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Aromatic amino acid biosynthesis and para-fluorophenylalanine resistance in *Aspergillus nidulans*

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1. INTRODUCTION

The pathways for the biosynthesis of aromatic amino acids have been extensively studied in a variety of micro-organisms and plants and have been found to be essentially the same (for reviews see Meister, 1965; Broquist & Trupin, 1966). In contrast to micro-organisms and plants which are able to synthesize aromatic amino acids from simpler compounds, animals require dietary phenylalanine (PHE) and tryptophan (TRY), but can synthesize tyrosine (TYR) by the hydroxylation of PHE (Udenfriend & Cooper, 1952; Kaufman, 1963). TYR synthesis by PHE-hydroxylation is also known to occur in some specialized micro-organisms like PHE-adapted pseudomonads (Mitoma & Leeper, 1954; Guroff & Ito, 1963) and in certain aromatic mutants of *Neurospora crassa* (Barratt *et al.*, 1956). Otherwise, in all organisms so far investigated, there is only one pathway for TYR synthesis—either by the transamination of *p*-hydroxy-phenylpyruvic acid (known in micro-organisms and plants) or by the hydroxylation of PHE (known in animals).

There is no report of any study concerned with PHE and TYR biosynthesis in *Aspergillus nidulans* although mutants at two loci (*phen2* and its alleles and *phen6*) have been found to require PHE for optimum growth. Some *p*-fluorophenylalanine-(FPA) resistant mutants have been found to have a partial requirement for TYR or PHE and all of them have been found to be selectively inhibited by indole or aminotyrosine + phenylanthranilic acid (Morpurgo, 1962; Calvori & Morpurgo, 1966), but the nature of these mutations has not been explained.

The observations reported in this paper suggest that both transamination and hydroxylation pathways for TYR synthesis occur in *A. nidulans* and that a metabolic block in the transamination pathway results in a partial TYR requirement which is associated with FPA resistance due to over-production of PHE. This paper also describes the formal and physiological genetics of TYR-requiring mutants and of an anthranilic acid-requiring FPA-resistant mutant of *A. nidulans*.

2. MATERIALS AND METHODS

The general techniques and terminology employed were those described by Pontecorvo (1949); Roper (1952); Pontecorvo *et al.* (1953); Pontecorvo & Käfer (1958) and Käfer (1958).

U.V. (Pontecorvo *et al.*, 1953) and N-methyl-N′-nitro-N-nitrosoguanidine (NTG) (Clutterbuck & Sinha, 1966), were used as mutagens. Production and replica-plating of microcolonies was done as described by Mackintosh & Pritchard (1963).

The use of 'master strain F' for assigning genes to linkage groups and for tests of translocations has been reported by McCully & Forbes (1965).

All the chemicals used were of analytical grade and concentrations are expressed in terms of weight per unit volume. The samples of phenylpyruvic acid used were found to be contaminated with PHE, when examined chromatographically.

All the strains used were from the stocks maintained in the Department of Genetics of the University of Glasgow, except *paba1,y ;trypA69*, which was kindly sent to me by Dr C. F. Roberts.

3. RESULTS

(i) *Phenylalanine-requiring mutants*

(*a*) *Isolation and growth characteristics of phenylalanine-requiring mutants*

Five PHE-requiring mutants (*phen2*, *3*, *4*, *5* and *6*) were available in existing strains and five more (*phen7*, *8*, *9*, *10* and *11*) were isolated after NTG treatment of *bi1;w6* and *bi1* conidia, out of 54,000 colonies tested—*phen7* and *8* from the former and *phen9*, *10* and *11* from the latter strain. All strains (*phen2–11*) were found to be slightly leaky—*phen6* being more leaky than the others.

(*b*) *Formal genetics of phenylalanine-requiring mutants*

Complementation tests between pairs of PHE-requiring mutants in diploids and heterokaryons gave identical results. *phen2*, *3*, *4*, *5*, *7*, *8*, *9*, *10* and *11* did not complement in any combination while *phen6* complemented with all the others. Previous workers had already located *phen2* in linkage group III (Käfer, 1958) and *phen6* in linkage group VII (McCully, 1964). The additional mutants did not, therefore, add to the number of loci already identified. These are hereafter designated *phenA* (*phen2* and its alleles) and *phenB* (*phen6*). The strains *bi1;w6;phenA7* and *bi1;phenB6* were found to be free of translocations. A I–IV translocation, found to be present in the strain *bi1;phenA3*, was eliminated by out-crossing.

(ii) *Partial tyrosine-requiring mutants*

(*a*) *Failure to isolate a tyrosine-requiring auxotroph in a* bi1 *strain*

U.V. or NTG treatment of *bi1* conidia did not yield any TYR auxotroph although 108,500 colonies, grown on minimal medium (MM) + biotin + TYR, were replicated on MM + biotin (Table 1).

(*b*) *The hypothesis of the existence of two pathways for tyrosine synthesis and the isolation of tyrosine-requiring mutants in a phenylalanine-requiring strain*

Failure to isolate a TYR-requiring auxotroph and the fact that during routine procedures for the isolation of auxotrophic mutants in this laboratory no TYR-

Table 1. *Failure to isolate a tyrosine-requiring mutant in a* bi1 *strain by replica plating*

Experiment number	Mutagen used	Percentage survival	No. of viable colonies tested	No. of tyrosine auxotrophs recovered
A	U.V.	12·0	16,500	none
B	U.V.	8·5	13,000	none
C	U.V.	6·2	12,500	none
D	NTG	20·0	12,000	none
E	NTG	20·0	13,500	none
F	NTG	18·0	15,000	none
G	NTG	18·0	12,000	none
H	NTG	25·0	14,000	none

requiring mutants have so far been recovered, suggested that both the transamination and the hydroxylation pathways for TYR synthesis are operative in *A. nidulans* and that a metabolic block in one pathway allows substantial growth, in the absence of exogenous TYR, because of the still functioning alternative pathway. On this hypothesis it should be possible to recognize a TYR-requiring mutant, blocked in the transamination pathway, in a *phen* strain by limiting PHE in the medium so as just to satisfy the PHE requirement without leaving sufficient for conversion to TYR. Under these conditions a *phen;tyr* double mutant might show a growth response related to the level of TYR supplied.

This approach was successful and two TYR-requiring mutants were recovered after plating NTG treated *bi1;phenA3* conidia on a medium containing biotin + TYR ($1{\cdot}4 \times 10^{-2}$M) + PHE ($3{\cdot}6 \times 10^{-3}$M) and replicating the colonies onto a medium containing biotin + PHE ($3{\cdot}6 \times 10^{-4}$M). Following a similar procedure but replicating the colonies onto a medium without either TYR or PHE (taking advantage of the fact that *phenA* strains are leaky), eight TYR-requiring auxotrophs were recovered (Table 2).

Table 2. *Isolation of tyrosine-requiring mutants by replica plating after NTG treatment of* bi1;phenA3 *conidia*

Experiment number	Percentage survival	No. of viable colonies tested	Grown on	Replicated on	Tyrosine auxotrophs: Total	Tyrosine auxotrophs: Isolation number
A	22·0	14,000	MM + biotin + tyrosine + optimum phenylalanine	MM + biotin + limiting PHE	1	*tyr1*
B	22·0	12,500			1	*tyr2*
C	20·0	15,000		MM + biotin	1	*tyr3*
D	30·0	9,000			2	*tyr4* *tyr5*
E	30·0	6,000			1	*tyr6*
F	40·0	8,500			2	*tyr7* *tyr8*
G	40·0	4,000			1	*tyr9*
H	40·0	3,000			1	*tyr10*

(*c*) *Growth patterns of* phenA;tyr1–10 *double mutants*

Auxanography of *bi1;phenA3;tyr1–10* strains revealed that TYR requirement of the mutants is satisfied by either TYR or *p*-hydroxyphenylpyruvic acid and that *phenA;tyr* double mutants were inhibited by excess of either TYR or PHE. Double mutants grew at a certain ratio of TYR and PHE, within a wide range of absolute concentrations of the two metabolites. TRY competitively inhibited the growth on TYR and PHE. Optimal growth of a *phenA3;tyr1* double mutant was obtained with the concentration of TYR between 4×10^{-2}M and 8×10^{-2}M and of PHE between 8×10^{-2}M and $3{\cdot}2 \times 10^{-3}$M. TYR-requiring mutations were noticed to be very leaky.

(*d*) *Growth patterns of* phenA3;tyr7, phenA3$^+$;tyr7, phenA3;tyr7$^+$ *and* phenA3$^+$;tyr7$^+$

The four types of segregants with respect to *phenA3* and *tyr7* from a cross *y;w2;arg1* × *bi1,tyr7;phenA3* were morphologically distinguishable on a medium devoid of PHE and TYR (Plate 1). Thus, partial TYR requirers are distinguishable from wild-type by their smaller colony size on a medium lacking TYR. It was noticed that although TYR had no growth-promoting activity for *phenA* strains, it had a sparing effect on the PHE requirement.

(*e*) *Formal genetics of partial tyrosine requirers*

All ten TYR-requiring mutants were phenotypically similar and did not complement each other either in heterokaryons or in diploids. Mutants *tyr1–10* are, therefore, allelic and thus define a single locus which is designated *tyrA*.

Mitotic and meiotic analysis revealed that the strain *bi1,tyrA8;phenA3* is free of translocations and *tyrA* is in linkage group I, tightly linked to *ribo1* on the left arm (Table 3).

Table 3. *Meiotic location of* tyrA

Type of cross	No. of colonies analysed from a hybrid perithecium	No. of recombinants recovered	Recombination fraction (per cent)
tyrA7 × *ribo1*	204	3	1·47 ± 0·84
tyrA8 × *ribo1*	191	none	—
tyrA9 × *ribo1*	103	none	—
tyrA10 × *ribo1*	95	none	—

(iii) *Exacting tyrosine-requiring mutants*

(*a*) *Isolation of non-leaky tyrosine-requiring mutants*

If *tyrA* mutants are blocked in one of the two pathways for TYR synthesis, it should be possible to obtain, by a further mutation, an exacting TYR-requiring strain, blocked in both pathways. Conidia of strain *bi1,tyrA7* were, therefore, treated with NTG, plated on MM + biotin + TYR and replicated on MM + biotin. Four exacting TYR requirers were recovered among $6{\cdot}4 \times 10^4$ colonies tested.

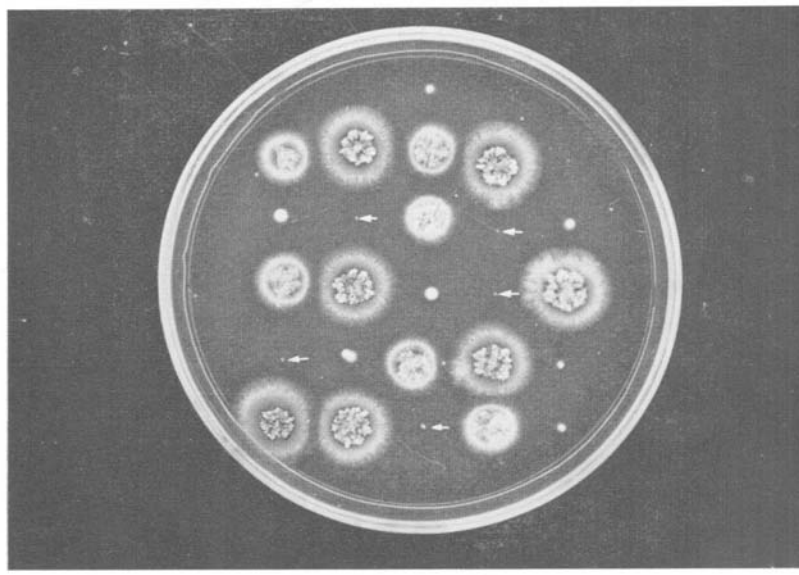

Plate 1. Segregants from a cross *y;w2;arg1* × *bi1,tyrA7;phenA3* on a medium without tyrosine and phenylalanine.

tyrA7;phenA3	=	not growing (→)
tyrA7⁺;phenA3	=	very small colonies
tyrA7;phenA3⁺	=	colonies of intermediate size
tyrA7⁺;phenA3⁺	=	large colonies

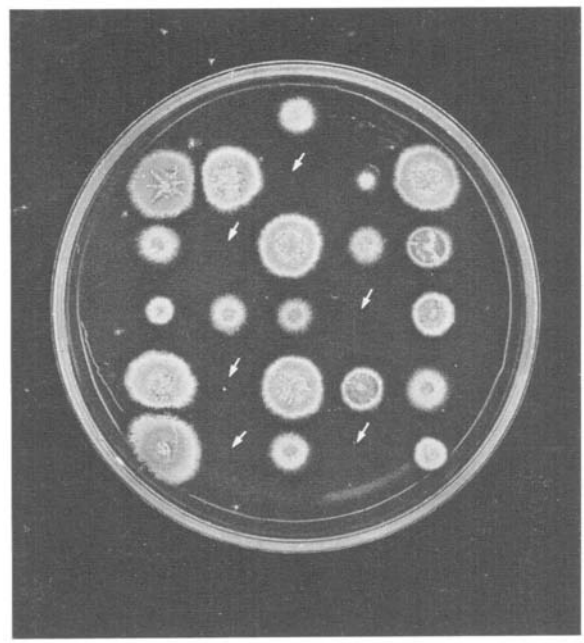

Plate 2. Segregants from a cross *tyrA7,bi1;tyrB1* × *y;s12;pyro4* on a tyrosine-less medium.

tyrA7;tyrB1	=	not growing (→)
tyrA7; tyrB1⁺	=	smaller colonies
tyrA7⁺;tyrB1	=	larger colonies
tyrA7⁺;tyrB1⁺		

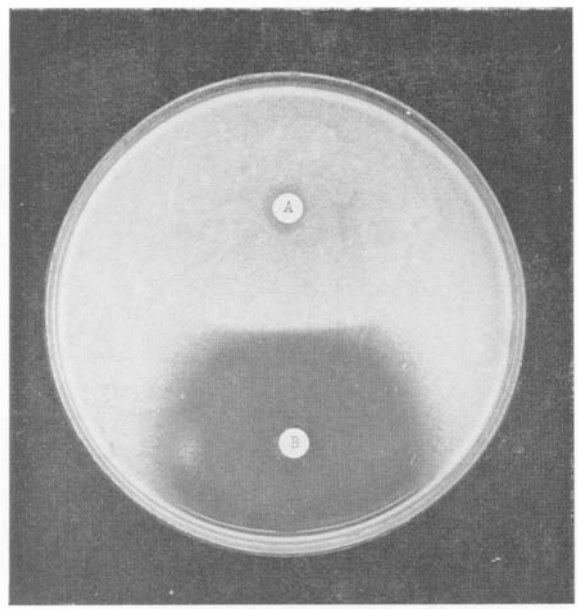

Plate 3. Auxanography of *bi1* showing competitive inhibition as revealed by a straight line which limits the area of growth from that of inhibition. Conidia were pre-germinated 6 hours at 37°C. in MM + biotin, phenylalanine and *p*-fluorophenylalanine were added at points A and B respectively and the plate was reincubated overnight.

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(*b*) *Growth characteristics of exacting tyrosine requirers*

If the absolute TYR requirement was due to mutations at two different loci, each showing a partial TYR requirement, these should be separable by recombination. The results from an appropriate cross to test this hypothesis are given in Table 4. Since *ribo1* and *tyrA7* were in *trans* in the cross and are very closely

Table 4. *Segregation of TYR requirement with respect to* ribo1 *in a cross* bi1,tyrA7; tyrB2 × ribo1,ad14,lu1,y

ribo1	103	*tyrA7*$^+$;*tyrB2* and *tyrA7*$^+$;*tyrB2*$^+$	Growth indistinguishable from wild-type in presence or absence of TYR
ribo1$^+$	102	49 = *tyrA7*;*tyrB2*	Exacting TYR requirers
		53 = *tyrA7*;*tyrB2*$^+$	Partial TYR requirers

linked, the great majority of the *ribo1*$^+$ colonies must carry the *tyrA7* allele and the great majority of *ribo1* colonies must carry the *tyrA7*$^+$ allele. Since about half the ribo$^+$ segregants are exacting TYR requirers and about half partial TYR requirers, one can deduce that two mutations are indeed involved in exacting TYR requirement, one of them being unlinked to *ribo1* and designated *tyrB*. Consistent with this, none of the *ribo1* segregants, which will be *tyrA*$^+$, are absolute TYR requirers. The failure to distinguish *tyrB2* and *tyrB2*$^+$ classes among the *ribo1* segregants suggested that *tyrB2* by itself is very leaky so as to overlap the wild-type. Thus, it is not possible to follow the segregation of *tyrB2* in the presence of *tyrA*$^+$. The three distinguishable phenotypes, on TYR-less medium, from a cross *tyrA*;*tyrB* × +;+ are shown in Plate 2.

(*c*) *Formal genetics of exacting tyrosine requirers*

Complementation tests in heterokaryons and diploids showed *tyrA7,bi1;tyr1–4* isolates to be allelic to each other, thus representing mutations in locus *tyrB*. The strain *tyrA1,bi1;tyrB1* was found to be translocation free and *tyrB* was located in linkage group III.

A difficulty in the meiotic location of *tyrB* is the scarcity of hybrid perithecia, which has turned out to be a feature of crosses involving *tyrA7,tyrB1–4* strains. A cross with a *phenA* strain poses another problem, i.e. the classification of *tyrA*, *tyrB* and *phenA* recombinants. However, *tyrB* and *phenA* mutants are not allelic to each other because the strains *tyrA7, bi1; tyrB1* and *tyrA7, ad14,lu1,y;phenA3* complemented both in heterokaryons and diploids. In a cross, *tyrA7, bi1;tyrB1* × *ribo1,ad14,lu1,y;arg2, tyrB* was found to be unlinked to *arg2*.

(iv) *Resistance to p-fluorophenylalanine* (*FPA*) *of partial tyrosine requirers* (*tyrA*)

(*a*) tyrA *mutants are FPA-resistant*

FPA-resistant mutants *fpA1* and *fpA12* were isolated by McCully and were found to be tightly linked to *ribo1* (McCully, 1964). The parent *bi1* strain was sensitive to FPA whereas *tyrA1*, *tyrA*7, *tyrA*8, *fpA1* and *fpA12* were all equally resistant to FPA. A *tyrA7,bi1;phenA3* strain was found to be FPA-sensitive.

All of the 103 *ribo1* segregants from the cross *ribo1,ad14,lu1,y* × *tyrA7,bi1;tyrB2* (Table 4) were found to be FPA-sensitive, indicating that *tyrB* mutants are FPA-sensitive.

(*b*) *Isolation of some more FPA-resistant mutants and tests for their linkage to* ribo1 *and for their TYR requirement*

Twelve FPA-resistant mutants were selected as fast growing sectors after point inoculation of *bi1* conidia on MM containing biotin and 0·05–0·1 % FPA. These, designated *fp46–57*, were tested for their TYR-requirements and were crossed to a *ribo1* strain to test for linkage of FPA resistance to *ribo1*. In ten strains the mutation determining FPA resistance was found to be closely linked to *ribo1* and nine out of these ten strains turned out to be partial TYR requirers (Table 5).

Table 5. *Linkage of* fp46–57 *to* ribo1

		Results of the cross *ribo1,ad14,1u1,y* × *bi1,fpX**		
Isolate	Growth response to TYR	No. of segregants analysed	*fpX*+ recombinants obtained	Percentage recombination between *ribo1* and *fpX*
fp46	+	206	0	—
fp47	+	208	0	—
fp48	−	208	95	45·7 ± 3·45
fp49	+	198	1	0·5 ± 0·5
fp50	−	187	0	—
fp51	+	200	3	1·5 ± 0·86
fp52	+	203	0	—
fp53	+	204	0	—
fp54	+	208	0	—
fp55	+	206	0	—
fp56	−	201	103	51·2 ± 3·52
fp57	+	205	0	—

FPA resistance was scored among prototrophic recombinants selected on MM.
\+ = Enhancement of growth by TYR.
− = No enhancement of growth by TYR.
X = Isolation numbers 46–57.

(*c*) *Tests of allelism between* tyrA, fpA *and the new FPA-resistant mutants that are closely linked to* ribo1

Diploids were synthesized between the strains *fpA1,paba1,y* and each of the following thirteen strains:

(1) *tyrA7,bi1;phenA3* (2) *ribo1,fpA1,bi1* (3) *ribo1,fpA12,bi1*
(4) *fp46,bi1* (5) *fp47,bi1* (6) *fp49,bi1*
(7) *fp50,bi1* (8) *fp51,bi1* (9) *fp52,bi1*
(10) *fp53,bi1* (11) *fp54,bi1* (12) *fp55,bi1*
(13) *fp57,bi1*

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All of them were found to be resistant to FPA and thus the *tyrA*, *fpA* and newly isolated FPA-resistant mutants that are closely linked to *ribo1*, are allelic to each other.

The diploids (*tyrA7,bi1;phenA3* + 'Master Strain F') and (*tyrA7,bi1;phenA*3 + *fpB37,ad14,paba1,y*) were found to be sensitive to FPA. Thus *tyrA7* and *fpB37* are non-allelic and recessive in heterozygous diploids.

(*d*) *Degree of FPA resistance of* tyrA7 *and* fpA50

In order to compare the degree of FPA resistance of *tyrA7* (partial TYR-requiring mutant at *fpA* locus) and *fpA50* (a mutant at *fpA* locus which has no requirement) with that of a FPA-sensitive wild-type strain, freshly harvested conidia of strains (1) *bi1*, (2) *tyrA7,bi1* and (3) *fpA50,bi1* were plated at densities estimated by haemocytometer counts on MM + biotin + different concentrations of FPA. The percentage survival of conidia, as revealed by the number of colonies visible after 48 hours' incubation, was determined.

The strain *bi1* was inhibited by 5–6 mg./l. of FPA whereas *fpA50,bi1* required 1000 times this concentration of FPA to produce the same degree of inhibition and the partial TYR-requiring strain *tyrA7, bi1* was found to be even more resistant (Fig. 1).

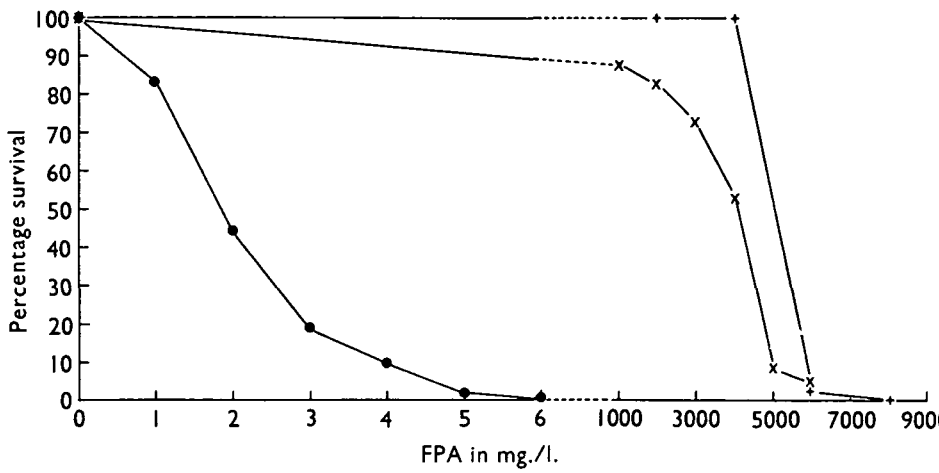

Fig. 1. Percentage survival of *bi1* (●), *fpA50, bi1* (×) and *tyrA7, bi1* (+) conidia on MM + biotin + FPA after 48 hours of incubation at 37°C.

(*e*) *Efficiency of PHE and other compounds in reversing FPA inhibition*

An auxanographic test (Pontecorvo, 1949) of *bi1* indicated that inhibition of growth by FPA is competitively reversed by PHE (Plate 3). Phenylpyruvic acid was found to be quite effective in reversing the inhibitory effect of FPA but its action was not competitive. TYR, TRY, *p*-hydroxyphenylpyruvic acid and anthranilic acid were found to be slightly effective but only when the relative concentrations of FPA were very low.

(v) *A FPA-resistant mutant which requires tryptophan*

(*a*) *Growth response of* fp48

The mutant *fp48* was found to grow very slowly either on MM or complete medium but quite well on both if supplemented with TRY, indole or anthranilic acid. Nicotinic acid had no such effect. In the CM, as routinely prepared, most of the TRY is destroyed.

(*b*) *Formal genetics of* fp48

The mutant *fp48* was found to be recessive in a heterozygous diploid and to be non-allelic to *fpA* or *fpB* mutants (isolated by McCully, 1964). It, therefore, defines a new locus, *fpE*. The mutant *fpE48* is allelic to *trypA* mutants of Roberts (1967) because the strains *paba1,y;tryA69* and *bi1;fpE48,Acr1,w3,thi4,ni3* do not complement either in heterokaryons or in diploids.

fpE was assigned to linkage group II and the strain *bi1;fpE48* was found to be free of translocations. Meiotic location of *fpE* was attempted by crossing *bi1;fpE48* to each of the strains *bi1;Acr1,w3,ab1,ni3,ad3* and *ribo1,bi1;Acr1,w3,thi4,ni3,ad3*. The results of the crosses were not conclusive because of distortions of the allele ratios, but suggested that the locus *fpE* may be located in the *ni3–ad3* interval on the right arm of linkage group II.

4. DISCUSSION

If the general scheme for aromatic amino acid biosynthesis in *A. nidulans* is the same as in other micro-organisms (Fig. 2), *phenA* and *phenB* mutants should

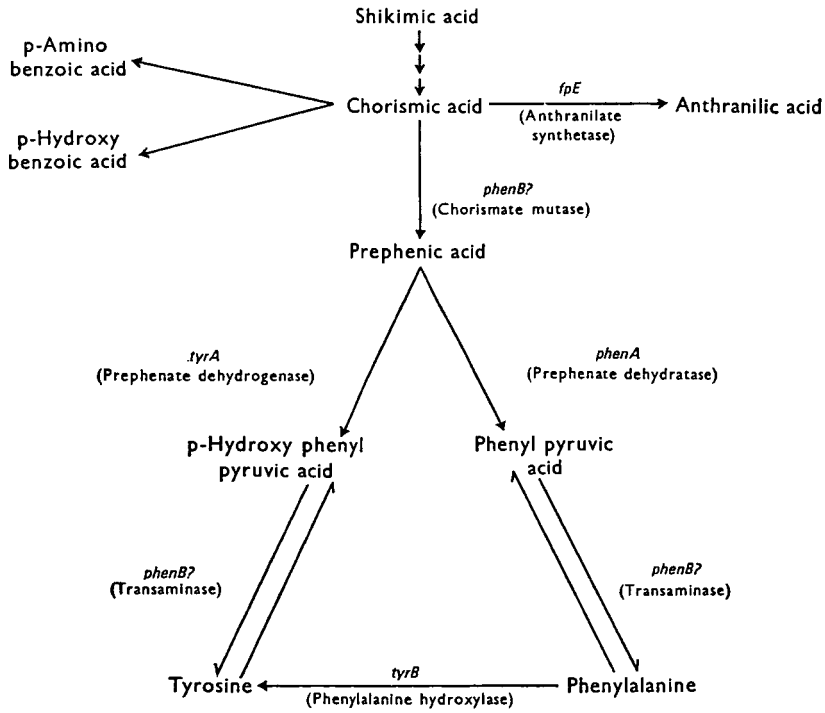

Fig. 2. Correlation of mutants with steps in aromatic amino acid biosynthesis.

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be blocked somewhere after chorismic acid. Mutants at the *phenA* locus are perhaps blocked between prephenic acid and phenylpyruvic acid. Mutations affecting this step are the only ones recovered as PHE-requirers in a variety of micro-organisms so far investigated (see, for review, Meister, 1965). It is possible that *phenA* mutants lack prephenate dehydratase activity and that they are leaky because some PHE can be made by prephenate dehydrogenase, which is present in *phenA ;tyrA*$^+$ strains. If this were true, a *phenA ;tyrA* double mutant, blocked at two homologous positions in the transamination pathways, should not be leaky and this is what has been found.

It is not known whether the blocks in *phenA* and *phenB* mutants are at different steps or at the same step for different reasons. But if they are at different steps, the block in the *phenB* mutant could be either at the chorismate mutase step or at the transamination step—in the former case leakiness of *phenB6* can be explained by lability of chorismic acid (Jensen & Nester, 1965) and in the latter case on the basis of non-specificities of transaminases (Rudman & Meister, 1953). In either case the *phenB6* mutant should require *PHE* + *TYR* but both these requirements are probably satisfied by exogenous PHE in *A. nidulans* because PHE can be converted to TYR in this mould. Leakiness of the mutants at *phenB* locus may be the reason why they are not likely to be frequently identified and isolated.

The results presented in this paper are consistent with the hypothesis that both transamination and hydroxylation pathways for TYR synthesis are operative in *A. nidulans* and suggest that *tyrA* mutants perhaps lack prephenate dehydrogenase activity whereas *tyrB* mutants are blocked in the PHE-hydroxylation step. The fact that *tyrB* mutants are not distinguishable from *tyrB*$^+$ in the presence of *tyrA*$^+$, indicates that if *tyrB* mutants are blocked in the hydroxylation of PHE, this is not a quantitatively important pathway in a *tyrA*$^+$ strain, although it may become decisive in a *tyrA* mutant. Thus it appears that the transamination pathway is the major pathway for TYR synthesis and when the mutants are blocked in this pathway, more PHE is converted to TYR through the hydroxylation pathway. This could be due to an increase in the activity or amount of PHE hydroxylase or simply due to an increased availability of the substrate. Mitchell has suggested that in man TYR can be formed by the hydroxylation of phenylpyruvic acid followed by transamination (Beadle, 1945). It is conceivable that *tyrA* mutants, if they lack prephenate dehydrogenase activity, channel prephenic acid to phenylpyruvic acid and PHE, which can be converted to TYR. As expected on the basis of the presence of PHE-hydroxylase activity, although TYR has no growth-promoting activity for *phenA* strains, at lower concentrations it (TYR) spares a part of the PHE requirement.

FPA-resistance of partial TYR-requiring mutants (*fpA* or *tyrA*) and of an anthranilic acid-requiring mutant (*fpE48*) suggests that these mutants either accumulate or overproduce PHE which competes against the antimetabolite. TYR-requiring mutants in *Escherichia coli* and *Micrococcus glutamicus* are known to accumulate up to 2 g./l. of PHE in the medium under optimal conditions of growth (Huang, 1964), but it is not known whether these mutants are FPA-resistant.

Antimetabolites have often been used for the isolation of mutants producing increased amounts of metabolites (Adelberg, 1958; Scherr & Rafelson, 1962) and PHE-excreting mutants of *E. coli*, derepressed in the PHE pathway, are known to be FPA-resistant (Adelberg, 1958). It has been suggested that FPA is inhibitory to *E. coli* primarily because it feed-back inhibits the activity of PHE-sensitive enzymes (Previc & Binkley, 1964*a*, *b*; Ezekiel, 1965). But it appears unlikely that FPA-resistance in *fpA* mutants in *A. nidulans* is due to a derepression (or loss of feed-back inhibition) of some enzyme specific for PHE synthesis, because on this hypothesis it is difficult to explain the growth responses of *phenA;tyrA* and *tyrA; tyrB* double mutants. On the other hand, all observations are compatible with the hypothesis that *tyrA* mutants are blocked in the transamination pathway and that this is one of the two alternative pathways for TYR synthesis in *A. nidulans*. Thus it appears that *A. nidulans* has a comparatively simple mechanism of over-synthesizing one metabolite (PHE) by cutting down the synthesis of the other (TYR).

In *A. nidulans*, all *tyrA* mutants tested so far are FPA-resistant but not all *fpA* mutants are partial TYR requirers. Most of the *fpA* mutants (nine out of ten isolated during the present investigation) are partial TYR requirers and one (*tyrA*7) which is a partial TYR requirer is more resistant to FPA than the one (*fpA*50) which does not require TYR for its optimal growth. This suggests that partial TYR requirers have lost the ability to synthesize an active enzyme, whereas little or non-requirers (but still FPA-resistant and by hypothesis over-synthesizing PHE) have only a partial defect, thus producing an enzyme which has reduced efficiency. In *fpA* mutants that apparently do not require TYR, the requirement is possibly below the level of detection. The observation that aminotyrosine + phenylanthranilic acid selectively inhibits all *fpA* mutants (Morpurgo, Sermonti, Petrelli & Ricci in Calvori & Morpurgo, 1966) also suggests that all *fpA* mutants represent metabolic blocks in TYR synthesis; and the possibility that *nic*8 is suppressed by *pf*21 because of an over-production of chorismic acid has been indicated by Warr & Roper (1965).

SUMMARY

1. Five additional phenylalanine (PHE)-requiring mutants have been isolated but they do not add to the number of loci already known which have been designated *phenA* (*phen2* and its alleles) and *phenB* (*phen6*).

2. Two pathways for tyrosine (TYR) synthesis in *A. nidulans* have been proposed: the well-known one by the transamination of *p*-hydroxyphenylpyruvic acid and an alternative one, as in animals, by the hydroxylation of PHE.

3. Ten allelic partial TYR-requiring mutants (*tyrA*), presumably blocked in the transamination pathway, have been isolated after N-methyl-N′-nitro-N-nitrosoguanidine (NTG) treatment of *bi1;phenA3* conidia.

4. Four partial TYR requirers (at another locus—*tyrB*) have been isolated after

NTG treatment of *tyrA7,bi1* conidia. They are presumably blocked in an alternative pathway for TYR synthesis, i.e. in the PHE-hydroxylation pathway.

5. *tyrA* mutants have been found to be *p*-fluorophenylalanine (FPA)-resistant and allelic to mutants at the *fpA* locus. *tyrB* mutants have been found to be very leaky and FPA-sensitive. *tyrA*;*tyrB* double mutants have been found to be exacting TYR requirers.

6. Mutants at loci *fpA* (*tyrA*) and *fpE* (anthranilic acid-requiring) have been interpreted to be *p*-fluorophenylalanine-resistant due to an oversynthesis of PHE.

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