*Genet. Res., Camb.* (1968), **12**, *pp.* 103–107
*Printed in Great Britain*



# A new locus in the tryptophan pathway of *Neurospora crassa*

BY MAJEED AHMAD AND A. MOZMADAR
*Department of Botany, University of Dacca, Pakistan*

SHELDON HENDLER
*Department of Biochemistry, College of Physicians and Surgeons, New York, U.S.A.*

(*Received* 5 *December* 1967)

## 1. INTRODUCTION

Tryptophan is metabolized from chorismic acid in five steps in *Neurospora crassa* (Fig. 1)

(1) CA + G $\xrightarrow[\text{anthranilate synthetase}]{\text{tryp-2 and tryp-1}}$ AA

(2) AA + PRPP $\xrightarrow[\text{PR-transferase}]{\text{tryp-4}}$ PRA

(3) PRA $\xrightarrow[\text{PRA isomernse}]{\text{tryp-1}}$ CDRP

(4) CDRP $\xrightarrow[\text{InGP synthetase}]{\text{tryp-1}}$ InGP

(5) InGP + serine $\xrightarrow[\text{tryptophan-synthetase}]{\text{tryp-3}}$ Tryptophan

Figure 1. Intermediates, genes and enzymes involved in tryptophan biosynthesis of *Neurospora crassa*. Abbreviations: CA = chorismic acid, G = glutamine, AA = anthranilic acid, PRPP = 5-phosphoribosyl-1-pyrophosphate, PRA = N-(5-phosphoribosyl) anthranilic acid, CDRP = 1-(0-carboxyphenylamino-1-deoxyribulose-5-phosphate, InGP = indole-3-glycerol phosphate, PR = phosphoribosyl.

These five steps are controlled by four genetic loci (Ahmad & Catcheside, 1960). An aggregate of proteins controlled by genes tryptophan-1 (tryp-1) and tryptophan-2 (tryp-2) catalyses steps 1, 3 and 4 (DeMoss, 1965). Tryp-1 specifies the component which shows anthranilate synthetase, PRA isomerase and InGP synthetase activities while tryp-2 specifies the component which catalyses the anthranilate synthetase reaction in association with the tryp-1 gene product. Tryptophan-4 (tryp-4) controls step 2 while tryptophan-3 (tryp-3) controls step 5.

The present paper reports a fifth locus in the tryptophan pathway and a new mutant for locus nicotinic-tryptophan in *Neurospora crassa*.

## 2. MATERIALS AND METHODS

Mutants were obtained by irradiating conidia of *N. crassa* strain *Emerson a* (*5297*) (*Ema*) with ultraviolet light. The procedure of Ahmad & Catcheside (1960) was followed for isolating them.

Histidine-2 (Y152M14); leucine-3 (R156); arginine-5 (27947); tryptophan-1 (A106);

leucine-1 (33757); pyrimidine-1 (H263); lysine-1 (33933); Lysine-2 (37101); tryptophan-2 (75001); adenine-1 (Y234M419); arginine-10 (B317) and nicotinic tryptophan (65001) were utilized as markers for the seven linkage groups in determining the positions of the two new mutants A370 and A420. Numbers in parentheses give the isolation number of the standard mutant of the different markers used.

Media and methods used for making heterocaryon tests and for making crosses were the same as utilized by Ahmad *et al.* (1964).

Mycelia were grown, harvested, lyophilized, powdered and extracted and fractions of crude extract were prepared according to the procedure of DeMoss (1965) except that cultures were not grown with forced aeration but on a shaker and the crude extract and fractions were not passed over a Sephadex-G25 column. Mutant A420 was grown by supplementing the minimal medium (Vogel, 1956) with 20 mg tryptophan per litre.

*Anthranilate synthetase assay.* The reaction mixture contained 50 $\mu$moles of Tris HCl buffer, pH 7·4, 5 $\mu$moles of $MgCl_2$, 0·5 $\mu$moles of chorismate, 25 $\mu$moles of l-glutamine and one tenth of the enzyme fraction as indicated in Table 2 in a final volume of 1 ml. After incubation at 37 °C for 90 min the reaction was terminated by the addition of 0·1 ml. of 27 % trichloroacetic acid. Proteins were removed by centrifugation and anthranilic acid was determined on samples of the supernatant solutions by the method of Bratton & Marshall (1939) as modified by Eckert (1943). Chorismic acid was prepared by the method of Gibson (1964) as modified by Edwards & Jackman (1965) and dissolved in 0·1 M-potassium phosphate buffer, pH 7·9, before use. Protein was determined according to the method of Warburg & Christian (1941).

## 3. RESULTS

A series of 391 tryptophan auxotrophs of *Neurospora crassa* was obtained. Tests with tryptophan and its precursors showed that fifty-five strains utilized anthranilic acid, indole or tryptophan, 126 utilized indole or tryptophan and 210 grew on tryptophan. Studies on the indole and tryptophan mutants will be reported elsewhere, while studies on the anthranilic acid mutants will be briefly reported here.

Heterocaryon tests among the fifty-five anthranilic acid mutants disclosed four groups. The first and second groups comprised forty-four and nine mutants respectively, while the third and fourth groups were represented by single mutants, *A370* and *A420*, respectively. Linkage studies revealed that the first group of mutants belonged to locus *tryp-2* and the second group of mutants belonged to locus *tryp-1* but the remaining two groups appeared to be new. Crosses of *A370* and *A420* were then made with markers for all the seven linkage groups to locate the two presumed new genes.

Spore counts from the crosses showed that *A370* was located in linkage group VII and occupied the same locus as *nicotinic tryptophan* (*nt*) while *A420* belonged to a new locus located in linkage group V. Crosses of *A370* with *arginine-1* (B317) gave nineteen recombinant spores out of 1522 spores counted. When *A370* was crossed with *nicotinic tryptophan* (65001) no recombinants were obtained out of a spore count of 2891. Since *A370* required nicotinic acid for growth it was a new mutant for the locus *nt*. It may be mentioned that anthranilic acid-utilizing mutants belonging to the loci *tryp-1* (*A106*), *tryp-2* (*A60*), and the new locus (*A420*) did not grow on nicotinic acid.

The exact position of *A420* in linkage group V was next determined through a three point linkage test. *A420* was combined with *lysine-2* (*37101*) (*lys-2*). The double mutant *A420 lys-2* was crossed with *iso-leucine-valine-1* (*16117*) (*iv-1*).

Classification of 1093 single spore cultures from this cross was undertaken as tabulated in Table 1. A linkage map was constructed from the data given in Table 1 and is shown in Fig. 2.

An attempt was made to establish the metabolic block in mutant *A420* by cross-feeding

Table 1. *Classification of 1093 single spore cultures from the cross* A420 lys-2 × iv-1

| Classes of spores | Number of spores |
|---|---|
| Tryptophan, lysine | 439 |
| Isoleucine-valine | 373 |
| Wild type | 19 |
| Tryptophan, lysine isoleucine-valine | 24 |
| Tryptophan, isoleucine-valine | 101 |
| Lysine | 131 |
| Tryptophan | 4 |
| Lysine, isoleucine-valine | 2 |
| Total | 1093 |

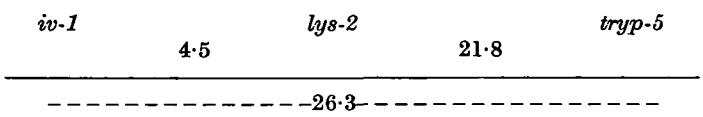


Fig. 2. Map of a section of linkage group V showing the position of the new locus *tryp-5*.

tests. *N. crassa* mutants of the previously known loci (*tryp-1* and *tryp-2*) required anthranilate for growth as does the new mutant *A420*. Hence cross-feeding tests were performed with mutants *A106* (*tryp-1*), *A60* (*tryp-2*) and *A420*. No growth was obtained in any case, although, as mentioned earlier, mutants for the three loci complement one another in heterocaryon tests.

Anthranilate synthetase activity was tested in various enzyme fractions of *A420* and the parent strain *Ema*. The activity of this enzyme (Table 2) was found to be similar in both strains, the highest activity occurring in the 40–50 % saturated ammonium sulphate fraction, as reported by DeMoss (1965).

## DISCUSSION

The data presented show that mutant *A420* occupies a new locus. Since only four loci are known in the tryptophan pathway in *N. crassa* (Ahmad & Catcheside, 1960; Barratt, Newmeyer, Perkins & Garnjobst, 1954), it is proposed to designate the locus occupied by *A420* as *tryptophan-5* (*tryp-5*).

With the establishment of locus *tryp-5*, the number of loci, mutants for which require anthranilate for growth, comes to three. Mutants for all three loci, although heterocaryon compatible, have no growth in cross-feeding tests. DeMoss & Wegman (1965) have shown that both *tryp-1* and *tryp-2* are involved in the control of anthranilate synthetase. The role of locus *tryp-5* remains enigmatic.

The various enzyme fractions of *A420* showed an anthranilate synthetase activity similar to the parent strain *Ema* (Table 2). Since *A420* responds to anthranilate and is found to have an active anthranilate synthetase this mutant is of considerable interest. Its metabolic role is not clear. It is probable, however, that locus *tryp-5* may control the utilization of tryptophan rather than its biosynthesis.

The biochemical studies also indicated the presence of an inhibitor of anthranilate synthetase activity in the crude extract. It is seen in Table 2 that protamine sulphate fraction has a higher specific activity and a higher total activity as compared with the crude extract. This can be explained by assuming that the crude extract had an inhibitor of anthranilate synthetase activity which fractionated out, at least to some extent, during

the protamine sulphate step. Evidence for the presence of an inhibitory factor for anthranilate synthetase activity in *N. crassa* was also found by DeMoss (1965).

A genetic locus analogous to locus *tryp-5* in *N. crassa* has not been reported in bacteria or yeast. It is possible, however, that locus E in *Aspergillus nidulans* (Roberts, 1967; Hütter & DeMoss, 1967) corresponds to locus *tryp-5* in *N. crassa*.

Table 2. *Anthranilate synthetase activity of enzyme fractions of parental wild type* Ema *and of mutant* A420

| Fraction | Specific activity (units/mg) | | Total activity (units)* | | Overall yield (%) | |
|---|---|---|---|---|---|---|
| | *Ema* | *A420* | *Ema* | *A420* | *Ema* | *A420* |
| Crude extract | 0·19 | 0·60 | 51·2 | 140·2 | 100 | 100 |
| Protamine sulphate supernatant | 1·00 | 1·69 | 117·0 | 182·5 | 229 | 130 |
| 0–40 % saturated ammonium sulphate | 0·49 | 1·36 | 16·6 | 33·1 | 32 | 24 |
| 40–50 % saturated ammonium sulphate | 1·52 | 1·89 | 61·1 | 61·2 | 119 | 44 |
| 50–60 % saturated ammonium sulphate | 0·13 | 0·14 | 3·2 | 6·7 | 6 | 5 |

* A unit of enzyme activity is defined as that amount of enzyme which will form 0·1 $\mu$mole of anthranilate in 90 min.

SUMMARY

A new locus, *tryptophan-5*, has been found in *Neurospora crassa*. Its position in linkage group V has been mapped. A single mutant *A420*, isolated for this locus, is not temperature sensitive. It requires anthranilate for growth, but has as much anthranilate synthetase activity as the parental strain.

Thanks are due to Dr P. R. Srinivasan for the facilities to carry out a part of this work, and to Dr Hadassah Tamir for useful discussions.

REFERENCES

AHMAD, M. & CATCHESIDE, D. G. (1960). Physiological diversity amongst tryptophan mutants in *Neurospora crassa*. *Heredity* **15**, 55–64.

AHMAD, M., KHALIL, MD., KHAN, N. A. & MOZMADAR, A. (1964). Structural and functional complexity at the tryptophan-1 locus in *Neurospora crassa*. *Genetics* **49**, 925–933.

BARRATT, R. W., NEWMEYER, D., PERKINS, D. D. & GARNJOBST, L. (1954). Map construction in *Neurospora crassa*. *Adv. Genetics* **6**, 1–86.

BRATTON, A. C. & MARSHALL, E. K. (1939). A new coupling component for sulfanilamide determination. *J. Biol. Chem.* **129**, 537–550.

DEMOSS, J. A. (1965). The conversion of shikimic acid to anthranilic acid by extracts of *Neurospora crassa*. *J. Biol. Chem.* **240**, 1231–1235.

DEMOSS, J. A. & WEGMAN, J. (1965). An enzyme aggregate in the tryptophan pathway of *Neurospora crassa*. *Proc. Natl. Acad. Sci. U.S.A.* **54**, 241–247.

ECKERT, H. W. (1943). Determination of *p*-aminobenzoic acid, conjugated *p*-aminobenzoic acid, and *p*-nitrogenzoic acid in blood. *J. biol. Chem.* **148**, 197–204.

EDWARDS, J. M. & JACKMAN, L. M. (1965). Chorismic acid. A branch point intermediate in aromatic biosynthesis. *Aust. J. Chem.* **18**, 1227–1239.

GIBSON, F. (1964). Chorismic acid: Purification and some chemical and physical studies. *Biochem. J.* **90**, 256–261.

HÜTTER, R. & DEMOSS, J. A. (1967). Enzyme analysis of the tryptophan pathway in *Aspergillus nidulans*. *Genetics* **55**, 233–239.

ROBERTS, C. E. (1967). Genetic analysis of the tryptophan pathway in *Aspergillus nidulans*. *Genetics* **55**, 233–239.

VOGEL, H. J. (1956). A convenient growth medium for *Neurospora* (Medium N). *Microbial Genetics Bull.* **13**, 42–43.

WARBURG, O. & CHRISTIAN, W. (1941). Isolierung und Kristallisation des Garungsferments Enolase. *Biochem. Z.* **310**, 382–421.