JOURNAL OF BACTERIOLOGY, Jan. 1971, p. 121-126

Vol. 105, No. 1
Printed in U.S.A.

Neurospora Mutant Deficient in Tryptophanyl-Transfer Ribonucleic Acid Synthetase Activity

M. NAZARIO, JOHN A. KINSEY, AND MAJEED AHMAD[1]

Department of Biochemistry and Department of Microbiology, University of Kansas Medical Center, Kansas City, Kansas 66103, and Department of Biology, University of Missouri, Kansas City, Missouri 64110

Received for publication 24 August 1970

A tryptophan auxotroph of *Neurospora crassa*, *trp-5*, has been characterized as a mutant with a deficient tryptophanyl-transfer ribonucleic acid (tRNA) synthetase (EC 6.1.1.2) activity. When assayed by tryptophanyl-tRNA formation, extracts of the mutant have less than 5% of the wild-type specific activity. The adenosine triphosphate-pyrophosphate exchange activity is at about half the normal level. In the mutant derepressed levels of anthranilate synthetase and tryptophan synthetase were associated with free tryptophan pools equal to or higher than those found in the wild type. We conclude that a product of the normal tryptophanyl-tRNA synthetase, probably tryptophanyl-tRNA, rather than free tryptophan, participates in the repression of the tryptophan biosynthetic enzymes.

Numerous reports have indicated that amino-acyl-transfer ribonucleic acid (tRNA) synthetases play an important role in the regulation of the amino acid biosynthetic enzymes in bacterial cells (18, 20). Recently McLaughlin et al. (14) have shown that a temperature-sensitive isoleucyl-tRNA synthetase mutant of yeast is derepressed for two isoleucine biosynthetic enzymes when the strain is grown at restrictive temperatures. In *Neurospora* evidence has been presented against the participation of the free amino acid in the repression of the arginine (19) and tryptophan (23) pathways. A mutant, *A420*, recently reported as representing a new tryptophan locus in *Neurospora crassa*, *trp-5* (1), is shown in this report to be deficient in tryptophanyl-tRNA synthetase [L-tryptophan: tRNA ligase(AMP), EC 6.1.12] activity. Results of experiments reported here indicate that the mutation at the *trp-5* locus impairs the ability of the organism to repress the tryptophan biosynthetic enzymes.

MATERIALS AND METHODS

Strains used. *Emerson a* 5297 (*Ema*) and *trp-5* were from the authors' stock collection. (Stocks of *trp-5* are being deposited with the Fungal Genetics Stock Center.) The biochemical assays were performed on strains reisolated from a cross of *Ema* × *trp-5*.

Chemicals. ^{14}C-tryptophan (specific activity, 23 mCi/mmole) was obtained from Amersham-Searle. ^{3}H-tryptophan was purchased from either Amersham-Searle (3,400 mCi/mmole) or from New England Nuclear (6,700 mCi/mmole). It was diluted with unlabeled tryptophan to specific activities of 190 or 335 mCi/mmole, respectively. ^{32}P-pyrophosphate was obtained from New England Nuclear.

Neurospora tRNA was isolated by a modification of the procedure of Zubay (27). Details of the procedure will be published elsewhere. Chorismic acid was isolated and assayed by the procedure of Gibson (7). All other biochemicals were obtained from Calbiochem or Sigma.

Growth conditions. (i) For stationary cultures, 125-ml flasks containing 25 ml of Vogel's medium N (24), supplemented as indicated, were innoculated with conidial suspensions made from 10-day-old cultures. The flasks were incubated for 48 hr at 25 C. Mycelia were then harvested, dried, and weighed. (ii) For shaking cultures, mycelia were grown in 250-ml Erlenmeyer flasks containing 40 ml of Vogel's medium N supplemented as indicated and innoculated with a fresh conidial suspension so as to give 10^6 conidia/ml. The flasks were incubated for 24 hr on a gyratory shaker at approximately 200 excursions/min at 30 C. The mycelia were harvested by filtration, washed with distilled water on Büchner funnels, frozen, and then lyophilized. All subsequent experiments were performed with extracts made with powdered lyophilized mycelia.

Preparation of extracts. Suspensions of powdered mycelia [60 mg per ml, in 0.03 M tris(hydroxymethyl) aminomethane (Tris)-acetate buffer (*p*H 8.0), made 1 mM in both mercaptoethanol and dipotassium ethylenediaminetetraacetic acid] were sporadically stirred for 45 min at 0 to 4 C and centrifuged for 40 min at 16,000 × *g*. Crude extracts were used in the tryptophan synthetase and formyl-kynureninase assays. Anthranilate synthetase, kynureninase, and tryptophanyl-tRNA synthetase were assayed on extracts filtered through columns (0.9 by 30 cm) of Sephadex G-25 (fine) equilibrated with the buffer used in the extraction.

Tryptophanyl-tRNA synthetase. (i) For the adenosine

[1] Senior Foreign Scientist Fellow of the National Science Foundation. On leave from the Department of Botany, University of Dacca, Pakistan.

triphosphate (ATP)-^{32}P-pyrophosphate exchange assay, the reaction mixture contained: Tris-acetate buffer (*p*H 8.0), 60 μmoles; $MgCl_2$, 4 μmoles; ATP, 1 μmole; ^{32}P-pyrophosphate (specific activity, 10^5 to 5×10^5 counts per min per μmole), 1 μmole; L-tryptophan, 0.1 μmole; KF, 5 μmole; and enzyme in a final volume of 0.50 ml. Incubations were for 20 or 30 min at 35 C. The reaction was stopped by addition of 0.6 ml of 0.5 M perchloric acid followed by 0.2 ml of a 10% suspension of acid-washed Norit. The samples were mixed, filtered through 2.4-cm Whatman GF/C glass-fiber circles and washed seven times with 4-ml portions of water. The filters were inverted on stainless-steel planchets, dried, and counted with a Nuclear-Chicago D-47 gas flow detector. Blanks were without tryptophan. One unit of activity is equivalent to 1 nmole of pyrophosphate exchanged per hr. (ii) For ^{3}H-tryptophanyl-tRNA formation, the reaction mixture contained: Tris-acetate buffer (*p*H 8.0), 60 μmoles; $MgCl_2$, 4 μmoles; ATP, 2 μmoles; *Neurospora* tRNA, 0.8 to 1.0 mg; ^{3}H-tryptophan, 6 to 11 nmoles, or ^{14}C-tryptophan, 8.7 nmoles; enzyme and 0.1% bovine serum albumin to a final volume of 0.50 ml. The reaction was started by the addition of the labeled tryptophan. After 5 or 10 min at 35 C, the reaction was stopped with 3.3 ml of 70% ethanol saturated with NaCl. After centrifugation at −10 C the pellet was washed twice by resuspension in 3.3 ml of the ethanol-NaCl solution and once in 3.3 ml of 70% ethanol. The tubes were drained thoroughly, and the pellets were then dissolved and transferred quantitatively to a scintillation vial with a total volume of 0.25 ml of water. Bray's solution (10 ml) was added, and the vials were counted in a Nuclear-Chicago liquid scintillation spectrometer with an efficiency of approximately 12% for ^{3}H and 67% for ^{14}C. Blanks were without enzyme or were zero-time controls.

One enzyme unit catalyzes the attachment of 1 nmole of tryptophan to tRNA per hr. Specific activities are expressed as units per milligram of protein. Protein was determined by the procedure of Lowry et al. (13) or by the biuret technique (17), by using bovine serum albumin as the standard.

Anthranilate synthetase. For anthranilate synthetase, the reaction mixture contained: potassium phosphate (*p*H 7.0), 100 μmoles; $MgCl_2$, 10 μmoles; L-glutamine, 20 μmoles; chorismic acid, 0.3 μmoles; enzyme and water to a final volume of 1.0 ml. The reaction was carried out at 37 C in the cuvette of an Aminco-Bowman spectrophotofluorometer. The reaction was started by the addition of chorismic acid. Increases in fluorescence were read at 1-min intervals for several minutes by using 315 and 400 nm as excitation and emission wavelengths, respectively. Specific activities are units (nanomoles of anthranilic acid formed per hour) per milligram of protein.

Kynureninase. For kynureninase, the incubation mixture contained: Tris-acetate buffer (*p*H 8.0), 50 μmoles; pyridoxal phosphate, 0.02 μmoles; $MgSO_4$, 2.5 μmoles; L-kynurenine, 0.16 μmoles; and enzyme in a final volume of 1.2 ml. The reaction was started by addition of kynurenine. Anthranilic acid formation was followed as described for anthranilate synthetase. Specific activities are units (nanomoles of anthranilic acid formed per hour) per milligram of protein.

Tryptophan synthetase. For tryptophan synthetase (EC 4.2.1.20), the method of Yanofsky (26) was used. Specific activities are units (micromoles of indole converted per hour) per milligram of protein.

Formyl kynureninase. For formyl kynureninase, the method of Knox (11) was used. Specific activities are units (micromoles of kynurenine formed per hour) per milligram of protein.

Tryptophan pool determination. Mycelial powder (50 mg) was extracted with 5 ml of water in a boiling-water bath for 15 min. The extract was cleared by filtration and centrifugation. Tryptophan content was determined by a modification of the tryptophanase technique of DeMoss (2). Hot-water extract (1 ml) was incubated for 1 hr at 37 C in a final volume of 1.3 ml with 1 mg of tryptophanase (Sigma), 0.08 μmole of pyridoxal phosphate, and 130 μmoles of potassium phosphate (*p*H 7.8). Toluene (2 ml) was added to each tube. The amount of indole produced was measured in portions of the toluene layer by the method of Yanofsky (26). The results of this assay were confirmed by the use of a bioassay utilizing *Pediococcus cerevisiae* (ATCC 8042, reference 8). The intracellular tryptophan concentration was calculated by the use of the formula of Slayman and Tatum (21).

RESULTS

Properties of the mutant. The *trp-5* mutant responds well to tryptophan, indole, or anthranilic acid, but not to shikimic acid (Table 1). From the observation that the mutant does not require tyrosine or phenylalanine, we may conclude that it is not blocked before the formation of chorismic acid. In addition, *trp-5* has elevated levels of anthranilate synthetase activity. Mixing of anthranilate synthetase extracts from *trp-5* and *Ema* gave an intermediate specific activity value. Preparations of anthranilate synthetase, obtained from *Ema* and *trp-5*, were purified through the ammonium sulfate step and analyzed by sucrose density gradient centrifugation by the method of DeMoss and Wegman (3). No differences were obtained in their sedimentation velocities (*unpublished data*).

These observations suggested the possibility that the metabolic step affected by mutation at *trp-5* could be beyond tryptophan formation. A direct test of this possibility revealed that the mutant had drastically reduced levels of tryptophanyl-tRNA synthetase activity.

When assayed for ^{3}H-tryptophanyl-tRNA formation, extracts of the mutant have less than 5% of the specific activity found in the parental wildtype, *Ema* (Table 2). In addition Table 2 shows that mixtures of extracts of *Ema* and *trp-5* gave no indication as to the presence, in *trp-5* extracts, of an inhibitor. The apparent K_m values for tryptophan (^{3}H-tryptophanyl-tRNA formation assay) were 6.7×10^{-7} M for *Ema* and 16.7×10^{-7} M for *trp-5*. By using the ATP-^{32}P-pyrophosphate exchange reaction, the specific activities in extracts of the mutant usually vary from

TABLE 1. *Growth response of trp-5 to different concentrations of shikimic acid, anthranilic acid, and tryptophan*[a]

Concn of supplement (mM)	Wt of mycelia (mg)		
	Shikimic acid	Anthranilic acid	Trypto-phan
0.10	Nil	14.1	20.9
0.25	Nil	13.4	18.3
0.50	Nil	12.0	16.8
1.00	Nil	6.6	16.0
2.50	Nil	4.1	16.9

[a] Stationary cultures grown as described in the text.

TABLE 2. *Tryptophanyl-tRNA synthetase activity in extracts of Ema and trp-5*[a]

Extract	^{14}C-trp-tRNA formed in 5 min (pmoles)
Ema, 52 μg of protein	56.2
trp-5, 120 μg of protein	5.4
Ema (52 μg) plus *trp-5* (120 μg)	65.3

[a] Both extracts were prepared from cultures grown in the presence of 0.1 mM L-tryptophan.

approximately one-third to two-thirds of the values in comparable preparations from the wild type. Table 3 shows the specific activities found in extracts of *Ema* and *trp-5* grown in the presence of different concentrations of tryptophan. The ratio of ATP-pyrophosphate exchange activity to tryptophanyl-tRNA formation activity for a given extract varied between approximately 5 and 14 for the wild type, whereas it was several hundred for the mutant.

Growth response to tryptophan. A growth response curve to tryptophan is shown in Fig. 1. At concentrations less than 0.25 mM the growth of *trp-5* is limited. It is interesting to note that tryptophan concentrations higher than 0.25 mM are inhibitory to *Ema*.

Biosynthetic enzymes. The specific activities of anthranilate synthetase and tryptophan synthetase were measured on extracts made from mycelia grown at different concentrations of tryptophan. The results are shown in Fig. 2a and b. At a growth-limiting tryptophan concentration (0.05 mM), the ratio of specific activities in *trp-5/Ema* was 7.6 for anthranilate synthetase and 4.0 for tryptophan synthetase. Even at a concentration of tryptophan several fold in excess of that required to allow maximal growth of the mutant (0.75 mM), the activities of both biosynthetic enzymes are not fully repressed in the mutant.

Tryptophan pools. It was of interest, in view of the derepressed levels of the two biosynthetic enzymes, to examine the intracellular concentrations of free tryptophan (Fig. 3). Of particular significance is the fact that, at an external tryptophan concentration of 0.05 mM, the intracellular concentration of tryptophan in *trp-5* and *Ema* is essentially the same.

Catabolic enzymes. The specific activities of two enzymes in the tryptophan-anthranilate cycle, kynureninase and formyl kynureninase, were determined. The results (Fig. 4) indicate different responses for the two enzymes.

DISCUSSION

Because of the indispensable function that aminoacyl-tRNA synthetases have in the formation of macromolecular intermediates of protein synthesis, the isolation of an aminoacyl-tRNA synthetase mutant, as an amino acid auxotroph with an absolute requirement for the amino acid,

TABLE 3. *Tryptophanyl-tRNA synthetase-specific activities in Ema and trp-5 grown at different concentrations of tryptophan*

Strain	Concn[a]	Tryptophanyl-tRNA synthetase-specific activity	
		Exchange assay[b]	Formation assay[c]
Ema	0	99	15.6
	0.05	142	11.7
	0.25	139	10.7
	0.75	78	16.4
Trp-5	0.05	114	0.20
	0.25	91	0.10
	0.75	35	0.09

[a] Concentration (millimolar) of tryptophan in growth medium.

[b] Adenosine triphosphate-^{32}P-pyrophosphate exchange assay.

[c] ^{3}H-tryptophanyl-tRNA formation assay.

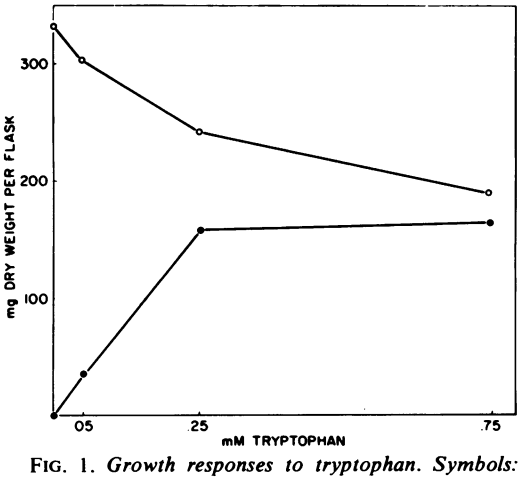

FIG. 1. *Growth responses to tryptophan. Symbols:* O, *Ema;* ●, *trp-5.*

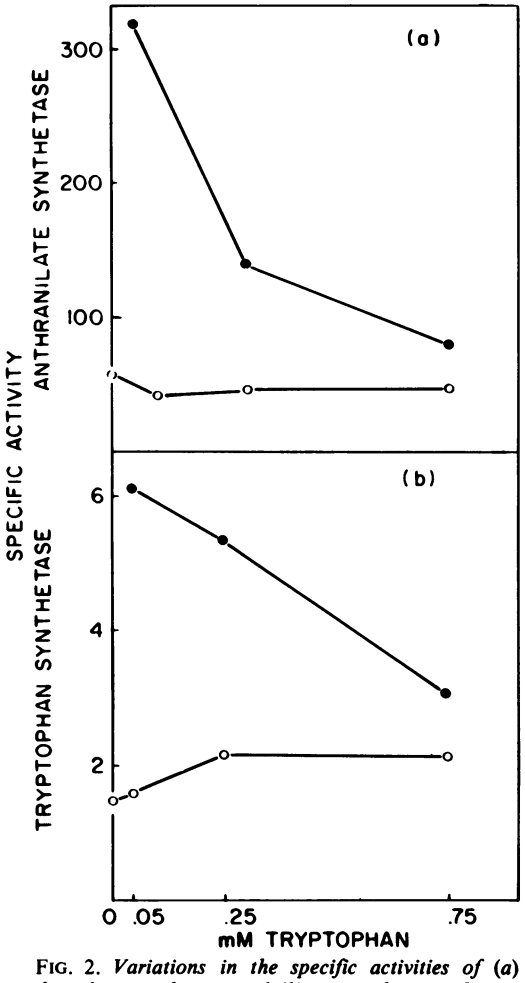

FIG. 2. *Variations in the specific activities of (a) anthranilate synthetase and (b) tryptophan synthetase as a function of the tryptophan concentration in the media. Symbols:* O, *Ema;* ●, *trp-5.*

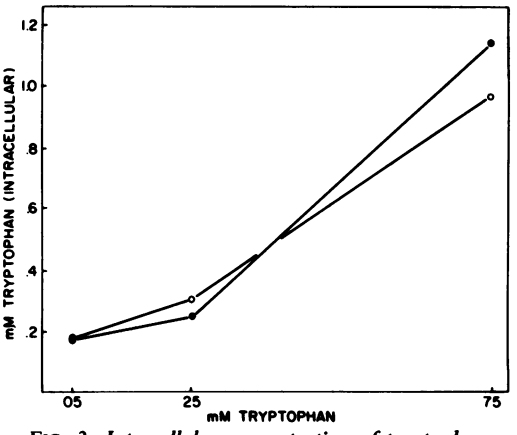

FIG. 3. *Intracellular concentration of tryptophan as a function of the concentration of tryptophan in the media. Symbols:*O, *Ema;*●, *trp-5.*

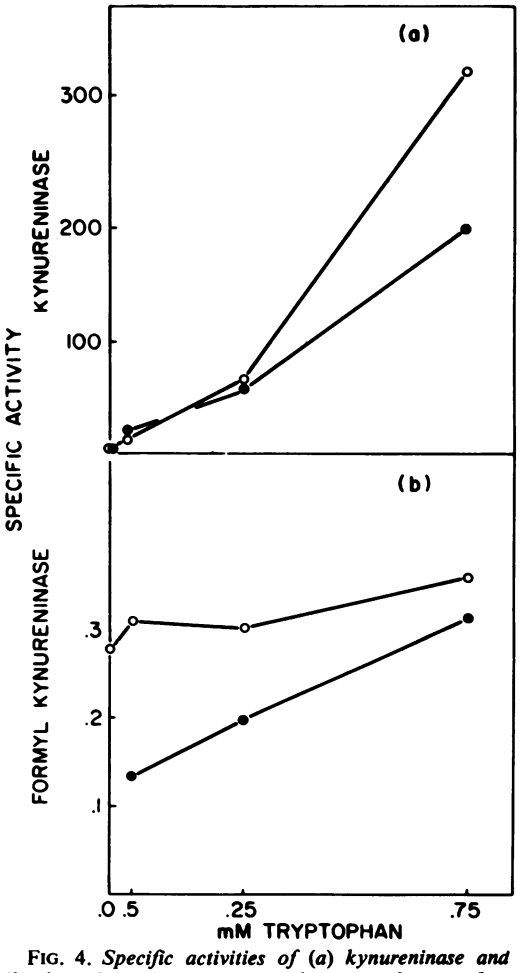

FIG. 4. *Specific activities of (a) kynureninase and (b) formyl kynureninase as a function of tryptophan concentration in the media. Symbols:* O, *Ema;* ●, *trp-5.*

was rather unexpected. However, such mutants have recently been reported in bacteria for histidine, glycine, tyrosine, methionine, and tryptophan (*see* reference 18).

The tryptophanyl-tRNA synthetase activity of *trp-5* resembles the enzyme of the mutants of the *trpS* locus of *Escherichia coli* isolated by Doolittle and Yanofsky (4) in that they have substantial activity in the ATP-pyrophosphate exchange reaction, whereas the activity in the overall reaction is less than 5% of the wild-type activity (Table 3). A similar situation was described earlier by Eidlic and Neidhardt (5) for a temperasensitive valyl-tRNA synthetase mutant of *E. coli.*

The apparent K_m value for tryptophan of 6.7×10^{-7} reported here for *Ema* agrees closely with the value, 6.5×10^{-7}, reported by Ito et al. (9)

for tryptophanyl-tRNA synthetase from *E. coli*. The K_m value for *trp-5* is 16.7×10^{-7}.

Doolittle and Yanofsky (4) and Ito et al. (9) have found that addition of tryptophan, along with ATP and Mg^{2+}, stabilizes the tryptophanyl-tRNA synthetase activity in extracts of *trpS* mutants. Our K_m determinations may undergo revision if conditions for stabilization of the tryptophanyl-tRNA synthetase from *trp-5* are found and purification of the enzyme becomes feasible. The difference between the K_m of the wild-type and the mutant tryptophanyl-tRNA synthetase presented here (2.5-fold higher) is of the same order as previously reported for other aminoacyl-tRNA synthetase mutants and their respective wild types (9, 22).

It appears likely that *trp-5* is the structural gene for tryptophanyl-tRNA synthetase. Demonstration of this point, however, will require the isolation, mapping and enzymatic characterization of partial revertants.

In bacterial cells aminoacyl-tRNA synthetases play a central role in metabolic regulation (18, 20). Recently, a similar role has been demonstrated in a eucaryote, yeast, by McLaughlin et al. (14). In *Neurospora*, however, there has been only indirect evidence for such a role (19, 23). In this report we have shown that two biosynthetic enzymes in the tryptophan pathway are derepressed four- to sevenfold under conditions where the mutant's growth is limited by low concentrations of tryptophan. Under the same conditions the pools of free tryptophan in the wild type and the mutant are essentially the same. The level of derepression that we have found is slightly higher than that which has been reported when tryptophan auxotrophs have been starved for tryptophan (12, 15). Perhaps even more significant is the fact that, at high external tryptophan concentrations, neither tryptophan synthetase nor anthranilate synthetase is fully repressed in the mutant—in spite of the fact that the pool of free tryptophan has increased substantially and that the mutant has attained its maximal growth. On the basis of these observations it appears highly unlikely that free tryptophan is involved in bringing about repression.

Kynureninase is known to be inducible in *Neurospora* (10, 25). It has been routinely used as an indication of the activity of the tryptophan-anthranilate cycle. In this study, as has previously been implied (10, 25), the activity of kynureninase increased with the intracellular concentration of free tryptophan. This is true both for *trp-5* and *Ema*. The activity of formyl kynureninase (Fig. 3b) shows a completely different pattern of response. It appears to be essentially constant in *Ema*, whereas it increases moderately in *trp-5*. We have no explanation at present for the difference in response to tryptophan of the two catabolic enzymes.

In considering any regulatory responses in the tryptophan pathway, it is necessary to take into consideration the existence of two physiologically separate pools of tryptophan in *Neurospora* (16). So far no quantitative data on the size of these two pools are available. However, unless the relative sizes of the two pools in *trp-5* are substantially different from the values found in either wild type or other tryptophan auxotrophs our conclusions should be valid.

Although tryptophanyl-tRNA synthetase from *trp-5* has very little activity in the overall reaction, it retains substantial activity for the ATP-pyrophosphate exchange reaction. This observation is in line with those of Eidlic and Neidhardt (5) and Freundlich (6) for valyl-tRNA synthetase in suggesting that it is the amino-acyl-tRNA or a derivative of it, rather than a product of the activation reaction, which acts in repression.

ACKNOWLEDGMENTS

We acknowledge the excellent technical assistance of Carol Heiman and Ruth Wallace. This work was aided by grant E-522 of the American Cancer Society.

LITERATURE CITED

1. Ahmad, M., A. Mozmadar, and S. Hendler. 1968. A new locus in the tryptophan pathway of *Neurospora crassa*. Genet. Res. **12**:103-107.
2. DeMoss, J. A. 1962. Studies on the mechanism of the tryptophan synthetase reaction. Biochim. Biophys. Acta **62**:279-293.
3. DeMoss, J. A., and J. Wegman. 1965. An enzyme aggregate in the tryptophan pathway of Neurospora crassa. Proc. Nat. Acad. Sci. U.S.A. **54**:241-247.
4. Doolittle, W. F., and C. Yanofsky. 1968. Mutants of *Escherichia coli* with an altered tryptophanyl-transfer ribonucleic acid synthetase. J. Bacteriol. **95**:1283-1294.
5. Eidlic, L., and F. C. Neidhardt. 1965. Protein and nucleic acid synthesis in two mutants of *Escherichia coli* with temperature-sensitive aminoacyl ribonucleic acid synthetases. J. Bacteriol. **89**:706-711.
6. Freundlich, M. 1967. Valyl transfer RNA: role in repression of the isoleucine-valine enzymes in *Escherichia coli*. Science **157**:823-824.
7. Gibson, F. 1968. Chorismic acid, p. 94-97. *In* W. E. M. Lands (ed.), Biochemical preparations, vol. 12. John Wiley & Sons Inc., New York.
8. Henderson, L. M., and E. E. Snell. 1948. A uniform medium for determination of amino acids with various organisms. J. Biol. Chem. **172**:15-29.
9. Ito, K., S. Hiraga, and T. Yura. 1969. Tryptophanyl transfer RNA synthetase and the expression of the tryptophan operon in the *tryp S* mutants of *Escherichia coli*. Genetics **61**:521-538.
10. Jacoby, W. B., and D. M. Bonner. 1953. Kynureninase from *Neurospora:* purification and properties. J. Biol. Chem. **205**:699-707.
11. Knox, W. E. 1955. Tryptophan oxidation, p. 242-253. *In* S. P. Colowick and N. O. Kaplan (ed.), Methods in enzymology, vol. 2. Academic Press Inc., New York.
12. Lester, G. 1961. Some aspects of tryptophan synthetase formation in *Neurospora crassa*. J. Bacteriol. **81**:964-973.
13. Lowry, O. J., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265-275.
14. McLaughlin, C., P. T. Magee, and L. H. Hartwell, 1969. Role of isoleucyl-transfer ribonucleic acid synthetase in ribonucleic acid synthesis and enzyme repression in yeast. J. Bacteriol. **100**:579-584.

15. Matchett, W. H., and J. A. DeMoss. 1962. Factors affecting increased production of tryptophan synthetase by a TD mutant of *Neurospora crassa*. J. Bacteriol. **83**:1294-1300.
16. Matchett, W. H., and J. A. DeMoss. 1964. Physiological channeling of tryptophan in *Neurospora crassa*. Biochim. Biophys. Acta **86**:91-99.
17. Mokrasch, L. C., and R. W. McGilvery. 1956. Purification and properties of fructose-1,6-diphosphatase. J. Biol. Chem. **221**:909-917.
18. Murgola, E. J., and E. A. Adelberg. 1970. Mutants of *Escherichia coli* K-12 with an altered glutamyl-transfer ribonucleic acid synthetase. J. Bacteriol. **103**:178-183.
19. Nazario, M. 1967. The accumulation of argininosuccinate in *Neurospora crassa*. II. Inhibition of arginyl-tRNA synthesis by argininosuccinate. Biochim. Biophys. Acta **145**:146-152.
20. Neidhardt, F. C. 1966. Roles of amino acid activating enzymes in cellular physiology. Bacteriol. Rev. **30**:701-719.
21. Slayman, C. W., and E. L. Tatum. 1964. Potassium transport in *Neurospora*. I. Intracellular sodium and potassium concentrations and cation requirements for growth. Biochem. Biophys. Acta **88**:578-592.
22. Szentirmai, A., M. Szentirmai, and H. E. Umbarger. 1968. Isoleucine and valine metabolism of *Escherichia coli*. XV. Biochemical properties of mutants resistant to thiaisoleucine. J. Bacteriol. **95**:1672-1679.
23. Turner, J. R., and W. H. Matchett. 1968. Alteration of tryptophan-mediated regulation in *Neurospora crassa* by indoleglycerol phosphate. J. Bacteriol. **95**:1608-1614.
24. Vogel, H. J. 1956. A convenient growth medium for *Neurospora (medium N)*. Microbial Genet. Bull. **13**:42-43.
25. Wainwright, S. D., and D. M. Bonner. 1959. On the induced synthesis of an enzyme required for biosynthesis of an essential metabolite: induced kynureninase synthesis in *Neurospora crassa*. Can. J. Biochem. Physiol. **37**:741-750.
26. Yanofsky, C. 1955. Tryptophan synthetase from *Neurospora*, p. 233-238. *In* S. P. Colowick and N. O. Kaplan (ed.), Methods in enzymology, vol. 2. Academic Press Inc., New York.
27. Zubay, G. 1962. The isolation and fractionation of soluble ribonucleic acid. J. Mol. Biol. **4**:347-356.