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Histidine-Mediated Control of Tryptophan Biosynthetic Enzymes in *Neurospora crassa*

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The formation of the five tryptophan biosynthetic enzymes of *Neurospora crassa* was shown to be derepressed in histidine-starved cells. This histidine-mediated derepression was not due to a lowered intracellular concentration of tryptophan in these cells. Furthermore, histidine-mediated derepression of tryptophan enzymes was found to be coordinate and not subject to reversal by tryptophan of either exogenous or biosynthetic origin. The synthesis of tryptophan enzymes also was found to be coordinate in cells which were not histidine-starved. Although histidine is clearly involved in regulating the synthesis of tryptophan enzymes, it did not prevent either tryptophan-mediated derepression of tryptophan enzymes or indole-3-glycerol phosphate-mediated derepression of tryptophan synthetase.

Tryptophan biosynthesis in *Neurospora crassa* involves the following five enzymatic steps (11, 37, 39): chorismic acid $\xrightarrow{I}$ anthranilic acid $\xrightleftharpoons{II}$ *N*-(5'-phosphoribosyl)anthranilic acid (PRA) $\xrightarrow{III}$ 1-(*o*-carboxyphenylamino)-1-deoxyribulose-5-phosphate (CDRP) $\xrightarrow{IV}$ indole-3-glycerol phosphate (InGP) $\xrightarrow{V}$ tryptophan. The five tryptophan enzymes indicated here are: I, anthranilate synthetase; II, anthranilate-5-phosphoribosylpyrophosphate phosphoribosyltransferase (PR transferase); III, *N*-(5'-phosphoribosyl)anthranilic acid isomerase (PRA isomerase); IV, InGP synthetase; V, tryptophan synthetase.

In *Neurospora*, four different types of control of formation of these enzymes have been discerned. (i) The existence of tryptophan-mediated control can be inferred from the two- to fivefold derepression of tryptophan enzymes which occurs in tryptophan mutants starved for tryptophan (21–25). This degree of derepression is about the same as that of other biosynthetic enzymes in *Neurospora;* e.g., aspartate carbamoyltransferase formation in *Neurospora* can be derepressed only two- to threefold (13), whereas a 1,000-fold derepression occurs in *Escherichia coli* (33). (ii) InGP, the physiological substrate of tryptophan synthetase (39), will cause derepression of tryptophan synthetase (34), but not of the other tryptophan enzymes (Lester, *personal communication*). InGP-mediated derepression is scarcely decreased by tryptophan (25, 34). (iii) The histidine intermediate imidazoleglycerol phosphate also derepresses the formation of tryptophan synthetase (34), but not of the other tryptophan enzymes (Cleary, Carsiotis, and Lester, *unpublished data*). Its derepressive effect also is scarcely decreased by tryptophan (34; T. Cleary, Ph.D. Thesis, Univ. of Cincinnati, Cincinnati, Ohio, 1969). (iv) Finally, we reported that derepression of InGP synthetase and tryptophan synthetase occurred in histidine auxotrophs grown in a histidine-limited medium (7). Evidence will be presented herein that the first three tryptophan enzymes are also derepressed in histidine-starved cells. We also report on studies of tryptophan-mediated, histidine-mediated, and InGP-mediated control of tryptophan biosynthetic enzymes in *Neurospora*.

MATERIALS AND METHODS

The histidine auxotrophs and their two parental wild-type strains 74A and Em a were identical to the strains used previously (7). Two heterocaryons, [(K80a) + (K595a)] and [(K80a) + (K153a)], composed of complementing intragenic (*his-2*) mutants (8), were formed by mixing conidia on agar slants prepared from unsupplemented growth medium (see below). His-3A, hlp is a mutant lacking the third enzyme of histidine biosynthesis (2), and is histidinol-permeable (hlp) because of another (unmapped) mutation (8, 36); it was obtained from M.E. Case. All other auxotrophs were obtained from the Fungal Genetics Stock Center, Dartmouth College, Hanover, N.H.

Growth procedures. The growth medium used was Vogel's medium N (35) containing 2% sucrose; any

supplements are indicated. After inoculation, all cultures were incubated at 30 C on a reciprocating shaker.

Samples of mycelia were obtained from cultures grown in three different ways, designated procedures A, B, and C. In procedure A, 50 ml of growth medium in a 250-ml flask was inoculated with 5×10^6 conidia; after growing for the periods of time indicated, the mycelia were collected. Procedure B, which permitted the determination of the differential rate of enzyme synthesis, was performed as follows. One liter of growth medium supplemented with 0.34 mM histidine was inoculated with 10^8 conidia of strain K85a and incubated for 21 hr. The mycelia from a 200-ml portion of the culture were filtered on a Büchner funnel and washed thereon with sterile water; the mycelia were not packed firmly or allowed to become dry. A sterile spoon was used to transfer the washed mycelia to a 1-liter flask containing 200 ml of unsupplemented growth medium. Two other flasks containing 200 ml of growth medium supplemented with either indole (0.27 mM) or tryptophan (0.76 mM) were inoculated in the same way. A fourth 200-ml portion of the culture was poured directly into an empty sterile flask. The mycelia contained in the remaining 200-ml portion of the culture were then collected as described below. They served as the zero-time sample for the ensuing incubation of the four newly inoculated flasks. The newly inoculated flasks were incubated for 6 hr, whereupon the mycelia were collected as described below. In procedure C, 600 ml of growth medium supplemented with 0.34 mM histidine was inoculated with 6×10^8 conidia. Mycelial samples were collected after 16, 24, and 42 hr of incubation.

All mycelial samples were collected by filtration on a Büchner funnel, washed with distilled water, frozen, and lyophilized.

Assay procedures. Extracts were prepared from lyophilized mycelia as before (7), with the use of 0.05 M potassium phosphate (*p*H 7.0) containing 10^{-4} M ethylenediaminetetraacetate. Two of the enzyme activities (anthranilate synthetase and PR transferase) in these extracts failed to give a linear response with increasing amounts of extract. After passage of 1 ml of extract through a column of Sephadex G-25 (1 by 6 cm), a linear response was obtained. The five tryptophan biosynthetic enzymes in Sephadex-treated extracts were assayed according to published methods: anthranilate synthetase (12), PRA isomerase (17), PR transferase and InGP synthetase (37), and tryptophan synthetase (38). All enzymes were assayed at 37 C except anthranilate synthetase (31 to 32 C). One unit of enzymatic activity catalyzes the formation of 1 μmole of product or the disappearance of 1 μmole of substrate per min. Protein concentration was measured by a modification of the biuret method (40). Specific activity is expressed as units of the respective enzymatic activity per milligram of protein.

The size of the mycelial tryptophan pool was determined by measuring the tryptophan, with tryptophanase (10), in extracts which had not been Sephadex-treated.

RESULTS

Anthranilate synthetase, PR transferase, and PRA isomerase in histidine auxotrophs. We measured the activity of the first three tryptophan enzymes in histidine mutants grown in a histidine-limited medium. The specific activity of anthranilate synthetase and PR transferase synthesized in the 22 mutants which were tested was two- to threefold higher than that of the same enzymes synthesized by their respective parental wild-type strains (Table 1). The specific activity of PRA isomerase synthesized in the 14 histidine mutants which were tested was also considerably higher than that of the enzymes synthesized by the parental wild-type strain (Table 1). The 22 histidine mutants tested included one or more mutants at each of the nine histidine structural genes. As expected, histidine starvation of histidine mutants also caused a fivefold derepression of histidinolphosphate aminotransferase, a histidine biosynthetic enzyme (Jones, *unpublished data*).

The observed increases in specific activities might have resulted from activation of the tryptophan enzymes during the assays by (unknown) material exclusively present in the mutant extracts. This possibility seemed remote because of the gel filtration step used in preparing these extracts. It was disproven by the close agreement of the calculated and observed activities obtained when extracts of three different classes of histidine mutants were mixed with that of the wild-type strain (Table 2).

Effect of histidine concentration. Our previous work (7) showed that the concentration of histidine in the growth medium affected the formation of InGP synthetase and tryptophan synthetase in histidine auxotrophs. An identical effect of histidine concentration on the formation of anthranilate synthetase, PR transferase, and PRA isomerase was observed (Fig. 1); the InGP synthetase and tryptophan synthetase activities in the same extracts are included in Fig. 1 for comparison. It is evident that the tryptophan enzymes were increased only in the histidine-starved cells. The intracellular tryptophan concentration was measured in the same experiment and showed little variation (Table 3). This implied that the increased synthesis of tryptophan enzymes was not due to a lowered concentration of endogenous tryptophan.

These data clearly established that a low level of exogenous histidine in the growth medium caused derepression of tryptophan enzymes in histidine auxotrophs. To prove that a low level of endogenously generated histidine would be

TABLE 1. *Enzymatic activities of histidine mutants and parental wild-type strains*[a]

Expt no.	Strain	His allele	Mycelial dry wt	Specific activity (10^{-3} units/mg)		
				Anthranilate synthetase	PR transferase[b]	PRA isomerase[c]
			mg/ml			
1[d]	K744a	1	3.4	3.0	6.7	24.7
	K85a	1	2.5	3.2	7.2	23.6
	K90a	1	2.4	3.5	9.8	19.8
	K74a	2	2.8	3.5	6.2	21.0
	K26a	3D	2.9	3.0	6.3	18.3
	K53a	3D	2.3	3.8	8.8	20.3
	K937a	4	4.0	2.8	5.8	13.0
	C141a	4	4.7	3.8	9.0	22.5
	K54a	5	2.2	3.3	5.7	15.8
	K52a	5	2.5	3.3	7.2	17.3
	K694a	6	3.8	3.3	6.0	15.0
	K692a	6	2.5	3.5	7.0	16.2
	K620a	7	2.4	3.7	7.3	12.8
	K277a	7	3.1	2.5	6.7	16.3
	Em a	—	7.6	1.3	3.0	4.5
	Em a[e]	—	8.2	1.3	3.3	ND[g]
2[f]	A-5	1	4.3	2.0	6.0	ND
	A-11	1	4.0	2.7	6.3	ND
	A-30	1	4.9	3.3	5.7	ND
	M43	2	4.1	2.5	6.7	ND
	M111	3A,B,D	4.3	2.3	6.8	ND
	M108	5	4.5	2.7	7.3	ND
	M105	6	5.2	2.5	7.8	ND
	M31	7	4.8	2.7	8.8	ND
	74A	—	8.7	1.0	3.5	ND
	74A[e]	—	9.6	0.8	3.8	ND

[a] All strains were grown by procedure A for 48 hr.

[b] Anthranilate-5-phosphoribosylpyrophosphate phosphoribosyltransferase.

[c] *N*-(5'-phosphoribosyl)anthranilic acid isomerase.

[d] Growth medium supplemented with 1.7×10^{-4} M L-histidine except where noted.

[e] No histidine in growth medium.

[f] Growth medium supplemented with 3.4×10^{-4} M L-histidine except where noted.

[g] Not done.

equally effective, we used two strains which synthesized histidine at a reduced rate.

The first strain was an intragenic (*his-2*) heterocaryon [(K80a) + (K595a)] capable of growing slowly on unsupplemented growth medium. Presumably, the slow growth rate was due to its limited ability to form functional adenosine triphosphate (ATP)-5-phosphoribosylpyrophosphate pyrophosphorylase, the histidine biosynthetic enzyme specified by the *his-2* gene (15). The specific activities of two tryptophan enzymes in this strain (Table 4) were two- to threefold higher than those of a second intragenic *his-2* heterocaryon [(K80a) + (K153a)] which grew as well as the wild-type strain in unsupplemeted growth medium. The second strain, *his-3A*, hlp (histidinol permeable), was a mutant blocked in the third step of histidine biosynthesis. This strain imbibes histidinol as a result of a second mutation (8, 36), and, because its histidinol dehydrogenase is functional, it will form histidine (8, 36). This strain was grown in growth medium supplemented with concentrations of histidinol which permitted either half-maximal or maximal growth rates. A comparison of the specific activities of two tryptophan enzymes formed under these growth conditions with those of the wild-type strain 74A is presented in Table 4. These data show that a low endogenous rate of histidine biosynthesis leads to increased formation of tryptophan enzymes.

Coordinate control of tryptophan enzymes in histidine-sufficient and histidine-starved cultures. Analysis of the data obtained in the experiment presented in Fig. 1 indicated that the five tryptophan biosynthetic enzymes were coordinately controlled in both wild-type strain Em a and mutant strain K85a (Fig. 2). This conclusion can be criticized on two grounds. First, the mycelial samples were obtained after 50 hr of growth during which noncoordinate derepression may have occurred. Second, the strain K85a (a *his-1* mutant) had accumulated imidazoleglycerol phosphate during its growth that may have contributed to the derepression of tryptophan synthetase (34). We therefore grew two *his-2* strains, K74a and M43, and their respective wild-type strains, Em a and 74A, in growth medium supplemented with 0.34 mM histidine. Since *his-2* mutants lack the first enzyme of histidine biosynthesis (15), no histidine intermediates can accumulate. The specific activities of four tryptophan enzymes in the wild-type strains after 24 hr of growth and in the *his-2* strains after 16, 24, and 42 hr of growth were measured. These data (Fig. 3) verified that the tryptophan enzymes are coordinately controlled. It is important to point out that after 16 hr of growth the *his-2* strains had not utilized all of the histidine supplement. Consonant with this, the specific activities of the tryptophan enzymes at that time were about equal to those in the 24-hr-old wild-type cells (Fig. 3). Nonetheless, the relative specific activities of the tryptophan enzymes which had been formed by 16 hr were the same as those formed after 24 and 42 hr of growth. At these later times the histidine supplement had been utilized. These experiments indicate that the synthesis of tryptophan enzymes is coordinate in histidine-sufficient

TABLE 2. *Additive effect of mixing Sephadex-treated extracts from wild-type and histidine mutant strains*[a]

Expt no.	Strain	ASU[b]/sample	ASU found upon mixing samples		PRtU[c]/sample	PRtU found upon mixing samples		PRAiU[d]/sample	PRAiU found upon mixing samples	
			Calculated	Observed		Calculated	Observed		Calculated	Observed
1	K74a[e]	1.23	1.91	1.77	2.17	4.00	4.34	ND[h]		
	Em a	0.68			1.83			ND		
2	K85a[f]	1.63	2.31	2.10	3.67	5.50	5.34	2.16	3.33	3.17
	Em a	0.68			1.83			1.17		
3	K26a[g]	0.72	1.40	1.32	1.50	3.33	3.50	ND		
	Em a	0.68			1.83			ND		

[a] Sephadex-treated extracts were prepared as described in Materials and Methods.
[b] Units of anthranilate synthetase (10^{-3} units/mg).
[c] Units of anthranilate-5-phosphoribosylpyrophosphate phosphoribosyltransferase (10^{-5} units/mg).
[d] Units of *N*-(5'-phosphoribosyl)anthranilic acid isomerase (10^{-3} units/mg).
[e] K74a is a *his-2* mutant and therefore blocked in the first step of histidine biosynthesis.
[f] K85a is a *his-1* mutant and therefore blocked in an intermediate step of histidine biosynthesis.
[g] K26a is a *his-3D* mutant and therefore blocked in the final step of histidine biosynthesis.
[h] Not done.

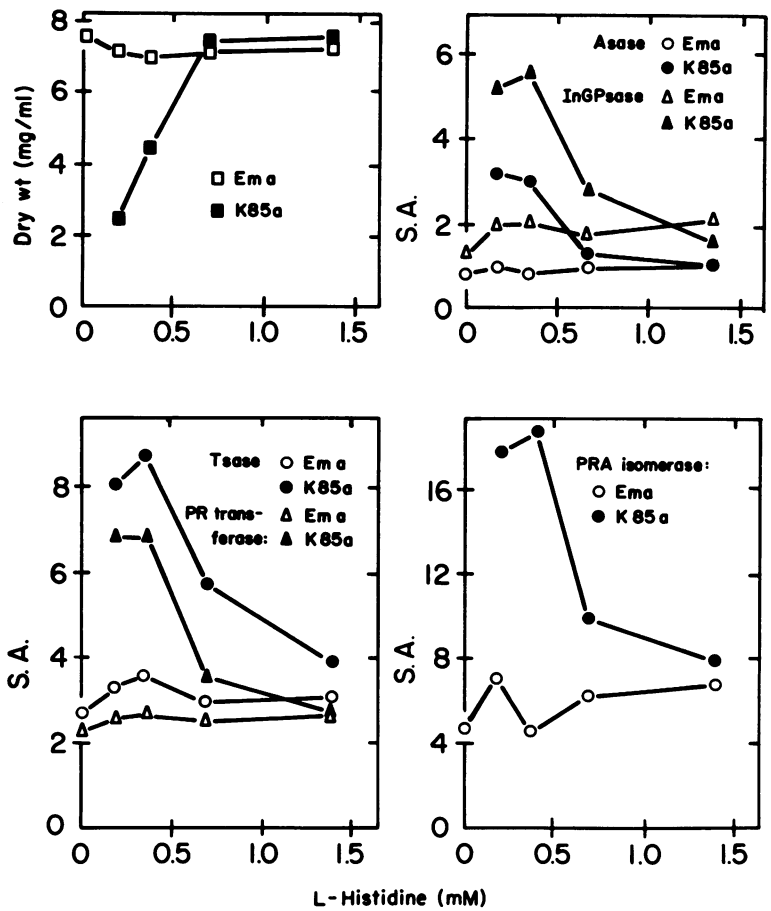

FIG. 1. *Effect of histidine concentration on growth, and the formation of the tryptophan biosynthetic enzymes in his-1 mutant K85a and wild-type strain Em a. All cultures were grown by procedure A for 50 hr in growth medium containing the indicated concentration of histidine. Abbreviations: Asase, anthranilate synthetase; PR transferase, anthranilate-5-phosphoribosylpyrophosphate phosphoribosyltransferase; PRA isomerase, N-(5'-phosphoribosyl)anthranilic acid isomerase; InGPsase, indole-3-glycerol phosphate synthetase; Tsase, tryptophan synthetase; S.A., specific activity, expressed as 10^{-3} units/mg of protein.*

cells of wild-type strains, histidine-sufficient cells of histidine auxotrophic strains, and histidine-starved cells of histidine auxotrophic strains.

Failure of tryptophan to prevent histidine-mediated derepression. To determine whether tryptophan prevented histidine-mediated derepression, mutant strain K85a was grown and sampled by procedure B (see Materials and Methods). This procedure permitted the addition of tryptophan or indole, which would serve as an endogenous source of tryptophan, to coincide with the onset of histidine starvation. The differential rate of enzyme synthesis in this experiment is shown in Table 5. The differential rate of tryptophan synthetase and InGP synthetase formation in the cells growing in unsupplemented medium was twice that of the cells growing in the histidine-supplemented medium. Furthermore, this higher differential rate also occurred in the cells grown in indole-supplemented and tryptophan-supplemented medium. Similar experiments have shown that the histidine-mediated derepression of anthranilate synthetase and PR transferase also is not overcome by either tryptophan or indole (Jones and Carsiotis, *unpublished data*).

TABLE 3. *Concentration of intracellular tryptophan in strain K85a group with various concentrations of histidine*[a]

Histidine in growth medium	Intracellular tryptophan[b] (μmoles/g of dry wt)
mM	
0.17	ND[c]
0.34	0.41
0.68	0.34
1.36	0.44

[a] Cultures grown by procedure A for 50 hr.
[b] Measured with tryptophanase.
[c] Not done.

Failure of histidine to prevent InGP-mediated derepression of tryptophan synthetase or tryptophan-mediated derepression of InGP synthetase. Whether or not histidine could prevent InGP-mediated or tryptophan-mediated derepression of tryptophan enzymes was studied next. The tryptophan synthetase mutants td141 and tdA78 used for this study retain the indole → tryptophan activity of tryptophan synthetase but have lost the InGP → tryptophan activity of this enzyme (1, 14, 29). Matchett and DeMoss (25) noted high tryptophan synthetase activity of such strains even before tryptophan or indole was exhausted from the growth medium. This could be due to the accumulation of InGP in such strains (1, 29), which causes InGP-mediated derepression of tryptophan synthetase (34). The accumulated InGP probably does not arise from chorismate (37), since the conversion of chorismate to anthranilate is inhibited by tryptophan (11). Rather the InGP probably is generated via the operation of the tryptophan-anthranilate cycle (26). Furthermore, upon exhaustion of the tryptophan supplement, derepression of the other tryptophan enzymes occurs (Lester, *personal communication*). When strains td141 and tdA78 were grown with a growth-limiting concentration of tryptophan, neither the InGP-mediated derepression of tryptophan synthetase nor the tryptophan-mediated derepression of InGP synthetase was prevented by the presence of 1.36 mM histidine (Table 6); this concentration of histidine is twice that required for maximal growth of histidine auxotrophs (Fig. 1). Identical results were obtained when this experiment was repeated with the use of a growth-limiting con-

TABLE 4. *Tryptophan enzymes in strains which synthesize histidine at a reduced rate*[a]

Expt no.	Strain	Supplement in growth medium	Growth period	Mycelial dry wt	Specific activity (10^{-3} units/mg)	
					Tryptophan synthetase	InGP[b] synthetase
			hr	*mg/ml*		
1	[(K80a) + (K595a)]	None	35	2.1	4.67	3.84
	[(K80a) + (K153a)]	None	35	8.1	1.33	1.83
2	His 3A, hlp[c]	Histidinol, 1 mM	24	3.7	4.84	3.17
	His 3A, hlp	Histidinol, 2 mM	24	7.4	1.50	1.17
	74A (wild-type)	None	24	7.3	1.50	1.17

[a] Cultures grown by procedure A.
[b] Indole-3-glycerol phosphate.
[c] Histidinol-permeable.

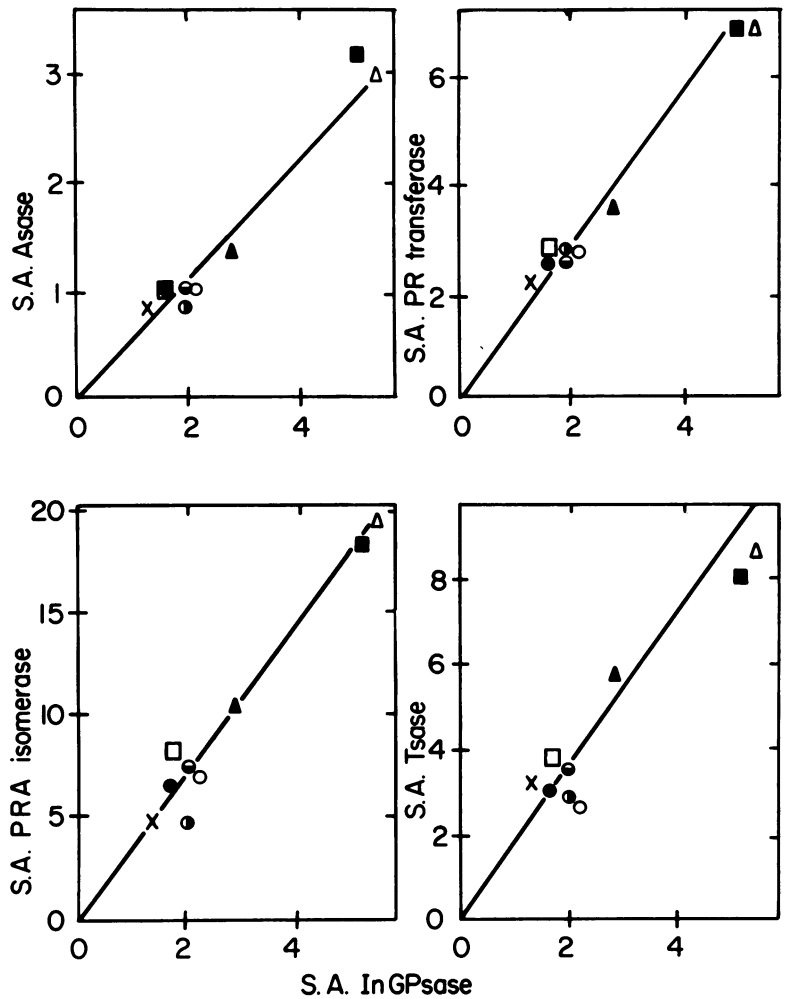

FIG. 2. *Coordinate control of tryptophan enzymes. All cultures were grown for 50 hr by growth procedure A Strains and initial histidine concentrations in growth medium: Em a (none, ○; 0.17 mM,×; 0.34 mM, ◒; 0.68 mM, ◑; 1.36 mM, ●); K85a (0.17 mM, ■; 0.34 mM, △; 0.68 mM, ▲; 1.36 mM, □). Abbreviations: S.A., specific activity, expressed as 10^{-3} units/mg of protein; Asase, anthranilate synthetase; PR transferase, anthranilate-5-phosphoribosylpyrophosphate phosphoribosyltranferase; PRA isomerase, N-(5'-phosphoribosyl)anthranilic acid isomerase; InGPsase, indole-3-glycerol phosphate synthetase; Tsase, tryptophan synthetase.*

centration of indole (0.2 mM) in place of tryptophan.

DISCUSSION

Our previous results (7) and those reported herein establish that the five tryptophan biosynthetic enzymes of *Neurospora* are derepressed in histidine auxotrophs when the auxotrophs are starved of histidine. The two- to threefold derepression which occurs in histidine auxotrophs is as high as that which occurs in tryptophan auxotrophs starved for tryptophan (21–24). Such modest degrees of derepression are common in fungi (16). Furthermore, histidine-mediated derepression is coordinate (Fig. 2 and 3), although the four structural genes for tryptophan biosynthesis are unlinked in *Neurospora* (5). Coordinate derepression of two unlinked leucine biosynthetic enzymes has been reported by Gross (16). We believe our failure to detect coordinate derepression in our previous study (7) was due to the longer growth period then used. Studies with other auxotrophs (amino acid, pyrimidine, and vitamin) have been complicated by the

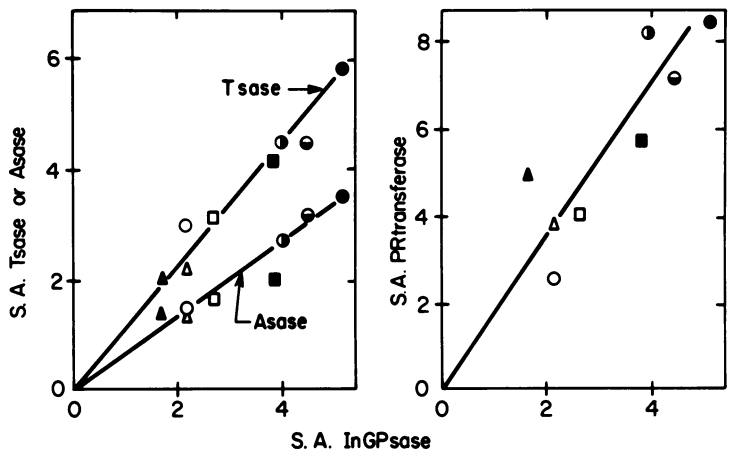

FIG. 3. *Coordinate control of tryptophan enzymes. All strains were grown in growth medium supplemented with 0.34 mM histidine by growth procedure C. Strains and time of growth: 74A (24 hr, ▲); Em a (24 hr, △); M43 (16 hr, ○; 24 hr, ◒; 42 hr, ●); K74a (16 hr, □; 24 hr, ◑; 42 hr, ■). Abbreviations: S.A., specific activity expressed as 10^{-3} units/mg of protein; Asase, anthranilate synthetase; InGPsase, indole-3-glycerol phosphate synthetase; PR transferase, anthranilate-5-phosphoribosylpyrophosphate phosphoribosyltransferase; Tsase, tryptophan synthetase.*

TABLE 5. *Differential rate of enzyme synthesis in strain K85a*[a]

Supplement in growth medium	Enzyme synthesized		Protein synthesized	Specific activity of enzyme synthesized (10^{-3} units/mg of protein)	
	InGPsase[b]	Tsase[c]		InGPsase	Tsase
	units	*units*	*mg*		
None	0.227	0.358	38	6.0	9.4
Histidine, 0.34 mM	0.333	0.486	128	2.6	3.8
Indole, 0.27 mM	0.130	0.380	26	5.0	14.6
Tryptophan, 0.76 mM	0.216	0.477	38	5.7	12.5

[a] Growth procedure B was used. The zero-time sample contains 0.13 units of indole-3-glycerol phosphate synthetase (InGPsase), 0.143 units of tryptophan synthetase (Tsase), and 30 mg of protein. After 6 hr of incubation, the total amount of InGPsase, Tsase, and protein in each culture was determined, and the amounts of enzymes and protein synthesized were calculated.

[b] Indole-3-glycerol phosphate synthetase.

[c] Tryptophan synthetase.

presence of proteolytic activity in the extracts of nutritionally starved cultures (Jones, *unpublished data*). Thus, the failure to detect derepression in starved auxotrophs could be misleading. Proteolytic activity, however, has not been detected in histidine-starved or tryptophan-starved cells (Jones, *unpublished data*). The development of autolytic enzymes in nutritionally starved cultures has been noted previously (6, 32).

The existence of histidine-mediated derepression raises two questions: by what mechanism does it operate, and is the histidine-tryptophan relationship in *Neurospora* a unique one? Apparently the mechanism is not a lowering of the concentration of intracellular tryptophan in histidine-starved cells (Table 3). In fact, the presence of tryptophan in the growth medium does not prevent derepression in histidine-starved cells (Table 5). The possibility that the derepression is caused

TABLE 6. *Effect of histidine on tryptophan enzymes in tryptophan mutants td141 and tdA78*[a]

Strain	Histidine in growth medium	Specific activity (10^{-3} units/mg)	
		Tryptophan synthetase	InGP[b] synthetase
	mM		
td141	0	15.7	7.33
	1.36	13.2	5.84
tdA78	0	9.33	4.33
	1.36	10.0	5.00

[a] The growth medium was supplemented with histidine as indicated and with a growth-limiting concentration of tryptophan (0.36 mM). The cultures were grown by procedure A for 60 hr.

[b] Indole-3-glycerol phosphate.

by the accumulation of histidine intermediates was considered, since it has been shown (34) that one such intermediate, imidazoleglycerol phosphate, causes derepression of tryptophan synthetase. However, we have shown (Table 1; also reference 7) that the derepression occurs in *his-2* mutants, which are blocked in the first step of histidine biosynthesis (15). Since mutants in the first step of a pathway are precluded from accumulating intermediates, we do not believe that this is the mechanism. We also dismiss the possibility that some interaction between tryptophan and histidine structural genes is involved, because tryptophan structural genes occur on linkage groups which lack histidine structural genes and vice versa (5). Another possibility is that the tryptophan biosynthetic enzymes which are derepressed by histidine starvation exist in the mitochondria (19) rather than the cytoplasm. This was dismissed because cycloheximide prevented histidine-mediated derepression (T. Cleary, M.S. Thesis, Univ. of Cincinnati, 1968), and protein synthesis in *Neurospora* mitochondria is insensitive to cycloheximide (20). Although the histidine and tryptophan biosynthetic pathways share no common intermediates (4), both 5-phosphoribosyl-1-pyrophosphate (PRPP) and glutamine are utilized in forming these two amino acids. Since starved histidine auxotrophs will accumulate intermediates (3), we considered whether the excessive utilization of PRPP or glutamine by starved histidine auxotrophs was causing the derepression of the tryptophan enzymes. However, *his-2* mutants, since they are blocked in the first step of histidine biosynthesis (15), will not "waste" PRPP or glutamine, because they accumulate no intermediates. Therefore, since *his-2* mutants derepress their tryptophan enzymes when starved of histidine, we dismissed the idea that excessive utilization of PRPP or glutamine was causing the derepression.

After discounting the above possibilities, we reconsidered the question of the mechanism on the basis of certain data presented herein plus a recent finding. First, there is the fact that histidine-mediated control is coordinate (Fig. 2 and 3). Since the four tryptophan structural genes in *Neurospora* are unlinked (5), the coordinate character of histidine-mediated control indicates that it involves a cytoplasmic factor and that each structural gene contains a control site equally sensitive to the factor. Although the cytoplasmic factor has not been identified, we found that treatment of wild-type cells with α-methylhistidine caused derepression of the tryptophan enzymes (M. Carsiotis and G. Lester, Bacteriol. Proc., p. 136, 1968). Since this compound is known to inhibit the charging of histidinyl-specific transfer ribonucleic acid (RNA) in *E. coli* (31), and because we found that the intracellular histidine concentration increased almost fourfold in the α-methylhistidine-treated cells, we concluded that it is not a shortage of free histidine itself which causes derepression of the tryptophan enzymes, but presumably a shortage of charged histidinyl-transfer RNA. The suggestion of such a role for histidinyl-transfer RNA is consistent with the postulated role of aminoacyl-transfer RNA derivatives in regulating amino acid biosynthetic enzyme systems (27, 30, 31), but is novel in assigning it a role in regulating the synthesis of two apparently unrelated enzyme systems. This suggestion recalls the hypothesis of Chapman and Nester (9, 28) that a common regulatory element is involved in the repression of both histidine and aromatic amino acid biosynthetic enzymes in *Bacillus subtilis*. Also, Jensen (18) has postulated the existence of interlocking control of enzyme *activity* in the histidine and aromatic amino acid biosynthetic pathways by PRPP, acting as a common allosteric effector.

Recent experiments suggest that the histidine-tryptophan relationship is not unique in *Neurospora*. Specifically, we have found (Carsiotis and Jones, *unpublished data*) that histidine starvation caused derepression of two arginine biosynthetic enzymes, arginine-specific carbamyl phosphokinase and ornithine transcarbamylase. Similarly, tryptophan starvation derepressed three histidine biosynthetic enzymes (R. F. Jones and M. Carsiotis, Bacteriol. Proc., p. 136, 1968) and the same two (as above) arginine biosynthetic enzymes (Carsiotis and Jones, *unpublished data*). We therefore suggest that histidine-mediated control of tryptophan enzymes may be only one facet of a superstructure of regulatory systems which coordinate amino acid biosynthesis in *Neurospora*. Further experiments are needed to determine its extent and mechanism of operation.

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