Metzenberg [7] postulated the possibility of mutations which might pleiotropically affect multiple transport systems and studied *un-3* as a candidate for such a mutant. They reported that protoplasts of *un-3* were more osmotically fragile than those of the wild type. This mutant strain was also shown to be a developmental mutant such that the amino acid transport deficiency was more fully expressed following germination of the conidia [13].

The *fpr-1* mutant has also been examined in some detail and it is presently thought to result in an overproduction of amino acids. The higher intracellular concentration(s) of amino acids apparently reduce amino acid transport by "trans-inhibition" [15, 16] of the individual amino acid transport systems (Sadler, R. and DeBusk, A. G., personal communication). To determine whether such a mechanism could be involved in the transport deficiency of *hgu-4*, the concentrations of endogenously synthesized amino acids were examined in the wild type and *hgu-4* strains. With the possible exception that the glutamate, alanine, and arginine pool values were somewhat lower than found in the wild type, no significant pool differences between wild type and *hgu-4* were found for mycelia grown for 6 (or 18) h in minimal medium. This finding would seem to rule out any possibility that *hgu-4* acts similarly to *fpr-1*.

The kinetic aspects of amino acid transport by *hgu-4* mycelia were examined. The transport of L-phenylalanine (Fig. 2) and L-arginine (Fig. 3) revealed dramatic changes in V_{max} values and only minor differences in apparent K_m values. The calculated V_{max} values for L-phenylalanine transport by wild type and *hgu-4* were 18.0 ± 0.9 and 5.5 ± 0.3 nmol $\cdot$ min^{-1} $\cdot$ mg dry wet cells^{-1}, respectively. The corresponding K_m values were 0.056 ± 0.006 and 0.027 ± 0.004 mM. The calculated V_{max} values for L-arginine transport were 6.8 ± 0.3 nmol $\cdot$ min^{-1} $\cdot$ mg dry wt. cells^{-1} for wild type and 3.8 ± 0.2 nmol $\cdot$ min^{-1} $\cdot$ mg dry wt. cells^{-1} for *hgu-4*. Again the corresponding values for the apparent K_m values were not substantially different, being 0.003 ± 0.0004 and 0.001 ± 0.0003 mM for wild type and *hgu-4*, respectively (Table III). Just the opposite results were obtained when the kinetics of L-aspartate transport (at pH 3.8, corresponding to the optimal pH for transport of this amino acid [17]) were examined (Fig. 4). For this amino acid the V_{max} values obtained for the wild type and *hgu-4* were identical: 12.3 nmol $\cdot$ min^{-1} $\cdot$ mg dry wt. cells^{-1}. The apparent K_m values however differed by an order of magnitude, i.e., 0.18 mM for wild type and 1.1 mM for *hgu-4* (Table III). The apparent lack of agreement between L-aspartate

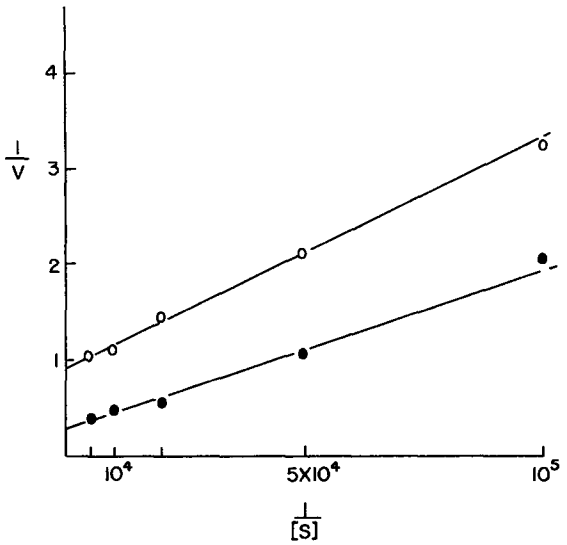

Fig. 2. Double reciprocal plot of L-phenylalanine transport, at pH 5.8, by wild type (●–●) and *hgu-4* (○–○). The cells have been germinated 6 h in potassium acetate minimal medium prior to the assays for transport activities (see Materials and Methods). Velocity (v) is nmol · min^{-1} · mg dry wt $cells^{-1}$ and substrate concentration [S] is molar.

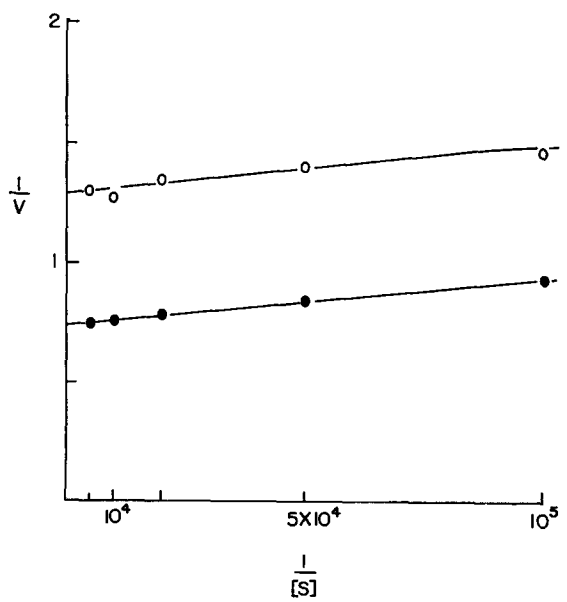

Fig. 3. Double reciprocal plot of L-arginine transport at pH 5.8 by wild type (●–●) and hgu-4 (○–○). Conditions of assay as described in the legend to figure 2.

TABLE III

KINETIC CONSTANTS (K_m AND V_{max}) OF AMINO ACID TRANSPORT BY WILD TYPE AND *hgu-4*.

	K_m**	V_{max}***
Wild Type*		
L-Phenylalanine	0.056 ± 0.006	18.0 ± 0.9
L-Arginine	0.003 ± 0.0004	6.8 ± 0.3
L-Aspartate	0.18 ± 0.02	12.3 ± 0.7
*hgu-4**		
L-Phenylalanine	0.027 ± 0.004	5.5 ± 0.3
L-Arginine	0.001 ± 0.0003	3.8 ± 0.5
L-Aspartate	1.1 ± 0.1	12.3 ± 1.0

* The cells were germinated for 6 h on acetate minimal medium prior to assay of transport activity. Transport of L-phenylalanine and L-arginine was assayed at pH 5.8 while transport of L-aspartate was assayed at pH 3.8. S.E. are given as ± values.

** The K_m values are expressed as mM.

*** The V_{max} values are expressed as nmol · min^{-1} · mg dry wt. cells^{-1}.

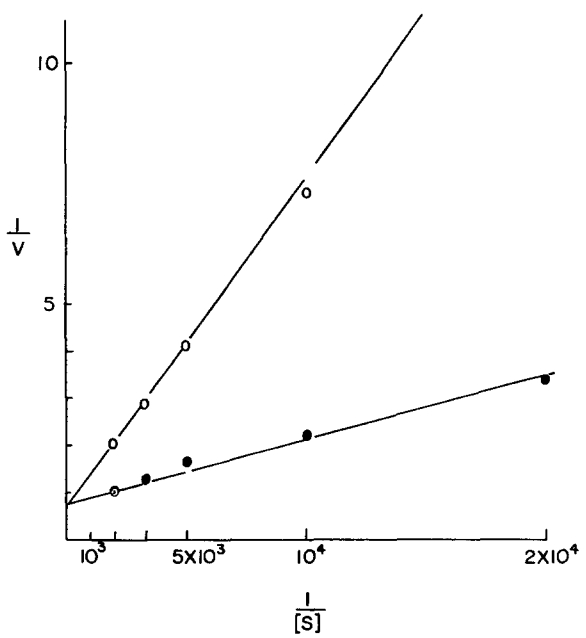

Fig. 4. Double reciprocal plot of L-aspartate transport, at pH 3.8, by wild type (●–●) and *hgu-4* (○–○). Conditions of assay as described in the legend to Fig. 2.

and L-arginine transport kinetics might be explained by the presence of an additional system, the basic amino acid transport system, by which L-aspartate is not transported. However, L-aspartate and L-phenylalanine are presumably [17] transported by the neutral and general transport systems and the dramatic change in K_m values for L-aspartate transport without a concomitant a large change in K_m for L-phenylalanine transport, is not at present clearly understood. The different K_m value (1.1 mM) found for L-aspartate transport by *hgu-4* does however explain the reduction in L-aspartate transport reported in Table II. The 0.1 mM concentration of L-aspartate employed in these transport assays would be significantly below saturation levels for transport activity.

The question now arises, how might a single gene mutation pleiotropically affect three genetically distinct amino acid transport systems? A plethora of published reports [7, 12, 18, 19] have suggested some "common component" that is required by the different transport systems in order to possess full activity. One report even suggested a single "allosteric" permease for amino acid transport in *Neurospora* [20].

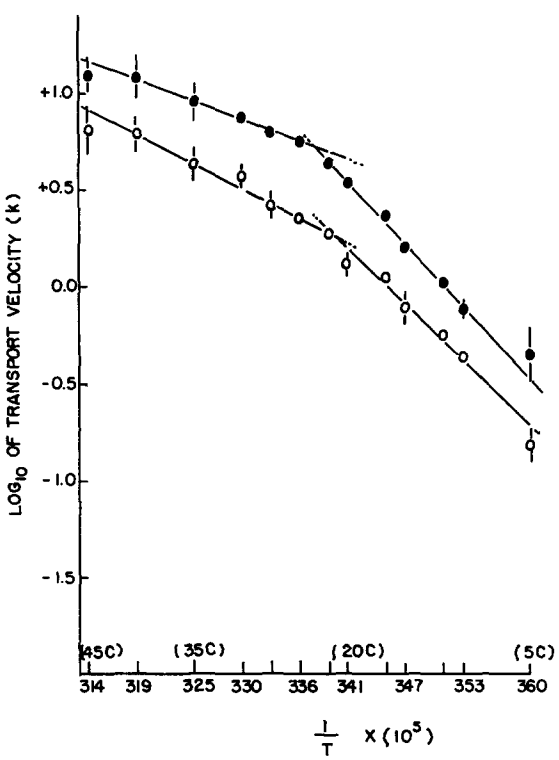

Fig. 5. Arrhenius plot of L-aspartate transport, pH 3.8, by wild type (●–●) and *hgu-4* (○–○) as a function of temperature. Transport velocity was nmol · min^{-1} · mg dry wt $cells^{-1}$. The temperatures in degrees centrigrade, given in parentheses were included for reference points only. The error bars passing vertically through the experimental points describe the deviation from the mean of two, or more, independent determinations. If a point does not possess an error bar, the circle size simply exceeded its length. The slope of the best-fit straight line was calculated by a least squares analysis using a Texas Instrument SR-51A pocket calculator. The cells were grown for 6 h at 28 °C prior to the assays (see text for details).

One component common to the three transport systems considered in this study is the membrane matrix within which each system must function. Alteration of a fatty acid desaturase in *hgu-4* might result in a greater percentage of saturated fatty acids in its membranes. One phenoytpic expression of such a lesion might be a phase transition [21, 22], i.e. order-disorder transition, occuring at higher temperatures [23]. A membrane in its "gel", as opposed to "fluid", state would presumably restrict activity of a membrane localized protein, particularly if that protein functioned by undergoing a conformational change to permit passage of a transported substrate. Such a restriction could lower transport velocities or cause an altered apparent affinity of the system for substrate.

Numerous studies [21, 24], primarily in bacterial systems, have described biphasic Arrhenius plots (logarithm of rate of substrate transport vs. the reciprocal of the absolute temperature of transport assay) with intersects (transition points) occuring at variable temperatures. Variations in the temperature at which the intersects occur may be correlated with the fatty acid supplement supplied to a fatty acid auxotroph [24]. If *hgu-4* were to differ from wild type in the chemical content of the lipid portion of its membrane, an Arrhenius plot for amino acid transport might reveal differing slopes or intersect(s).

Fig. 5, 6 and 7 present Arrhenius plots for L-aspartate transport by the wild type and various transport deficient mutants (comparative transport velocities for L-aspartate transport by each of these strains are given in Table IV). The plots for wild-type and *hgu-4* (Fig. 5) are clearly biphasic, having two slope components, with

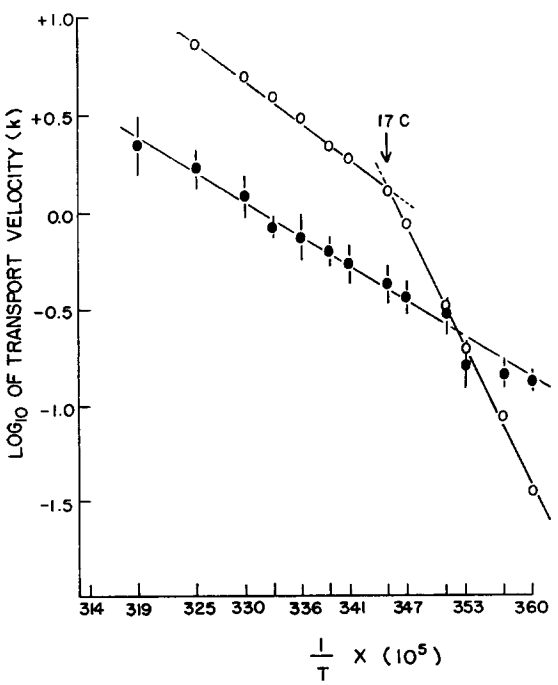

Fig. 6. Arrhenius plot of L-aspartate transport, pH 3.8, by *pm-n* (○–○) and *pm-g* (●–●) as a function of temperature. Assay conditions as described in the legend to Fig. 5.

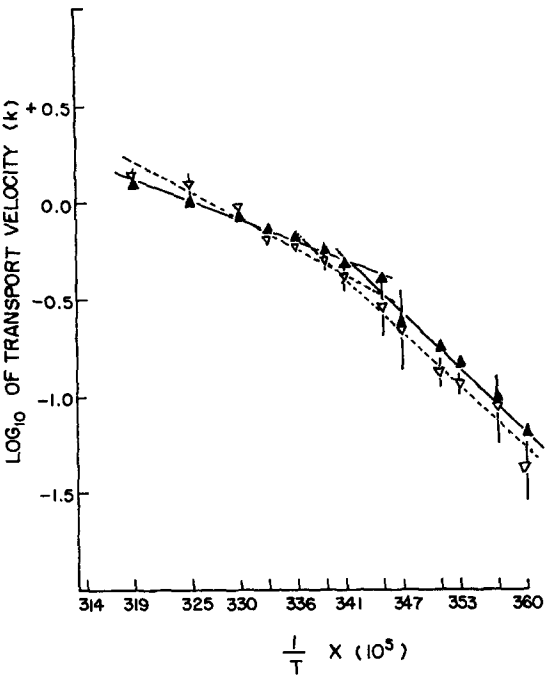

Fig. 7. Arrhenius plot of L-aspartate transport, pH 3.8, by *nap* (▽–▽) and *un-3* (▲–▲) as a function of temperature. Assay conditions as described in the legend to Fig. 5.

intersects occuring at 20–22 °C. These results are similar to those of previously reported bacterial studies [21, 24] and the transition point may represent a membrane phase transition. Alternatively, because of the presence of two separate transport systems the transition point may be attributable to changes in relative transport rate of aspartate by these systems as the temperature changes. If the former interpretation is correct, Arrhenius plots of L-aspartate transport by the neutral or general amino acid transport systems alone should also exhibit two slope components with an intersect (presumably at the same temperature as observed with the wild type). Transport

TABLE IV

TRANSPORT VELOCITIES (nmol/min/mg DRY WT. CELLS) OF L-[^{14}C]ASPARTATE (0.1 mM) at pH 3.8 BY WILD TYPE* AND VARIOUS TRANSPORT DEFICIENT MUTANTS

Strain	Velocity
Tatum (wild type)	5.95
hgu-4(4)a	1.90
nap	0.93
un-3	0.87
pm-n	4.96 ≈ 83 % of total
pm-g	0.95 ≈ 17 % of total

* Cells preincubated for 6 h on acetate minimal medium prior to transport assays (see Materials and Method section).

of L-aspartate by the neutral amino acid transport system (assayed in the *pm-g* mutant strain) as a function of temperature reveals a single slope component in an Arrhenius plot (Fig. 6). Thus, the energy of activation for L-aspartate transport by the neutral system does not change noticeably from 5 to 35 °C. Transport of L-aspartate by the general amino acid transport system, however, (assayed in the *pm-n* strain) exhibits two slope components with an apparent intersect at 17 °C (Fig. 6).

Before returning to *hgu-4* (and the other pleiotrophic transport-reduced mutants) we might first consider the implications of the data obtained with wild type, *pm-n* and *pm-g*. Over the temperature range 5–15 °C (used for determination of slope) the energies of activation for L-aspartate transport are 25.6 kcal mol^{-1} for wild type, 49.5 kcal mol^{-1} for the general amino acid transport system (*pm-n*) and 13.6 kcal mol^{-1} for the neutral amino acid transport system (Table V). Thus, over this temperature range it takes roughly 4-times the energy to activate transport of L-aspartate by the general system as opposed to transport by the neutral system. The presence of both systems in wild type results in an intermediate energy of activation.

Over the temperature range 25–35 °C (again the 10 °C differential used for slope determination) the energies of activation for aspartate transport are 8.7 kcal mol^{-1} for the wild type, 16.8 kcal mol^{-1} for the general amino acid transport system (*pm-n*) and 13.4 kcal mol^{-1} for the neutral amino acid transport system (*pm-g*) (Table V). In this instance the energy required to activate transport by the general and neutral transport systems is greater when the systems are separate (via mutation) than when they are both present, as in the wild type. One might imagine some degree of direct (common protein component) or indirect (perturbation of membrane structure) interaction between the components of the individual systems.

Analysis of the *hgu-4* mutant (as well as *nap* and *un-3*) reveals the same type of Arrhenius plot for L-aspartate transport as found for the wild type (Figs. 5 and 7). Although the log k values (i.e. transport velocities) are lower, the energies of activation at the lower temperature range, i.e. slope components, (with some exceptions) and the points of intercept (20–22 °C) are similar in wild type and the three pleiotropic transport-reduced mutants. At the higher temperatures (25–35 °C) *hgu-4* and *nap* require greater energy to activate L-aspartate transport than does wild type (or *un-3*)

TABLE V

ENERGIES OF ACTIVATION* (kcal mol^{-1}) FOR L-ASPARTATE (0.1 mM) TRANSPORT (pH 3.8) BY VARIOUS STRAINS OF *N. CRASSA* GERMINATED 6 h ON ACETATE MINIMAL MEDIUM PRIOR TO ANALYSIS OF TRANSPORT ACTIVITY

*The energies of activation were calculated by determining the slopes of the straight lines, in the Arrhenius plots, between 5 and 15 °C and 25 and 35 °C and fitting the value into general equation $e = 4.56 \cdot \text{slope}$.

	5–15 °C	25–35 °C
Wild type ($SY4f_8a$)	25.65	8.82
hgu-4(4)a	29.31	11.76
nap	23.81	12.60
un-3	22.72	9.24
pm-n	49.46	16.80
pm-g	13.56	13.44

(Table V). The similarity in Arrhenius plots for wild type, *hgu-4*, *nap* and *un-3* clearly suggest, however, that one or the other systems, i.e. neutral or general amino acid transport systems, are not preferentially reduced or missing in these strains. Possible interaction(s) between these systems (as mentioned above) may be changed, however.

Although the data from the Arrhenius plots suggested that the point of intersect of the two slope components in wild type (and the various pleiotropic mutants), was attributable to the combined activity of two systems rather than to a membrane phase transition, the Arrhenius plot of L-aspartate transport by the general amino acid transport system (Fig. 6) might be interpreted as due to a membrane phase transition.

In an attempt to pursue this point further we compared the temperature(s) at which the intersect point occurred when *pm-n* conidia were germinated at two separate temperatures prior to assaying transport activity at different temperatures. It has been suggested that organisms adapt to lower temperatures by appropriately changing the degree of saturation of the fatty acids present in their membranes. If such is true of *Neurospora*, germination of cells at 15 °C, as opposed to 28 °C, should result in a greater percentage of unsaturated fatty acids in its membranes with the result that the transition point in an Arrhenius plot of L-aspartate transport should occur below the temperature (17 °C) observed with cells grown at 28 °C. Conidia of *pm-n* were germinated for 8 h (longer germination times were required to approximate the transport rates of cells germinated 6 h at 28 °C) at 15 °C and assayed for L-aspartate transport

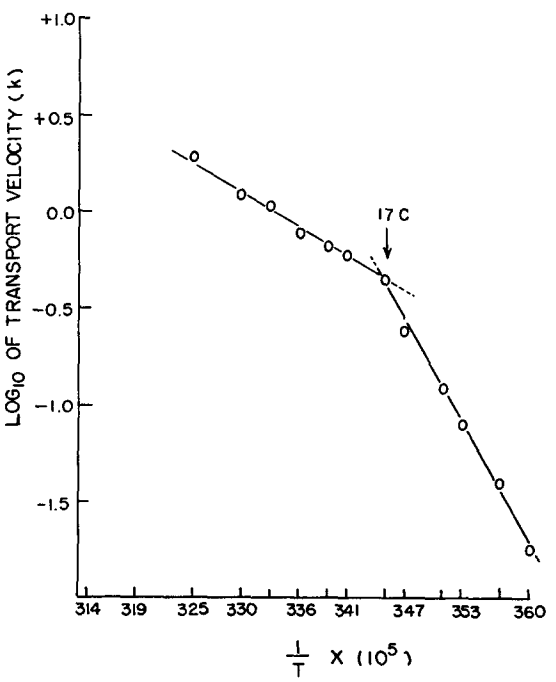

Fig. 8. Arrhenius plot of L-aspartate transport, pH 3.8, by *pm-n* (○–○) as a function of temperature. Assay conditions as described in the legend to fig. 5, except that the cells were grown for 8 h at 15 °C prior to the transport assays. The experimental points represent the average of two determinations and thus no error bars were drawn.

as a function of temperature. Essentially the same results were obtained under both conditions of germination (compare Figs. 6 and 8). The point of intersect occured at at 17 °C regardless of the temperature of germination.

DISCUSSION

It is obvious that although phenotypically similar in their resistance to amino acid analogs, the mutants *fpr-1*, *nap*, *un-3* and *hgu-4* differ in the mechanism of resistance. We have shown here that the *hgu-4* mutant is pleiotropically reduced in amino acid transport and the means by which the reduction in transport occurs is different for the structural classes of amino acids. If amino acid transport in *Neurospora* is mediated by genetically (and biochemically) distinct transport systems we must assume that these systems contain some transport component(s) in common, otherwise, we should not have been able to isolate the mutants described above.

The acidic amino acid L-aspartate is transported in conidia and mycelia by the neutral and general amino acid transport systems. During germination of conidia to mycelia the two transport systems appear to differentially undergo transitions in relative transport activities, in substrate specificities, and in kinetic constants. In conidia the general amino acid transport system constitutes approximately 40 % of the total L-aspartate transport activity with the neutral amino acid transport system transporting the remaining 60 % [17]. The reported K_m and V_{max} values for L-aspartate transport in conidia by the general and neutral transport systems are 3.4 mM (K_m) and 20.0 nmol · min^{-1} · mg cells^{-1} (V_{max}) and 0.08 mM (K_m) and 1.50 nmol · min^{-1} (V_{max}), respectively [20]. In conidia germinated for 6 h the general system now constitutes approximately 83 % of the total L-aspartate transport activity with the neutral system transporting the remaining 17 %. Germination of conidia for 9–10 h appears to complete the transition as the general system now appears to predominate, transporting greater than 95 % of the total L-aspartate accumulated [25]. In addition a dramatic change has occurred in the general system itself. First of all, it now recognizes both the D- and L-stereoisomers of aspartate and glutamate. Secondly, it appears to transport both the zwitterionic and anionic species of L-aspartate and thirdly, the apparent K_m of the general system for L-aspartate is 0.04 mM [25]. We have discussed these developmental changes in some detail because the *hgu-4* lesion may result in an inability of this strain to undergo the postulated transition(s) with germination.

The published K_m values for L-phenylalanine and L-arginine transport in conidia and mycelia are very nearly equal [2, 26] and thus the observed V_{max} differences in the *hgu-4* mutant, without a concomitant change in K_m values, could be explained as a failure of this strain to increase its V_{max} (and K_m?) potential during germination. Examination of the K_m values for L-aspartate transport by wild type and *hgu-4* reveals an orderly developmental transition by wild type, i.e., 0.54 mM (apparent) in conidia [20], 0.18 mM in 6 h mycelia, and 0.07 mM in 9–10 h mycelia [25], while the *hgu-4* strain remains at 1.1 mM in 6 h mycelia (note that the K_m value for L-aspartate transport by the general system in conidia is 3.4 mM [20]).

That the *hgu-4* lesion is affecting both the neutral and general amino acid transport systems is suggested by the energies of activation required for L-aspartate transport at the higher temperatures. Between 25 and 35 °C the energy of activation is

8.82 kcal mol^{-1} for wild type and 13.4 and 16.8 kcal mol^{-1} in *pm-g* and *pm-n*, respectively. This observation would seem to suggest that mutational modification of either transport system affects the remaining system. That the *hgu-4* strain (and *nap*) requires a comparable energy of activation of 11.8 (and 12.6) kcal mol^{-1}, over the same temperature range, supports the proposition that the lesion is exerting effects similar to the mutational modification of the individual systems, i.e. affecting some component common to the two systems. The identity of the postulated common component is unknown, although the membrane matrix is a strong candidate.

The data from the Arrhenius plots suggest that the point of intersect* of the two slope components in wild type (and the various pleiotropic mutants) is not directly attributable to a membrane phase transition, as has been reported in various studies in bacterial systems. Instead it is suggested to be due to differential transport activity for L-aspartate by two "separate" systems.

The biphasic character of the Arrhenius plot of L-aspartate transport by the general system, however may be due to a membrane phase transition. In the *pm-n* strain, there should be only one transport system functioning and any deviation from a straight line plot would imply a temperature effect on the system. That no such deviation occurs when L-aspartate transport is examined in *pm-g* (neutral system alone) would then have to be interpreted as independence of this system from a phase transition in the membrane or that or no phase transition occurs. The failure of the point of intersect for L-aspartate transport in *pm-n* to shift to a temperature lower than 17 °C when the cells were germinated at lower temperatures, suggests that either the transition points seen in these Arrhenius plots are not due to membrane phase transitions or that the conditions of germination were not sufficient to dramatically change the composition of the membrane. It would seem therefore, that the complexity of the *Neurospora* membrane will make it difficult to assess, using transport assays, the physical state of the membrane matrix. Spin labeling studies in whole cells of *Neurospora* [27] suggested that there was no membrane phase transition between 30 and 50 °C. The lower of these two temperatures, however, is still at least 13 °C above where we would expect any phase transition to occur. Indeed it is not certain that dramatic changes in membrane fluidity will be observable in *Neurospora*, although the fatty acid auxotroph (*cel*) [27–29] might be useful in future studies.

In summary, we have been seeking mutants affected in some protein (or glycoprotein) component of the various amino acid transport systems. Mutations affecting components of individual transport systems have been widely studied with considerable success. Mutations affecting components shared by systems have been more difficult to analyze, however, as evidenced by the similarity in phenotypes of *fpr-1* and *hgu-4*, and care will have to be exercised in studies in this area. Perhaps we might acquire a new perspective on an old problem, transport mechanisms, if we also turn our attention to that structure in which these components must reside and function, the membrane matrix.

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* The decision to draw two straight lines through the experimental points in some of the Arrhenius plots, instead of a curved line, was based on the literature precedents in bacterial systems (see refs. 21–24). In all probability, the system under study is more complex (see ref. 21) than indicated by the straight line plots, but the desire to compare data between the wild type and mutants prompted the connecting of experimental points by straight lines.

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