Cross-pathway Regulation of Ornithine Carbamoyltransferase Synthesis in *Aspergillus nidulans*

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Regulation of the cross-pathway type was found in *Aspergillus nidulans*. Ornithine carbamoyltransferase, a biosynthetic enzyme of the arginine pathway, is derepressed as a result of deprivation not only of arginine but also of histidine, proline and tryptophan. Cross-pathway regulation operates in the mutant *suD19* in which the ornithine carbamoyltransferase level is insensitive to arginine. The spectrum of the amino acids involved in this type of regulation seems to be different from those described for other fungi.

INTRODUCTION

The phenomenon of general or cross-pathway regulation of the biosynthesis of amino acids has been described for the fungus *Neurospora crassa* (Carsiotis *et al.*, 1974) and for yeasts (Schürch *et al.*, 1974; Wolfner *et al.*, 1975). Starvation for a given amino acid results in simultaneous derepression not only of its own biosynthetic pathway, but also of several unrelated pathways. In *N. crassa*, starvation for either histidine, arginine or tryptophan results in a simultaneous derepression of biosynthetic enzymes for all of them. In *Saccharomyces cerevisiae*, the enzymes of the biosynthetic pathways for arginine, lysine, leucine, isoleucine/valine, histidine, tryptophan and probably some others are derepressed during limitation of any of these amino acids.

We have shown previously that in *Aspergillus nidulans* ornithine carbamoyltransferase (OCT), an enzyme of the arginine biosynthetic pathway, is derepressed under conditions of arginine limitation (Cybis *et al.*, 1970). OCT appears to be under the control of the same regulatory gene *suD* as the arginine inducible enzymes of arginine catabolism (Cybis *et al.*, 1972). The results presented here indicate the existence of a cross-pathway control of OCT synthesis.

METHODS

Strains. These were all from the collection of the Department of Genetics, University of Warsaw. The symbols *arg*, *bi*, *his*, *lu*, *lys*, *paba*, *pro* and *pyro* denote auxotrophy for arginine, biotin, histidine, leucine, lysine, *p*-aminobenzoic acid, proline and pyridoxine, respectively; *w* and *y* indicate white and yellow conidia, respectively.

Media. The minimal medium (MM) of Cove (1966) was used.

Methods of culture, harvesting of mycelia and preparation of extracts. These were as described previously (Cybis *et al.*, 1970). Starvation experiments were performed in three ways. (a) About 20 h-old mycelium grown on medium with all the required supplements was collected, washed with MM, transferred to medium without amino acid supplements and harvested after 8 h. (b) An amino acid-requiring non-leaky auxotroph was cultured on the appropriate amino acid at a growth-rate limiting concentration. The growth with the amino acid at different concentrations had previously been established by following the increase in mycelial dry weight. (c) The leaky auxotroph was grown on medium without amino acid supplements.

Enzyme assay. Ornithine carbamoyltransferase (OCT; carbamoylphosphate:L-ornithine carbamoyltransferase; EC 2.1.3.3) activity was determined as described previously (Cybis *et al.*, 1970).

Table 1. *Effect of amino acid starvation on OCT activity*

Strain	Amino acid added to medium	Expt*:time (h)	OCT activity [μ mol citrulline min ⁻¹ (mg protein) ⁻¹]
<i>biA1 pabaA9</i>	None	—	0.20
<i>biA1 argA1</i>	None	a:8	1.40
<i>biA1 lysB5</i>	None	a:8	0.23
<i>biA1 lysB5</i>	Lysine (0.1 mM)	b:24	0.45
<i>biA1 lys-231</i>	None	a:8	0.25
<i>biA1 luA1</i>	None	a:8	0.30
<i>biA1 luA1</i>	Leucine (0.1 mM)	b:36	0.36
<i>biA1 pyroA4 w-3 his-1</i>	None	c:24	0.70–1.3
<i>biA1 phenA2</i>	None	a:8	0.27
<i>biA1 phenA2</i>	Phenylalanine (0.05 mM)	b:24	0.36
<i>proA6 pabaA9 biA1</i>	None	a:8	1.40
<i>proA6 pabaA9 biA1</i>	Proline (0.2 mM)	b:24	0.80–1.5
<i>pabaA9 yA2 trypB403</i>	None	a:8	1.30
<i>pabaA9 yA2 trypB403</i>	Tryptophan (0.05 mM)	b:30	1.30

* a, b and c denote the starvation technique used, see Methods.

Estimation of arginine pool. Extraction was performed in a boiling water bath (5 min). Insoluble material was removed by filtration and arginine in the extract was determined by the method of Van Pilsum *et al.* (1956).

Chemicals. All chemicals used were of reagent grade from commercial sources, with the exception of carbamoylphosphate which was synthesized according to the method of Spector *et al.* (1957).

RESULTS

The OCT activity observed in the wild-type strain grown on MM represents a state of full repression of this enzyme. It cannot be repressed further by the addition of arginine. Arginine starvation results in derepression of OCT. A maximum sevenfold increase in enzyme activity took place within about 9 h after transferring mycelium of the arginine auxotroph *argA1* to arginine-free medium (see Cybis *et al.*, 1970).

Mutant *his-1* is a leaky auxotroph. When grown on medium without histidine, its OCT activity increased reaching a maximum in about 24 h. The maximum activity obtained varied from one experiment to another, but was lower than the maximum reached in the arginine-starved *argA1* strain. The presence of histidine but not arginine prevented this derepression (Tables 1 and 3).

3-Amino-1,2,4-triazole, a histidine analogue, inhibits histidine biosynthesis, causing a shortage of histidine a few hours after it is added to the medium of *N. crassa* (Carsiotis *et al.*, 1974). The addition of 10 mM-3-aminotriazole for 2.5 to 3 h to the growth medium of *A. nidulans* (prototrophic with regard to amino acids) resulted in a marked increase in OCT activity. This effect could be reversed by simultaneous addition of 10 mM-histidine, but not by arginine, even at 50 mM (Table 2).

Tryptophan starvation of the *trypB403* mutant and proline starvation of the *proA6* mutant both resulted in similar high OCT activities, regardless of the starvation technique used (Table 1). For the *proA6* mutant, limitation of proline at a concentration which caused only a slight decrease in the mycelium weight was sufficient to enhance OCT activity. In all other mutants tested, much more drastic changes in the growth rate were necessary for the derepression to occur.

Lysine starvation of mutants *lysB5*, *lysD7* and *lys-231*, leucine starvation of *luA1* and phenylalanine starvation of *phenA2* mutants had little or no effect on their OCT activity. Slight derepression of OCT was sometimes observed with growth rate-limiting concentra-

Table 2. *Effect of 3-aminotriazole on the OCT activity of the pabaA9 biA1 strain*

The supplements indicated were added simultaneously to a 20 h culture, and mycelium was harvested 3 h later.

Medium supplementation (mM)			OCT activity [μmol citrulline min^{-1} (mg protein) $^{-1}$]
3-Aminotriazole	Histidine	Arginine	
—	—	—	0.20
5	—	—	0.50
10	—	—	0.75
50	—	—	0.48
10	10	—	0.25
10	—	10	0.77

Table 3. *Effect of various amino acids on OCT activity in starved mycelium*

Strain	Starvation conditions	Amino acid supplementation (5 mM)	OCT activity [μmol citrulline min^{-1} (mg protein) $^{-1}$]
<i>biA1 pyroA4 w-3 his-1</i>	MM 24 h	Arginine	0.65
		Ornithine	0.90
		Proline	0.80
		Histidine	0.25
<i>proA6 pabaA9 biA1</i>	Proline (0.2 mM) 24 h	None	1.1 (0.8–1.5)
		Proline (10 mM)	0.20
		Arginine	0.21
		Ornithine	0.24
		Lysine	0.58
		Glutamate	0.92
		Alanine	1.2
		Leucine	1.0
<i>proA6 biA1 agaA65</i>	Proline (0.2 mM) 24 h	Tryptophan	1.0
		None	1.2
		Arginine	0.75
		Ornithine	0.25
<i>proA6 biA1 ap-2</i>	Proline (0.2 mM) 24 h	None	1.2
		Arginine	1.5
		Ornithine	1.4

tions of these amino acids, but no derepression occurred under conditions of complete starvation (Table 1).

The effect of addition of several amino acids on the OCT activity under conditions of amino acid deprivation is shown in Table 3. Tryptophan- or histidine-mediated derepression of OCT could not be reversed by arginine, even at high concentrations, or by any of the other amino acids tested. Repression was obtained only with the limiting amino acid.

Under proline limitation, arginine and ornithine maintained the normal repressed level of OCT. The results obtained could be attributed to the fact that the *proA6* mutant can use these two amino acids as good proline sources, via arginine catabolism (Weglenski, 1966), and so there may be no proline shortage. Indeed, growth of the *proA6* mutant on 0.2 mM-proline plus 10 mM-arginine (measured as mycelial mass obtained from equal inocula) was the same as on 10 mM-proline. The double mutant *proA6 agaA65*, which lacks arginase (Bartnik *et al.*, 1977), is unable to use arginine, but can still use ornithine as a proline source. With this strain, arginine did not support growth on 0.2 mM-proline and OCT was derepressed (Table 3). Neither arginine nor ornithine supported growth of a strain carrying, in addition to *proA6*, the mutation *ap-2* affecting basic amino acids transport (unpublished) and they did not cause repression of OCT in this strain (Table 3).

Table 4. *Pools of arginine under different growth conditions*

Cultures were grown for 20 h and then samples were withdrawn for extraction, except for experiment (b) in which a culture grown initially on 10 mM-proline was transferred to medium without proline for 8 h.

Strain	Amino acid supplementation	Arginine [nmol (mg dry wt) ⁻¹]
<i>pabaA9 biA1</i>	None	8
<i>proA6 pabaA9 biA1</i>	Proline (10 mM)	10
	Proline (0.2 mM)	95
	None (8 h, expt b)	62
<i>pabaA9 yA2 trypB403</i>	Tryptophan (5 mM)	15
	Tryptophan (0.05 mM)	75
	Tryptophan (0.05 mM)	
	+ arginine (5 mM)	88

The presence in the growth medium of 3-aminotriazole during starvation for proline or tryptophan did not cause any further increase in the OCT activity.

Assay of the arginine pool under different growth conditions showed that the total pool of free arginine present in proline- or tryptophan-starved cells was very high – about as high as the maximum found in mycelium grown in the presence of 5 mM-arginine (Table 4).

It was possible that the observed increase in OCT activity was not actually a derepression of OCT synthesis but a change in its activity. However, in a starvation experiment with the *proA6* mutant in medium without amino acid supplements but containing cycloheximide at 10 µg ml⁻¹, there was no change in OCT activity during at least 7 h, indicating that OCT derepression required protein synthesis.

The *suD19* mutant is a pleiotropic regulatory mutant which, among other characteristics, is insensitive to arginine starvation. In an *suD19 argA1* strain there was no difference in OCT activity whether or not arginine was present in the medium (Cybis *et al.*, 1972). Therefore, it was interesting to see whether cross-pathway regulation operated in this mutant. 3-Aminotriazole was used to produce a shortage of histidine. The results are obscured by the fact that the OCT level in *suD19* is higher than in the wild-type and increases with the age of the mycelium. Addition of 10 mM-3-aminotriazole to exponentially growing mycelium of *suD19* for 2.5 to 3 h resulted in an OCT specific activity of 0.80, compared with 0.50 in the absence of the analogue.

DISCUSSION

The results presented show that OCT is derepressed not only under conditions of arginine starvation but also under histidine, proline or tryptophan starvation. Little or no effect was obtained with lysine, leucine or phenylalanine deprivation. It is possible that the spectrum of amino acids participating in this phenomenon is different in different organisms, since, for example, in yeasts deprivation of practically any amino acid produces a general derepression effect.

Simultaneous starvation for different amino acids did not result in enhanced derepression, as may be concluded from the results of combined proline-histidine and tryptophan-histidine starvation (obtained with the use of the histidine analogue). The basic rule of general cross-pathway regulation is that derepression of enzymes caused by shortage of a given amino acid may be reversed only by the same amino acid. The character of OCT derepression described here fits this pattern. The results obtained with arginine and ornithine for proline-starved cells can be explained by the role of these amino acids as proline precursors. The behaviour of the *proA6 agaA65* and *proA6 ap-2* strains supports this conclusion. The repression is brought about by the limiting amino acid, regardless of the intracellular arginine content (see Table 4).

Two levels of OCT regulation, general and specific, have been demonstrated in yeasts (Delforge *et al.*, 1975). Only an approximately twofold increase in OCT activity is obtained by means of the cross-pathway regulation in the wild-type; maximum levels – about 50-times higher – are reached when the mutant *argR*, relieved from specific repression, is the subject of non-specific amino acid starvation. The same is observed in the starved arginine auxotroph. In *A. nidulans* there was no pronounced difference in OCT activity between arginine-, proline- and tryptophan-starved cells. The activities varied from one starvation experiment to another but were all within the same range. This could be interpreted as indicating that derepression in the *argA1* mutant is not the arginine-specific response, but is brought about by the cross-pathway regulation mechanism. On the other hand, the increase in OCT activity produced by 3-aminotriazole in the mutant *suD19* indicates that two independently acting regulatory systems are involved.

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REFERENCES

- BARTNIK, E., GUZEWSKA, J., KLIMCZUK, J., PIOTROWSKA, M. & WEGLENSKI, P. (1977). Regulation of arginine catabolism in *Aspergillus nidulans*. In *Genetics and Physiology of Aspergillus*, pp. 243–254. Edited by J. E. Smith & J. A. Pateman. London: Academic Press.
- CARSOTIS, M., JONES, R. F. & WESSELING, A. C. (1974). Cross-pathway regulation: histidine-mediated control of histidine, tryptophan and arginine biosynthetic enzymes in *Neurospora crassa*. *Journal of Bacteriology* **119**, 893–898.
- COVE, D. J. (1966). The induction and repression of nitrate reductase in the fungus *Aspergillus nidulans*. *Biochimica et biophysica acta* **113**, 51–56.
- CYBIS, J., PIOTROWSKA, M. & WEGLENSKI, P. (1970). Control of ornithine transcarbamylase formation in *Aspergillus nidulans*. *Bulletin de l'Académie polonaise des sciences, Série des Sciences biologiques* **18**, 669–672.
- CYBIS, J., PIOTROWSKA, M. & WEGLENSKI, P. (1972). Genetic control of the arginine pathways in *Aspergillus nidulans*. Common regulation of anabolism and catabolism. *Molecular and General Genetics* **118**, 273–277.
- DELFORGE, J., MESSENGUY, F. & WIAME, J. M. (1975). The regulation of arginine biosynthesis in *Saccharomyces cerevisiae*. The specificity of *argR*[–] mutations and the general control of amino acid biosynthesis. *European Journal of Biochemistry* **57**, 231–239.
- SCHÜRCH, A., MIOZZARI, J. & HÜTTER, R. (1974). Regulation of tryptophan biosynthesis in *Saccharomyces cerevisiae*: mode of action of 5-methyl-tryptophan and 5-methyl-tryptophan-sensitive mutants. *Journal of Bacteriology* **117**, 1131–1140.
- SPECTOR, L., JONES, M. E. & LIPMANN, F. (1957). Carbamyl phosphate. *Methods in Enzymology* **3**, 643–655.
- VAN PILSUM, J. F., MARTIN, R. P., KITO, E. & HESS, J. (1956). Determination of creatine, creatinine, arginine, guanidoacetic acid, guanidine and methylguanidine in biological fluids. *Journal of Biological Chemistry* **222**, 225–236.
- WOLFNER, M., YEP, D., MESSENGUY, F. & FINK, G. R. (1975). Integration of amino acid biosynthesis into the cell cycle of *Saccharomyces cerevisiae*. *Journal of Molecular Biology* **90**, 273–290.