Cross-Pathway Regulation: Tryptophan-Mediated Control of Histidine and Arginine Biosynthetic Enzymes in *Neurospora crassa*

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In *Neurospora crassa*, the starvation of tryptophan mutants for tryptophan resulted in the derepression of tryptophan, histidine, and arginine biosynthetic enzymes. This tryptophan-mediated derepression of histidine and arginine biosynthetic enzymes occurred despite the fact that the tryptophan-starved cells had a higher intracellular concentration of histidine and arginine than did nonstarved cells.

Starvation of histidine, tryptophan, and arginine auxotrophs of *Neurospora crassa* leads to a two- to fourfold derepression of histidine, tryptophan, and arginine biosynthetic enzymes, respectively (1–3, 10, 14–19, 21). We reported (7, 9) that histidine starvation of *Neurospora* also causes derepression of tryptophan and arginine biosynthetic enzymes. We use the term cross-pathway regulation for this phenomenon wherein starvation for a single amino acid causes derepression of biosynthetic enzymes of other amino acids as well as those of the deficient amino acid. To determine whether other amino acids are involved in cross-pathway regulation, we measured the production of histidine and arginine biosynthetic enzymes in tryptophan mutants grown on limiting and excess concentrations of tryptophan. Our results show that tryptophan-mediated control of histidine and arginine biosynthetic enzymes occurs in *Neurospora*.

MATERIALS AND METHODS

The tryptophan mutants *trp*-4-9A and *trp*-1-13A were obtained from J. A. DeMoss; wild-type strain 74A was from A. M. Lacy. Mutant *trp*-1-13A lacks three enzymatic activities required for tryptophan formation in *N. crassa* (12), one of which is indoleglycerolphosphate synthetase.

Growth and assay procedures. The growth medium, Vogel medium N (25), contained 2% domestic sugar; further additions are indicated in the tables. Cultures were inoculated with conidia to a final concentration of 2.5×10^7 conidia per ml and were incubated on a rotary shaker at 30 C for 18 h. Mycelia were harvested by filtration on a Buchner funnel, washed with distilled water, frozen, and lyophilized. The lyophilized mycelia were stored at −15 C. Extracts from lyophilized mycelia were prepared as

before (6). The enzymes assayed were: tryptophan synthetase (EC 4.2.1.20); indoleglycerolphosphate synthetase; histidinolphosphate transaminase (EC 2.6.1.9); histidinolphosphate phosphatase (EC 3.1.3.15); histidinol dehydrogenase (EC 1.1.1.23); carbamylphosphate synthetase (EC 2.7.2.5; arginine specific); ornithine transcarbamylase (EC 2.1.3.3); and aspartyl transcarbamylase (EC 2.1.3.2). Except for histidinol dehydrogenase, all enzymes were assayed at 37 C by previously described procedures (9). Histidinol dehydrogenase was assayed at 25 C in extracts made with 0.05 M tris(hydroxymethyl)aminomethane, pH 9.1, which were passed through a Sephadex G-25 column prepared in the same buffer. A portion of the extract was added to a cuvette containing 50 μmol of glycine-sodium hydroxide buffer, pH 9.7, 15 μmol of 2-mercaptoethanol, 5 μmol of nicotinamide adenine dinucleotide, 2.5 μmol of histidinol, and water to yield a final volume of 1 ml. The increase in absorbancy at 340 nm was measured for 6 min and the nanomoles of histidine formed per minute were calculated; 1 nmol of histidine formed requires the reduction of 2 nmol of nicotinamide adenine dinucleotide.

For all enzymes, 1 mU catalyzes the formation of 1 nmol of product or the disappearance of 1 nmol of substrate per min. Protein concentration was measured by a modification of the biuret method (26). Specific activity is expressed as milliunits per milligram of protein.

Amino acid pool size determinations. An aqueous extract of the lyophilized mycelia was prepared (11), and the concentration of histidine and arginine was determined by use of an amino acid analyzer. Sometimes the histidine content in the aqueous extract was measured by microbiological assay.

RESULTS

Tryptophan, histidine, and arginine biosynthetic enzymes in tryptophan mutants. The effect of starvation of the tryptophan mutants for tryptophan was the formation of higher

levels of the three histidine biosynthetic enzymes measured (Table 1), as well as higher levels of tryptophan synthetase. The level of tryptophan and histidine biosynthetic enzymes in the wild-type strain was unaffected by tryptophan supplementation of the medium. Previous work (7, 9) had shown that arginine biosynthetic enzymes are subject to histidine-mediated cross-pathway regulation. Therefore, in another experiment, we measured the level of two arginine (carbamylphosphate synthetase, ornithine transcarbamylase), two histidine (transaminase, phosphatase), and two tryptophan (tryptophan synthetase, indoleglycerolphosphate synthetase) biosynthetic enzymes, as well as the intracellular concentration of histidine and arginine. In the tryptophan-starved cells, the level of the two arginine biosynthetic enzymes was fourfold higher than in the nonstarved cells (Table 2); as before, the two tryptophan and two histidine biosynthetic enzymes were also elevated in the tryptophan-starved cells (Table 2). In the experiments reported above, exogenous tryptophan served as the source of tryptophan in starved and non-starved cells. Matchett et al. have suggested (20, 21) that tryptophan of biosynthetic origin is more effective in causing repression than is exogenous tryptophan. In an experiment with the mutant trp-1-13A (data not shown), indole was added to the culture as a source of biosynthetic tryptophan; the amount of indole added permitted the mutant to grow at the wild-type growth rate. In these cells none of the three enzymes (tryptophan synthetase, histidinol-phosphate transaminase, ornithine transcarbamylase) were derepressed. This result indicated that endogenously synthesized tryptophan was also effective in preventing the derepression of histidine and arginine biosynthetic enzymes due to tryptophan-mediated cross-pathway regulation.

However, the pyrimidine biosynthetic enzyme aspartyl transcarbamylase, which is subject to regulation in Neurospora (13), was not derepressed in the tryptophan-starved mutants (Table 2). This indicated that the elevated levels of histidine and arginine biosynthetic

TABLE 1. Enzymatic activities of tryptophan mutants and wild type

Strain	Tryptophan in growth medium (mM)	Mycelial dry wt (mg/ml)	Sp act[a]					Intracellular histidine (μmol/g [dry wt])
			Tsase	InGPsase	HDH	HPpase	HPtr	
trp-1-13A	0.1	2.1	8.3	NP	28.3	541	33.3	16.5
	0.5	6.4	4.3	NP	13.3	273	7.2	6.6
trp-4-9A	0.1	1.7	10.3	ND	35.0	575	30.8	21.0
	0.5	5.4	4.7	ND	16.7	333	7.8	8.0
74A	0	6.4	2.8	2.3	10.0	250	7.0	3.2
	0.1	6.0	3.0	2.3	11.7	241	5.3	5.7
	0.5	5.8	2.8	1.8	11.7	225	7.3	5.4

[a] Expressed as nanomoles per minute per milligram of protein. Abbreviations: Tsase, Tryptophan synthetase; InGPsase, indoleglycerolphosphate synthetase; HDH, histidinol dehydrogenase; HPpase, histidinolphosphate phosphatase; HPtr, histidinolphosphate transaminase; NP, not present in trp-1-13A; ND, not done.

TABLE 2. Enzymatic activities and amino acid pools of tryptophan mutants and wild type

Strain	Tryptophan in growth medium (mM)	Mycelial dry wt (mg/ml)	Sp act[a]							Intracellular amino acid pools (μmol/g [dry wt])	
			Tsase	InGPsase	HPtr	HPpase	CPsase	OTCase	ATCase	His	Arg
trp-1-13A	0.1	2.8	10.3	NP	41.7	400	11.0	2950	200	18.5	92.0
	0.5	8.9	4.5	NP	13.3	167	2.3	633	200	8.3	44.2
trp-4-9A	0.1	2.8	10.3	3.0	36.7	483	13.8	3733	143	22.5	96.0
	0.5	8.2	3.7	1.5	9.7	185	3.2	850	217	3.1	10.8
74A	0.1	9.0	2.0	2.0	6.2	150	1.0	350	183	3.7	20.0

[a] Expressed as nanomoles per minute per milligram of protein. Abbreviations: Tsase, Tryptophan synthetase; InGPsase, indoleglycerolphosphate synthetase; HPtr, histidinolphosphate transaminase; HPpase, histidinolphosphate phosphatase; CPsase, carbamylphosphate synthetase (arginine specific); OTCase, ornithine transcarbamylase; ATCase, aspartyl transcarbamylase; NP, not present in trp-1-13A.

enzymes were not due to a general derepression of regulated enzymes.

The increased specific activity might have been due to activation in vitro of the enzymes by metabolites accumulated in the starved mutants. This was shown to be unlikely since mixtures of extracts prepared from the tryptophan-starved and nonstarved cells had the expected additive activity.

Amino acid pools in tryptophan mutants. Amino acid pools were measured in order to determine whether the derepression of the histidine and arginine biosynthetic enzymes was a consequence of lowered pools of these amino acids in tryptophan-starved cells. The results (Tables 1 and 2) showed, in fact, that the histidine and arginine pools were considerably higher in the tryptophan-starved cells. Furthermore, addition of histidine to a culture of tryptophan-starved cells failed to prevent tryptophan-mediated derepression of histidinolphosphate transaminase, although the histidine pool was elevated 15-fold (R. F. Jones, unpublished data).

The presence of the elevated amino acid pools was a curious finding since one expects that feedback inhibition of enzyme activity will prevent accumulation of the end-product amino acid. It was thought possible that proteases, induced upon amino acid starvation (23), were responsible for the elevated amino acid pools. However, extracts prepared from the two tryptophan mutants grown on limiting (0.1 mM) tryptophan had only marginal amounts of proteolytic activity (Jones, unpublished data). Whether this in vitro assay is a true reflection of conditions in vivo, however, is unknown.

DISCUSSION

It is clear from the data presented herein that tryptophan-mediated control of histidine and arginine biosynthetic enzymes occurs in *Neurospora*. The magnitude of derepression of the histidine and arginine biosynthetic enzymes in tryptophan-starved cells was about the same (two- to fourfold) as occurs in histidine- and arginine-starved cells (1–3, 10). The mechanism of tryptophan-mediated control is open to speculation. It is apparently not due to the existence of enzyme activators in the extracts of tryptophan-starved cells (see above). Nor are the total histidine and total arginine pools lowered in tryptophan-starved cells (Tables 1 and 2); a lowering of the intracellular concentraion of histidine and arginine would presumably cause derepression of their respective biosynthetic

enzymes (1–3, 10). However, as already discussed previously (9), the existence of multiple pools (4, 20, 24) leaves open the possibility that tryptophan starvation lowers the specific histidine and arginine pools that regulate synthesis of the biosynthetic enzymes.

Several studies (e.g., ref. 6) have implicated transfer ribonucleic acid (tRNA) in the control of enzyme synthesis. A regulatory role for tRNA in *Neurospora* is also suggested by the finding that a mutant with a defective tryptophanyl-tRNA synthetase has elevated levels of biosynthetic tryptophan enzymes (22). We reported (M. Carsiotis, Abstr. Annu. Meet. Amer. Soc. Microbiol. 1973, P70, p. 152) that the histidine and arginine biosynthetic enzymes are also derepressed in this mutant. It still does not seem appropriate, however, on the basis of available data, to attribute cross-pathway regulation to a shortage of aminoacyl-tRNA.

Glutamine is used in the synthesis of tryptophan, histidine, and arginine, thereby raising the possibility that cross-pathway regulation involves glutamine or some enzyme of glutamine metabolism. A direct role for glutamine, however, seems unlikely for the following reasons. Anthranilate synthetase, the first enzyme in tryptophan biosynthesis, is inhibited by tryptophan (16, 17) and is the only tryptophan biosynthetic enzyme that utilizes glutamine as a substrate. The mutant *trp*-1-13A used in these studies lacks anthranilate synthetase (17). Therefore, glutamine is presumably spared in *trp*-1-13A, regardless of whether it is grown with limiting or excess tryptophan. In contrast, the mutant *trp*-4-9A, blocked in the second step of tryptophan biosynthesis (17), presumably wastes glutamine when starved for tryptophan since there is no inhibition of anthranilate synthetase. Thus, since tryptophan-mediated derepression occurs under conditions where glutamine is either spared or wasted, it seems unreasonable to ascribe a regulatory role to glutamine. However, a regulatory role for glutamine synthetase in cross-pathway regulation, similar to its regulatory role in *Klebsiella aerogenes* (5), remains a possibility.

Cross-pathway regulation in *Neurospora* encompasses histidine-mediated and tryptophan-mediated control of histidine, tryptophan, and arginine biosynthetic enzymes. The occurrence of arginine-mediated control of the same enzymes has been reported (A. C. Wesseling and M. Carsiotis, Abstr. Annu. Meet. Amer. Soc. Microbiol. 1973, P66, p. 152) and will be the subject of a forthcoming publication.

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