# GENETIC CONTROL OF PHENYLALANINE AND TYROSINE BIOSYNTHESIS IN *NEUROSPORA CRASSA*[1,2]

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THE present paper reports genetical and biochemical studies of phenylalanine and/or tyrosine-requiring mutants of *Neurospora crassa* blocked after chorismate in the aromatic synthetic pathway. METZENBERG and MITCHELL (1958) previously suggested that in *N. crassa* prephenic acid is a precursor of phenylalanine but not of tyrosine. On the other hand, COLBURN and TATUM (1965) isolated a class of mutants (*pt*) which required both phenylalanine and tyrosine and which appeared to accumulate prephenic acid. They concluded that prephenic acid is a precursor, but not the immediate precursor of the keto acid analogues of these two aromatic amino acids (cf. Figure 4).

Comparable investigations of similar mutants in *Escherichia coli* and *Aerobacter aerogenes* by COTTON and GIBSON (1965) and in *Bacillus subtilis* by NASSER and NESTER (1967) indicated that prephenic acid is the immediate precursor of the keto acids of phenylalanine and tyrosine in these bacteria. In view of these differences and because of certain difficulties of interpretation in the prior studies with *N. crassa*, a reinvestigation of the pathway in this organism appeared desirable.

A brief resumé of certain of these results has been published previously (EL-ERYANI 1967). While the manuscript for this paper was being prepared, a paper by BAKER (1968) appeared which reports certain findings basically in agreement with the major results reported here.

## MATERIALS AND METHODS

*Strains:* Previously isolated strains of interest were obtained in 1965 and 1966 from the Fungal Genetics Stock Center and are described in Table 1. In addition, 87 new mutants were isolated (following ultraviolet irradiation or treatment with ethyl methanesulfonate) by filtration enrichment (WOODWARD, DE ZEEUW, and SRB 1954) using wild type strain 74A: 78 requiring tyrosine, 6 phenylalanine, and 3 both phenylalanine and tyrosine. All mutants including those previously isolated, were identified initially with respect to the biochemical position of genetic blocks by complementation with two test strains: an *arom* gene cluster mutant of the polar, non complementing type, and an *arom-3* mutant not exhibiting allelic complementation (GILES, CASE, PARTRIDGE, and AHMED 1967). Mutants which complemented both testers were presumed to be blocked beyond chorismic acid and the complementation tests served to differentiate post-chorismic acid mutants from a similar class of pre-chorismic acid mutants which require either phenyl-

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TABLE 1

*Phenylalanine-requiring and/or tyrosine-requiring strains obtained from the Fungal Genetics Stock Center*

| Strain designation | Isolation No. | Origin | Requirement | Linkage group |
|---|---|---|---|---|
| *tyr-1** | Y-6994 | Nitrogen mustard | tyrosine | III R |
| *tyr-2*† | STL-1 | spontaneous | tyrosine | I R |
| *tyr-3* | UT-145 | | tyrosine | .... |
| *tyr* | NM-160 | | tyrosine | I |
| *phen-1*‡ | H-6196 | S35 | phenylalanine‡ | I L |
| *phen-2* | E-5212 | | phenylalanine | .... |
| *phen-3* | Y-16329 | X ray | phenylalanine | .... |
| *pt*§ | S-4342 | X ray | phenylalanine and tyrosine | IV R |

* BARRATT, NEWMEYER, PERKINS, and GARNJOBST (1954).
† PERKINS, GLASSEY, and BLOOM (1962).
‡ BARRATT and OGATA (1954). This mutant and its $F_1$ isolates also grow slowly on tyrosine or leucine.
§ COLBURN and TATUM (1965); BAKER (1968).

alanine, or tyrosine, or both. The latter class is known to occur in other microorganisms as well, and has been designated "leaky polyaromatic". Other strains used in genetic analyses were *vel* (B18), *al-2,os* (P641,B135) and the alcoy system designed for the detection of linkage (PERKINS 1964).

*Preparation of substrates and enzyme extracts:* Chorismic acid was obtained from the culture filtrate of *A. aerogenes* 62–1 in a procedure similar to that outlined by EDWARDS and JACKMAN (1965). Prephenic acid was obtained by heating a solution of chorismic acid at 70°C for 60 min and the barium salt was prepared according to the method of METZENBERG and MITCHELL (1956).

Growth of mycelia and enzyme extractions were performed according to the procedure described by GILES *et al.* (1967). Mycelium was routinely grown for 72 hrs at 25°C in standing culture on 200 ml Fries minimal in 2.5 l Fernbach flasks. Fries minimal was supplemented with 80 μg/ml of phenylalanine, or tyrosine, or both, depending on the mutant requirement. Harvested mycelium was lyophilized, powdered, and extracted in 0.1 M $KPO_4$ buffer pH 8, containing $1 \times 10^{-4}$M EDTA and $3 \times 10^{-3}$M α-thioglycerol. This buffer will be referred to subsequently as phosphate buffer. The extract was cleared by centrifugation and then treated with excess protamine sulfate, as indicated by lack of further precipitation on addition of more protamine sulfate to the supernatant solution. Following centrifugation, the supernatant solution was used to obtain a precipitate in the 0–55% $(NH_4)_2SO_4$ saturation range. All enzyme preparations were dissolved in the above buffer. Protein determinations were made using a biuret reagent (MOKRASCH and MCGILVERY 1956).

*Enzyme assays:* Chorismic mutase and prephenic dehydratase were assayed according to the procedure of COTTON and GIBSON (1965). The mutase assay contained 0.1 ml enzyme extract, 0.1 ml of 10 mM chorismic acid and 0.2 ml phosphate buffer. The reaction mixture was incubated for 30 min at 37°C and the reaction was stopped by adding 0.4 ml of 1 N HCl. Further incubation at 37°C for 10 min converted the prephenate formed to phenylpyruvic acid. After the addition of 3.2 ml of 1 N NaOH, the absorbance was read at 320 mμ. A molar extinction coefficient of 17,500 was used to convert absorbancy of μmoles of phenylpyruvate. Specific activities are expressed as mμmoles of phenylpyruvate formed/30 min/mg protein.

The assay for prephenic dehydratase contained 0.1 ml enzyme, 0.1 ml of 14 mM potassium prephenate in 0.05 M pH 8.1 Tris HCl, and 0.4 ml phosphate buffer. After incubation at 37°C for 30 min, the reaction was terminated by adding 2.4 ml of 1 N NaOH and absorbance was read at 320 mμ. Specific activities were calculated in the same manner as for chorismic mutase.

Prephenic dehydrogenase was assayed by either the Millon reaction (SCHWINCK and ADAMS 1959; E. W. NESTER, personal communication) or by monitoring the formation of $NADH_2$ at 340 mμ. The latter assay requires the use of standing cultures which apparently lack $NADH_2$ oxidase. The assay mixture for the Millon reaction contained 0.1 ml potassium prephenate (prepared as above), 0.2 ml enzyme, 0.04 ml of 20 mM NAD+, and 0.26 ml phosphate buffer. The reaction mixture was incubated at 37°C for 30 min and the reaction was stopped by adding 0.25 ml of 6 N $H_2SO_4$ followed by 0.9 ml of $H_2O$. The amount of 4-OH-phenylpyruvate formed was calculated from a standard curve of OD at 490 mμ using tyrosine. Specific activities are expressed as mμmoles of 4-OH-phenylpyruvic acid formed/30 min/mg protein.

The assay for monitoring $NADH_2$ formation consisted of 0.1 ml potassium prephenate (prepared as above), 0. 2ml enzyme, 0.04 ml of 20 mM NAD+, and 0.06 ml phosphate buffer. The reaction was run at 37°C and specific activities were expressed as the change in OD/min/mg protein.

*Sucrose density gradient centrifugation and Sephadex gel filtration:* Sucrose density gradients, 5–20%, were prepared and utilized according to the method of MARTIN and AMES (1961) employing a Beckman SW 41 rotor. A Sephadex G-200 column was prepared as described by ANDREWS (1962). The column was packed to a bed of 2.5 × 70 cm and equilibrated overnight with phosphate buffer. A 10 ml sample of ammonium sulfate fraction desalted on Sephadex G-25 and mixed with standard marker proteins was applied to the column and the flow rate of phosphate buffer as eluant was adjusted to 12 ml/hr. Two ml fractions were collected.

## RESULTS

*Genetic and complementation studies:* Genetic and complementation analyses demonstrated that all 78 of the tyrosine-requiring mutants isolated in the course of the present investigation are allelic with *tyr-1* and that *tyr-1* and *tyr-3* (Table 1) are alleles. No mutants similar to *tyr-2* were recovered. No evidence was obtained for intragenic complementation among the *tyr-1* alleles by plate tests or by genetic crossing and screening for pseudo-wild types. However, it is possible that in both tests complementation was obscured by the leakiness of the strains.

No complementation was observed among the four available *pt* mutants which were isolated in four independent experiments. However, a conclusion that allelic complementation is lacking in the *pt* locus is unwarranted in view of the small size of the sample.

A three-point cross involving *tyr* strain No. NM-160 and an *al-2,os* double mutant indicated that NM-160 is 17 map units to the left of *al-2*, and the prototroph frequency from a cross between NM-160 and *tyr-2* was about 12% (Figure 1).

Genetic analysis of *phen-2*, *phen-3*, and the six newly obtained phenylalanine-requiring mutants showed that all are in linkage group IIIR, closely linked to *vel* (Table 2). Data obtained by PERKINS and ISHITANI (1959) established that *vel* is about five map units to the left of *tyr-1*. The map distance between *tyr-1* and *phen-2* was estimated by random plating of ascospores from a cross of these two mutants. The number of colonies in supplemented plates was 356 as compared with four in minimal plates. Thus *phen-2* appears to be situated approximately two map units from *tyr-1*. In order to establish the order of the three genes (*vel*, *phen-2*, and *tyr-1*) more directly, a cross between a *phen-2*, *vel* double mutant and *tyr-1* was prepared and a large number of ascospores were isolated. However,

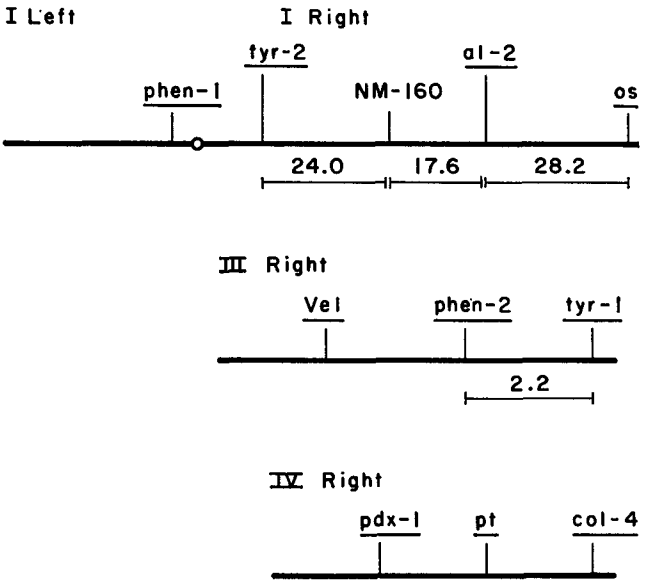


FIGURE 1.—Partial genetic maps of linkage groups I, III, and IV of *N. crassa* showing the distribution of phenylalanine-requiring and/or tyrosine-requiring mutants.

a clear ordering of the three mutants was not possible due to the apparent absence of the class with a phenylalanine and tyrosine double requirement. Consequently, a large number of ascospores from this cross was plated on minimal medium and 400 colonies were isolated and scored for the *vel* and *vel*$^+$ morphology. The ratio of *vel*$^+$ to *vel* was 393 : 77. Thus the order is *vel–phen-2–tyr-1*, with *tyr-1* the most distal locus known in linkage group IIIR.

The apparent absence of a *tyr-1, phen-2* double mutant class was unexpected. However, tetrad analysis from a cross involving *tyr-1* and *phen-2* has established that this class of mutants has an ambiguous nutritional requirement due to the extreme leakiness of *phen-2*. The *tyr-1, phen-2* double mutant class was scored, by nutritional tests, simply as a tyrosine-requirer because of the leakiness of the phenylalanine requirement. However, enzymatic analyses of cultures of the four meiotic products of a recombinant ascus made it possible to detect the double mutant class by the simultaneous absence of prephenic dehydrogenase and prephenic dehydratase activities.

*Biochemical studies:* Enzymatic assays for chorismic mutase, prephenic dehydrogenase, and prephenic dehydratase performed on wild-type Neurospora and all the strains listed in Table 1 confirmed the results of the genetic analyses and showed that the *pt* locus lacks chorismic mutase, and that prephenic dehydrogenase and prephenic dehydratase are lacking in *tyr-1* and *phen-2* mutants, respectively (Table 3). However, *phen-3*, which is genetically allelic with *phen-2*, does produce a partially active prephenic dehydratase. This mutant is also appreciably more leaky on minimal medium than *phen-2*.

TABLE 2

*Linkage of phenylalanine-requiring strains to* vel *in linkage group III R. Cross:* vel *(B18-A)* × *Phenylalanine strain-a.*

(Random single spore isolates)

| Cross | Distribution of isolates: Noncrossovers P1 (*vel*) | Noncrossovers P2 | Crossovers: Doubles | Crossovers: Wild type | Percent recombinants | Percent viability |
|---|---|---|---|---|---|---|
| *vel* × E5212 | 60 | 72 | 0 | 2 | 1.5 | 65 |
| *vel* × Y16329 | 73 | 88 | 0 | 0 | 0 | 81 |
| *vel* × 1 | 62 | 73 | 0 | 0 | 0 | 82 |
| *vel* × 2 | 89 | 88 | 1 | 2 | 1.7 | 80 |
| *vel* × 3 | 55 | 70 | 0 | 0 | 0 | 63 |
| *vel* × 128 | 184 | 150 | 1 | 5 | 1.8 | 78 |
| *vel* × 130 | 91 | 82 | 0 | 0 | 0 | 87 |
| *vel* × 131 | 63 | 71 | 0 | 0 | 0 | 67 |

TABLE 3

*Specific activities of post-chorismic acid, phenylalanine and tyrosine biosynthetic enzymes in wild-type and in phenylalanine-requiring and/or tyrosine-requiring strains of* N. crassa

| Strain | | Enzymatic activities: Chorismic mutase | Prephenic dehydratase | Prephenic dehydrogenase: Million reaction | O.D.$_{340}$ |
|---|---|---|---|---|---|
| Wild type | (74A) | 354.3 | 308.5 | 73.70 | 0.050 |
| *pt* | (S-4342) | 0.0 | 272.7 | 63.9 | 0.063 |
| *tyr-1** | (Y-6994) | 305.7 | 290.3 | 0 | 0 |
| *tyr-3* | (UT-145) | 368.5 | 368.8 | 0 | 0 |
| *phen-2* | (E-5212) | 408.7 | 0 | 59.9 | 0.051 |
| *phen-3*† | (Y-16329) | 346.3 | 62.8 | 64.0 | 0.045 |
| *phen-1* | (H-6196) | 265.5 | 244.9 | 57.6 | 0.052 |
| *tyr* | (NM-160) | 262.7 | 220.6 | 97.0 | 0.066 |
| *tyr-2* | (STL-1) | 356.6 | 170.1 | 8.3 | 0.019 |

* Three additional alleles were also found to lack the dehydrogenase.
† Prephenic dehydratase activity detected in *phen-3* was observed in two different extractions but was lost in both cases after storing the extract at —15°C overnight.

The one available *tyr-2* mutant, isolated by ST. LAWRENCE (PERKINS *et al.* 1962), differs from *phen-2* and NM-160 in its specific tyrosine requirement and in the reduced activity of prephenic dehydrogenase (Table 3). Further studies of the *tyr-2* dehydrogenase have shown that this enzyme is similar to wild-type in its apparent $K_m$, substrate inhibition, and feedback inhibition by the end product, tyrosine. In fact, the only difference so far detected between wild type and *tyr-2* is the low level of enzyme activity.

The possibility that *tyr-2* may be producing an inhibitor of enzyme activity was investigated, utilizing a balanced heterokaryon between a *tyr-1* mutant and *tyr-2*. Heterokaryons involving *tyr-1* plus *phen-2* and *tyr-2* plus *phen-2* served as controls. Heterokaryotic cultures were grown on minimal media in standing cul-

tures. The period of incubation had to be extended to four days due to the poor growth rate of heterokaryons involving *tyr-2*. Enzyme assays of these cultures showed that a heterokaryon between *tyr-1* (having no detectable activity) and *tyr-2* possesses an enzyme activity three times as great as that produced by *tyr-2* alone (Table 4). The results given in Table 4 demonstrate that the *tyr-2* mutant does not produce an inhibitor of prephenic dehydrogenase by a dominant mechanism.

Evidence relating to the physical properties, especially possible aggregation, of the three specific enzyme activities involved in post-chorismic acid phenylalanine and tyrosine biosynthesis in *N. crassa* was sought, utilizing Sephadex gel filtration and sucrose density-gradient centrifugation. Figures 2 and 3 show that the three enzymes, chorismic mutase, prephenic dehydratase, and prephenic dehydrogenase, are separable by both techniques and that there is only one molecular species of chorismic mutase resolvable by these methods.

BAKER (1966) showed that chorismic mutase obtained from *N. crassa* is activated by tryptophan and feedback-inhibited by phenylalanine and by tyrosine. A preliminary investigation of the regulation of prephenic dehydrogenase and prephenic dehydratase (Table 5) suggests that both enzymes are feedback-inhibited by their end products, tyrosine and phenylalanine, respectively.

## DISCUSSION

Genetic, complementation, and biochemical studies of the phenylalanine and/or tyrosine-requiring mutants in *Neurospora crassa* have demonstrated that at least three genetic loci are concerned with the biosynthesis of these two aromatic amino acids from chorismic acid. The *pt* locus appears to be the structural gene for chorismic mutase, while the *tyr-1* and *phen-2* loci apparently encode prephenic dehydrogenase and prephenic dehydratase, respectively. The most conclusive evidence has been obtained for the *phen-2* locus, since mutants either lacking entirely, or possessing only partial, activity for prephenic dehydratase have been demonstrated at that locus. (The mutant strain Y-16329, designated originally *phen-3*, has been shown to be allelic to *phen-2* mutants and to possess a reduced level of prephenic dehydratase which is much less stable than the wild-type enzyme). The present investigation has also demonstrated that the intermediates in the phenylalanine-tyrosine pathway of *N. crassa* (Figure 4) are

TABLE 4

*Enzymatic analyses of heterokaryons involving* tyr-2, phen-2, *and* tyr-1

| Type of heterokaryon | Specific activities | | |
|---|---|---|---|
| | Chorismic mutase | Prephenic dehydratase | Prephenic dehydrogenase (Millon reaction) |
| *tyr-1* + *tyr-2* (Y-6994-A) + (STL-1-A) | 286.9 | 199.1 | 24.0 |
| *tyr-2* + *phen-2* (STL-1-A) + (E-5212) | 367.2 | 215.2 | 43.9 |
| *tyr-1* + *phen-2* (Y-6994-A) + (E-5212-A) | 474.8 | 243.0 | 63.6 |
| *tyr-2* (STL-1) | 356.6 | 170.1 | 8.3 |

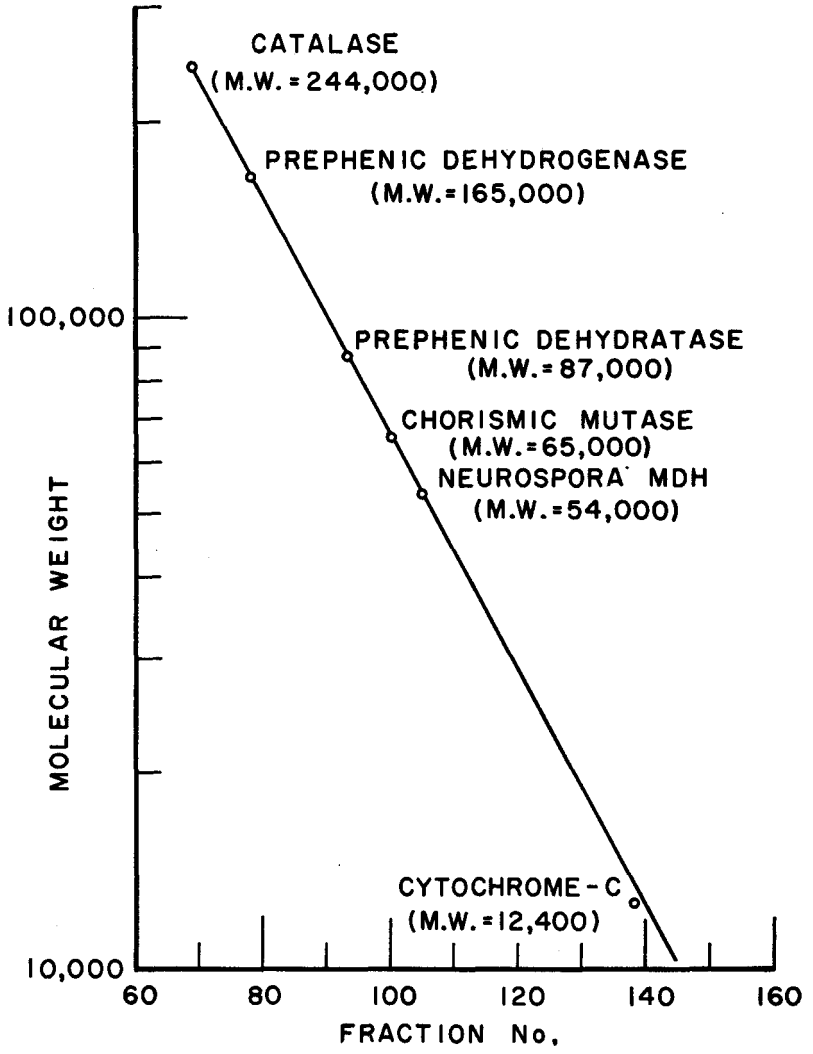


FIGURE 2.—Estimation of molecular weights of the post-chorismic, phenylalanine and tyrosine biosynthetic enzymes of *N. crassa*. The reference markers were catalase (SAMEJIMA, KAMATA, and SHIBATA 1962), Neurospora malate dehydrogenase (MUNKRES 1965), and cytochrome-*c* (NARITA, MURAKAMI, and TITANI 1964).

identical with those known in *E. coli, A. aerogenes* (COTTON and GIBSON 1965), yeast (LINGENS, GOEBEL and UESSELER 1966) and the higher plant Phaseolus (COTTON and GIBSON 1967; GAMBORG and KEELEY, 1966).

The conclusion of METZENBERG and MITCHELL (1958) that prephenic acid is a precursor of phenylalanine but not of tyrosine was based on accumulation studies; i.e., when the phenylalanine-requiring strain No. 5212 (now designated *phen-2*), which accumulates prephenic acid, was crossed with another strain (C-165) which does not, the resulting double mutant did not accumulate pre-

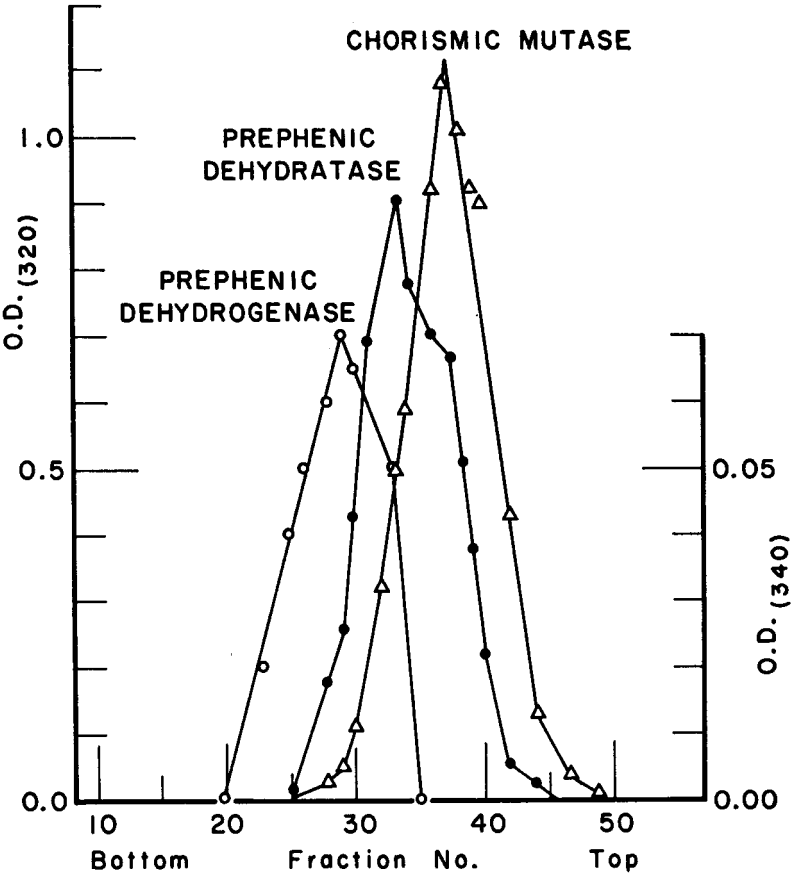


FIGURE 3.—Distribution of activities after centrifugation in a 5–20% sucrose gradient of the post-chorismic, phenylalanine and tyrosine biosynthetic enzymes obtained from wild-type *N. crassa*.

phenic acid. However, when strain No. C-165 was obtained from DR. MARY MITCHELL in 1965 and tested by complementation with the standard testers described in MATERIALS AND METHODS, it was found to be a "leaky polyaromatic" since it did not complement the tester for the *arom* gene cluster.

COLBURN and TATUM (1965), on the other hand, argued that prephenic acid is a precursor of both phenylalanine and tyrosine but they postulated a hypothetical intermediate located between prephenic acid and the keto acids of these two aromatic amino acids. This was based on the finding that the *pt* class of mutants accumulated prephenic acid. However, the conditions of prolonged incubation used to isolate prephenic acid from the culture filtrate of *pt* mutants favors the chemical transformation of chorismate to prephenate (COTTON and GIBSON 1965).

In *E. coli* W and *A. aerogenes* one protein or protein complex carries out both the chorismic mutase activity and the subsequent activity in the biosynthetic pathway (COTTON and GIBSON 1965, 1967). Thus "chorismic mutase P" also

TABLE 5

*Feedback inhibition of prephenic dehydrogenase and prephenic dehydratase by tyrosine and phenylalanine, respectively*

| Concentration of end product added at start of assay | Percent inhibition |
|---|---|
| tyrosine | Prephenic dehydrogenase |
| $2 \times 10^{-6}$M | 13 |
| $2 \times 10^{-5}$M | 45 |
| $1 \times 10^{-4}$M | 61 |
| $1 \times 10^{-3}$M | 87 |
| phenylalanine | Prephenic dehydratase |
| $4 \times 10^{-5}$M | 0.0 |
| $3 \times 10^{-4}$M | 37 |
| $2 \times 10^{-3}$M | 62 |
| $1 \times 10^{-2}$M | 70 |

shows prephenic dehydratase activity and "chorismic mutase T" also shows prephenic dehydrogenase activity. In both cases a single mutational event leads to the loss of one of the mutase activities and the corresponding physically associated activity. Therefore, it appears probable that in these two organisms only two genetic loci are concerned with the synthesis of the keto acid analogues of phenylalanine and tyrosine from chorismic acid.

The *N. crassa* system is clearly different from that found in these two organisms. The *pt* locus, which specifies chorismic mutase, is located in a separate linkage group from the *phen-2* and *tyr-1* loci and a mutational event in this locus leads to the loss of chorismic mutase only (Table 3). Furthermore, in *N. crassa* there is only one molecular species of chorismic mutase (EL-ERYANI 1967; BAKER 1968). BAKER (1968) has also shown that the dehydratase and the mutase are separable by Sephadex gel filtration. However, no active dehydrogenase was recovered. The latter enzyme is quite unstable under a variety of conditions, including the buffer system used by BAKER. It has, nevertheless, been possible in the present studies to recover this enzyme from sucrose density-gradient and Sephadex gel filtration fractions using a phosphate buffer system. However, a loss of more than 50% of the activity occurred. The results illustrated in Figures 2 and 3 demonstrate that prephenic dehydrogenase and prephenic dehydratase are not associated with the single molecular species of chorismic mutase found in *N. crassa.*

The difference between *N. crassa* and the two bacterial species *E. coli* and *A. aerogenes* probably does not signify an evolutionary divergence separating eukaryotes from prokaryotes. The *N. crassa* system is, in essence, similar to that in strain 168 of *B. subtilis* (LORENCE and NESTER 1967). In this organism there is only one chorismic mutase which is specified by a unique genetic locus and there is no evidence that this enzyme is associated with succeeding enyzmes of the pathway. A second strain of *B. subtilis* does, on the other hand, possess two

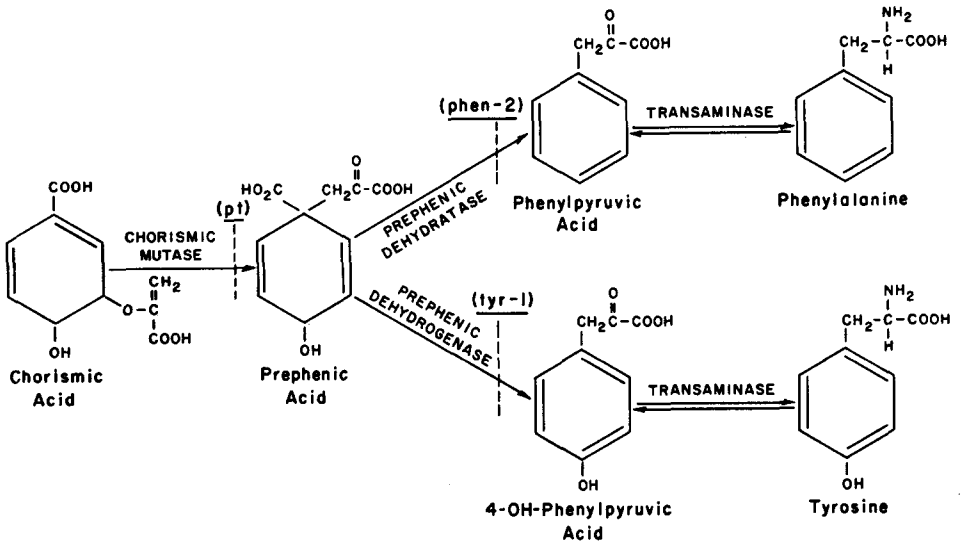


FIGURE 4.—Intermediates and gene-enzyme relationships in the post-chorismic, phenylalanine and tyrosine biosynthetic pathway in *N. crassa*.

additional species of chorismic mutase and these are both encoded by a second single genetic locus, or at least share a common polypeptide. Neither one of the two species is complexed with prephenic dehydrogenase or prephenic dehydratase. The *N. crassa* system appears to be essentially identical to that in *Saccharomyces cerevisiae* (LINGENS, GOEBEL and UESSELER 1966) and Phaseolus (COTTON and GIBSON 1967).

Probably the most interesting finding from genetic analyses of phenylalanine-requiring and tyrosine-requiring mutants of *N. crassa* is the close linkage between *tyr-1* and *phen-2*. These two structural genes encode two separate enzymes which utilize the same substrate, prephenic acid. The two loci may not be contiguous in view of the high prototroph frequency in crosses between the two strains (1–2%). This range is certainly higher than that observed for most complex genetic regions. There is, however, no known genetic marker between *tyr-1* and *phen-2*. This situation suggests a common evolutionary origin for the two genes. One could have arisen from the other by gene duplication followed by divergence.

An intriguing question remains unanswered with regard to the *tyr-2* locus. Like *phen-1* and strain No. NM-160, *tyr-2* is the only mutant of its kind. However, it differs radically from these two strains in that it produces a dehydrogenase with markedly low activity. This low activity cannot be explained as a result of the accumulation of an easily dissociable, reversible inhibitor acting on a normal enzyme, since the enzyme preparations had been subjected to precipitation and dialysis. The observation of a normal inhibitory response to tyrosine argues against the location of the mutant damage in a regulatory subunit.

The patterns of feedback inhibition for phenylalanine and tyrosine biosynthesis appear to be quite different in Neurospora and bacteria. In *N. crassa* tyrosine is a

potent feedback inhibitor of prephenic dehydrogenase, while prephenic dehydratase is inhibited by phenylalanine (Table 5). However, the latter enzyme is only inhibited to 60% by $2 \times 10^{-3}$M phenylalanine. This is in marked contrast with prephenic dehydrogenase which is inhibited more than 80% by $1 \times 10^{-3}$M tyrosine. Chorismic mutase is inhibited by phenylalanine and by tyrosine (BAKER 1966). By contrast, in *B. subtilis* although phenylalanine and tyrosine feedback-inhibit prephenic dehydratase and prephenic dehydrogenase, respectively, they have no appreciable effect on the mutase (NESTER and JENSEN 1966). Furthermore, NESTER (1968) has shown that histidine is an inhibitor of both the dehydrogenase and the dehydratase obtained from *B. subtilis*. By contrast, $1 \times 10^{-2}$M histidine had no effect on the *N. crassa* enzymes.

Despite the phenylalanine requirement of *phen-1* and the tyrosine requirement of strain No. NM-160 (*tyr*), both have essentially wild-type levels of chorismic mutase, prephenic dehydrogenase, and prephenic dehydratase. The reasons for the particular requirements exhibited by these strains have not been clarified.

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## SUMMARY

Genetic and biochemical investigations of phenylalanine-requiring and/or tyrosine-requiring mutants of *Neurospora crassa* have revealed that at least three genetic loci are concerned with the biosynthesis from chorismic acid of the keto acids of these two aromatic amino acids. The following conclusions concerning these three loci have been established: mutants at the *pt* locus lack activity for the enzyme chorismic mutase which converts chorismic acid to prephenic acid; *tyr-1* mutants lack activity for prephenic dehydrogenase, the enzyme which catalyzes the conversion of prephenic acid to 4-OH-phenylpyruvic acid; *phen-2* mutants lack activity (or in the case of the allele previously designated *phen-3* have reduced and unstable activity) for the enzyme prephenic dehydratase which transforms prephenic acid to phenylpyruvic acid. The two loci *tyr-1* and *phen-2* are closely adjacent in linkage group IIIR. Unlike the situation in *E. coli* and *A. aerogenes*, in *N. crassa* these three post-chorismic acid enzymes are not aggregated. They are separable by sucrose density-gradient centrifugation and by Sephadex gel filtration. The activities of prephenic dehydrogenase and prephenic dehydratase are regulated by their end products, tyrosine and phenylalanine, respectively. On the basis of comparisons with wild type, the one available *tyr-2* mutant possesses a prephenic dehydrogenase with relatively low activity. The strains *phen-1* and NM-160 (*tyr*) have essentially wild-type levels of chorismic mutase, prephenic dehydrogenase and prephenic dehydratase. The reasons for the particular requirements exhibited by these two strains have not yet been clarified.

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