General Control of Arginine Biosynthetic Enzymes in *Neurospora crassa*

By HARRY J. FLINT*† AND BARBARA F. KEMP‡

Department of Genetics, University of Nottingham, University Park, Nottingham NG7 2RD

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The response of six amino acid synthetic enzymes, including four concerned with arginine synthesis, one with histidine synthesis and one with lysine synthesis, to conditions of histidine and arginine limitation and to exogenously provided amino acids is described in *Neurospora crassa*. The activities of all these enzymes increased in response to lowered levels of histidine or arginine, but showed little or no repression in wild-type cultures supplemented with casein hydrolysate. The activity of glucose-6-phosphate dehydrogenase (an enzyme not concerned with amino acid synthesis) remained unaffected by any of these conditions. In the case of the four arginine synthetic enzymes evidence is presented that suggests the 'general' system implicated in the 'cross-pathway' response to histidine is also entirely responsible for the derepression occurring under conditions of arginine limitation. Further investigations into the control of one enzyme, ornithine carbamoyltransferase, are also reported. These show that the enzyme is stable, and suggest that its derepression in response to histidine limitation entails new protein synthesis and involves control at a stage prior to translation.

INTRODUCTION

The regulation of arginine biosynthesis in *Neurospora crassa* has been the subject of extensive investigations (Cybis & Davis, 1975; Davis *et al.*, 1978; Flint *et al.*, 1980) but the mechanisms that control the levels of enzymes in this pathway have remained obscure. The activities of the eight enzymes involved have been shown to increase progressively with decreasing mycelial arginine content in arginine bradytrophic strains (Barthelmess *et al.*, 1974; Cybis & Davis, 1975). In addition, there is evidence to show that the activities of at least two arginine synthetic enzymes become elevated, together with many enzymes involved in histidine and tryptophan synthesis, under conditions of histidine or tryptophan limitation (Carsiotis & Jones, 1974; Carsiotis *et al.*, 1974). Such 'cross-pathway' regulatory phenomena have also been observed in *Saccharomyces cerevisiae* (Schürch *et al.*, 1974; Delforge *et al.*, 1975) and in *Aspergillus nidulans* (Piotrowska, 1980) and have been taken to imply the existence of a 'general' regulatory system whereby the enzymes of many different amino acid pathways respond to the limitation of any one of a number of amino acids. This contrasts with the 'pathway-specific' control that predominates in bacteria, whereby the enzymes of a particular pathway respond only to the end-product of that pathway.

This paper reports the response of a number of amino acid synthetic enzymes, including four of the enzymes involved in arginine synthesis, to conditions of histidine and arginine limitation, and to the presence of exogenously provided amino acids. This allows an assessment of the role of general control in governing enzyme levels in the arginine pathway. Further studies on one of the enzymes (ornithine carbamoyltransferase) are also described that examine enzyme stability, the requirement of *de novo* synthesis for derepression and the

† Present address: Department of Biology, Cavehill Campus, University of the West Indies, Barbados, West Indies.

‡ Present address: Department of Genetics, University of Leeds, Leeds LS2 9JT.

level at which general control of enzyme synthesis is exerted. These are based on the change in enzyme activity observed in derepressed cultures following the inhibition of protein synthesis or the restoration of repressive conditions.

METHODS

Strains. The St. Lawrence wild-type strain of *N. crassa* and the 'leaky' arginine double mutant *arg-1r3 arg-10r1* were obtained from H. Kacser. The origin and properties of the latter strain were described by Barthelmess *et al.* (1974). The arginine auxotroph *arg-5* and the histidine auxotroph *his-2* were obtained from the Fungal Genetics Stock Center, Humboldt State University Foundation, Arcata, California, U.S.A.

Growth conditions and harvesting. Mycelium was grown in 1 l conical flasks under constant agitation (at 200 rev. min⁻¹) in a Gallenkamp orbital shaker at 29 °C. Each flask contained 200 ml Vogel's minimal medium and 4.5 g glucose as a carbon source, and was inoculated with about 10⁷ conidia from a suspension freed of mycelial debris by passage through a glass wool filter. Mycelium was harvested at least 16 h after inoculation by filtering the contents of complete flasks under suction through three layers of filter paper in a Buchner funnel. The mycelial pads were removed, rapidly frozen to -30 °C and then lyophilized. In this form mycelium could be stored under desiccation at -30 °C for several weeks without loss of enzyme activity. The number of harvests taken for assay at each time in the different experiments is given in Results.

Enzyme assays. All enzyme assays were performed on dialysed extracts prepared from lyophilized mycelium. Powdered mycelium (10 or 20 mg) was homogenized in 1 ml 0.05 M-Tris/HCl buffer (pH 7.5) containing 0.1 mM-EDTA and 0.5 mM-dithiothreitol, and the homogenate was centrifuged at 15 min at 20000 g in a refrigerated centrifuge. The supernatant was then dialysed overnight against 0.01 M-Tris/HCl buffer (pH 7.5) at 4 °C. Assays were carried out immediately after dialysis using appropriate dilutions of the extract and were done at 37 °C unless stated otherwise.

Ornithine carbamoyltransferase (EC 2.1.3.3) was assayed by the method of Davis (1962) but using the procedure of Marsh *et al.* (1965) to measure the citrulline formed. Acetylornithine aminotransferase (EC 2.6.1.11) was assayed by the method of Vogel & Jones (1970).

Glutamate acetyltransferase (EC 2.3.1.35) was assayed by measuring the formation of acetyl[¹⁴C]glutamate from [¹⁴C]glutamate as described by Denes (1970). Argininosuccinase (argininosuccinate lyase; EC 4.3.2.1) activity was determined by the formation of [¹⁴C]argininosuccinate from [¹⁴C]arginine. The assay mixture (final volume 0.3 ml) contained 20 mM-sodium fumarate, 20 mM-L-[guanido-¹⁴C]arginine (specific activity 8×10^4 c.p.m. μmol^{-1}), 0.033 M-phosphate buffer (pH 7.4) and sufficient dialysed extract to give a protein concentration of about 1.2 mg ml⁻¹. The reaction was initiated by the addition of extract, and stopped after 20 min incubation by the addition of 0.05 ml 30% (w/v) sulphosalicylic acid. The resulting mixture was centrifuged (1000 g for 10 min) and the supernatant was passed through a Dowex 50-X8 (H⁺) column (a Pasteur pipette plugged with glass wool and half filled with resin) that had been pre-equilibrated at pH 5.0 with sodium citrate buffer (0.2 M-Na⁺). The same buffer was used for elution. Arginine was retained, while argininosuccinate was eluted and collected in the first 4 ml of eluant to pass through the column after discarding the sample volume. Tubes that received either no fumarate, no extract or boiled extract were used as controls. The production of [¹⁴C]argininosuccinate was linear with time of incubation and protein concentration under the conditions used. It proved necessary to purify both the [¹⁴C]arginine used for this assay and the [¹⁴C]glutamate used in the acetyltransferase assay prior to use by passage through Dowex columns. Radiochemicals were obtained from The Radiochemical Centre, Amersham.

Saccharopine dehydrogenase (NAD⁺, lysine-forming; EC 1.5.1.7) was assayed at 28 °C by the method of Broquist (1971) and glucose-6-phosphate dehydrogenase (EC 1.1.1.49) was assayed at 25 °C by the method of Scott (1975). Histidinol dehydrogenase (EC 1.1.1.23) was assayed using the tetrazolium dye method of Martin (1971).

A unit of enzyme activity represents the formation of 1 μmol product min⁻¹ in the case of ornithine carbamoyltransferase, argininosuccinase, glutamate acetyltransferase and acetylornithine aminotransferase. For the remaining enzymes a unit is equivalent to a rate of change of 0.01 absorbance units min⁻¹. Protein was determined by the Lowry method, using bovine serum albumen for calibration.

Amino acid pools. Amino acid analysis was performed using an autoanalyser on the supernatants obtained after homogenizing lyophilized mycelium in 5% (w/v) sulphosalicylic acid, as described previously (Flint *et al.*, 1980). In some cases the method of Van Pilsum *et al.* (1965) was used to determine mycelial arginine. For this purpose, 10 mg of powdered, lyophilized mycelium was homogenized in 1.2 ml 5% (w/v) trichloroacetic acid, kept on ice for 30 min, and then centrifuged at 1000 g for 15 min. A 5- or 10-fold dilution of the supernatant was then used for the determination of arginine, employing standard solutions containing the appropriate concentrations of trichloroacetic acid. Estimates obtained by this method were entirely consistent with those obtained using the autoanalyser.

Table 1. *Amino acid pools in cultures treated with 3-amino-1,2,4-triazole*

3-Amino-1,2,4-triazole (3AT; final concentration 2.5 mM) was added to wild-type cultures after 17 h growth on minimal medium. The values represent the means of two replicate harvests.

Amino acid	Pool size [$\mu\text{mol (g dry wt)}^{-1}$]		
	Minimal medium	1 h after 3AT addition	6 h after 3AT addition
Histidine	4.2	1.2	1.1
Arginine	31	66	84
Ornithine	29	25	37
Citrulline	5.2	3.2	2.1
Lysine	5.7	9.9	30.1
Leucine	1.1	1.9	3.6
Valine	6.4	8.3	17.1
Isoleucine	1.1	1.2	1.7
Glutamate	131	109	149
Glutamine	56	84	87
Alanine	82	95	124
Tyrosine	1.3	ND	1.4
Phenylalanine	0.5	ND	1.0
Tryptophan	0.3	ND	0.2

ND, Not determined.

RESULTS

Histidine-mediated control

The control of arginine synthetic enzymes through histidine-mediated cross-pathway regulation was investigated in wild-type cultures treated with 3-amino-1,2,4-triazole (3AT). 3AT is known to inhibit the histidine synthetic enzyme imidazoglycerol-phosphate dehydratase; this in turn causes reductions in mycelial histidine content and in the state of charging of histidyl tRNA (Spurgeon & Matchett, 1977). Table 1 shows the effect of 3AT on amino acid pools in wild-type cultures. Histidine decreased fourfold; the arginine precursors ornithine and citrulline showed, respectively, no change and a twofold decrease. The other amino acids examined either increased or remained constant. Arginine, lysine, leucine and valine had all increased some threefold or more after 6 h exposure.

Derepression of the arginine synthetic enzymes ornithine carbamoyltransferase and argininosuccinase followed the addition of 3AT to cultures growing on minimal medium (Fig. 1 *a, b*). ('Derepression' and 'repression' are used in this paper in the operational sense, to refer to an increase in enzyme activity consequent upon decreasing an amino acid level and a decrease in enzyme activity consequent upon raising an amino acid level, without implying a particular mechanism.) In addition, the presence of 3AT inhibited growth (Fig. 1 *c*). The addition of histidine [which rapidly restores normal mycelial histidine levels (see Fig. 4)] 1 h before 3AT addition abolished derepression and almost completely eliminated the effect of 3AT on growth. The addition of arginine at the same stage failed to abolish the derepression and only slightly alleviated the effect of 3AT on growth. The activities of five other enzymes, determined in the same experiment for harvests taken 6 and 23 h after 3AT addition, are shown in Table 2. In addition to the two arginine synthetic enzymes discussed already, glutamate acetyltransferase and acetylornithine aminotransferase also showed histidine-mediated derepression. Saccharopine dehydrogenase (involved in lysine synthesis) and histidinol dehydrogenase (involved in histidine synthesis) also responded to 3AT. Glucose-6-phosphate dehydrogenase, on the other hand, did not, confirming that histidine limitation does not cause indiscriminate changes in all enzyme activities.

The increase noted in the mycelial arginine pool, and in certain other amino acids, following 3AT addition (Table 1) could be due to increased endogenous synthesis resulting

Table 2. *Enzyme derepression in cultures treated with 3-amino-1,2,4-triazole*

3-Amino-1,2,4-triazole (3AT; final concentration 2.5 mM) was added to wild-type cultures after 17 h growth on minimal medium; where indicated, arginine or histidine (final concentration 1.2 mM) was added 1 h before 3AT. The values for the cultures with no addition and with the addition of 3AT plus histidine represent the means of four harvests taken at the times shown (standard errors in parentheses); the other values are for single harvests.

Addition to growth medium	Time of harvest after 3AT addition (h)	Enzyme activity [milliunits (mg protein) ⁻¹ h ⁻¹]					
		Acetylornithine aminotransferase	Glutamate acetyltransferase	Ornithine carbamoyltransferase	Argininosuccinase	Saccharopine dehydrogenase	Histidinol dehydrogenase
None	0, 3, 6, 9	11.4 (0.9)	26.0 (1.3)	391 (9)	13.7 (0.6)	23.8 (2.0)	3.7 (0.4)
3AT + Histidine	3, 6, 9, 23	9.2 (1.3)	36.1 (4.0)	444 (25)	13.2 (1.1)	23.6 (1.3)	2.4 (0.4)
3AT	6	42.3	51.1	2179	51.7	76.2	8.1
	23	46.1	86.8	2062	76.2	64.2	7.4
3AT + Arginine	6	ND	67.9	2083	65.7	ND	ND
	23	46.4	93.8	2882	93.5	89.6	9.8

ND, Not determined.

* See Methods for definition of units.

dehydrogenase
Glucose-6-phosphate

Fig. 1. Enzyme derepression and growth in wild-type cultures treated with 3-amino-1,2,4-triazole (3AT). Specific activity ratios (enzyme activities expressed relative to those for minimal-grown cultures) of ornithine carbamoyltransferase (a) and argininosuccinase (b), and growth (c) are shown for cultures treated with 3AT (final concentration 2.5 mM) (○), 3AT (2.5 mM) plus arginine (1.2 mM) (●) or 3AT (2.5 mM) plus histidine (1.2 mM) (■). 3AT was added after 17 h growth on minimal medium; arginine or histidine was added 1 h before 3AT. The values for minimal-grown cultures (□) represent the mean of four harvests, except in (c) where they are for single harvests; the values for treated cultures are for single harvests. The numbers of doublings in (c) were derived from $\log_2(P_t/P_0)$ where P_0 is the total culture protein at the time of 3AT addition and P_t is the total culture protein at a subsequent time t .

from enzyme derepression. However, it could also be attributable to the reduced utilization of amino acids for protein synthesis that must accompany the inhibition of growth by 3AT. It is not possible to decide between these two explanations on the evidence available.

Enzyme levels under repressive conditions

The previous section examined the derepression of amino acid synthetic enzymes in response to a decrease in the mycelial histidine pool. In order to determine whether an increase in mycelial histidine, or in any other amino acid, caused repression, enzyme activities were determined for cultures supplemented with casein hydrolysate (Table 3). The activities of all the enzymes during growth with casein hydrolysate were close to their fully repressed levels during growth on minimal medium, although a slight, but statistically significant ($P < 0.01$), repression (of about 40%) was observed for glutamate acetyltransferase and acetylornithine aminotransferase. Thus, increases in amino acid pools to levels above those found for minimal-grown cultures (as occur in histidine-limited cultures – see Table 1) are expected to have only a small effect on enzyme levels.

Arginine-mediated control

In the absence of a suitable inhibitor of arginine synthesis, enzyme derepression in response to arginine limitation was examined in an arginine auxotrophic (*arg-5*) strain grown on low-arginine medium (Table 4). Arginine limitation resulted in the cross-pathway derepression of histidinol dehydrogenase and saccharopine dehydrogenase, in addition to causing the derepression of arginine synthetic enzymes. On high-arginine medium (2 mM) the *arg-5* strain showed activities close to those observed for the wild-type strain grown on minimal medium, although the mycelial arginine level was about fourfold higher in the auxotroph. Previous studies have shown that growth of the wild-type strain on medium containing arginine does not cause the four arginine enzymes to become repressed below their minimal growth levels (Cybis & Davis, 1975; Barthelmess *et al.*, 1974).

The specific activity ratios (i.e. the activities expressed relative to those for minimal-grown wild-type cultures) of argininosuccinase, saccharopine dehydrogenase and histidinol dehydrogenase in mycelium harvested at various times after the onset of histidine-

Fig. 2. Relationships between the activity of ornithine carbamoyltransferase and the activities of three other amino acid synthetic enzymes under conditions of histidine and arginine limitation. Specific activity ratios are shown, taking the values for minimal-grown wild-type cultures (see Table 2) as 1.0 ($\square$). Each point represents a single harvest for the wild-type grown in the presence of 3AT ($\bullet$) or for *arg-5* grown on low levels (0.12 or 0.04 mM) of arginine ($\circ$). Data obtained from the growth of a histidine auxotroph (*his-2*) on low levels (0.06 or 0.03 mM) of histidine ($\blacksquare$) are also included for the argininosuccinase/ornithine carbamoyltransferase plot.

arginine-mediated derepression are plotted against ornithine carbamoyltransferase activity in Fig. 2. In all three cases the relationships for arginine- and histidine-mediated derepression are similar. This is consistent with the derepression of these four enzymes being governed by a single common system of control, irrespective of which amino acid is responsible.

The data for glutamate acetyltransferase are less extensive, but the specific activity ratios observed for this enzyme were between 50% and 66% of those found for ornithine carbamoyltransferase both for the *arg-5* strain grown on low-arginine medium and after prolonged exposure of the wild-type strain to 3AT (Table 4). Acetylornithine aminotransferase could not be determined in the *arg-5* strain, as *arg-5* is its structural locus. The specific activity ratios for this enzyme in arginine bradytrophic strains are about 30% higher than those shown by glutamate acetyltransferase. Thus there is no evidence to suggest that the derepression of these two enzymes, relative to that of the other amino acid synthetic enzymes studied here, differs under conditions of histidine and arginine limitation.

Stability of ornithine carbamoyltransferase

Ornithine carbamoyltransferase (OCTase) was chosen for further study because of its ease of assay and high activity in mycelial extracts. In order to determine whether this enzyme was subject to degradation in growing cultures the decline in derepressed OCTase activity was followed in an arginine bradytrophic strain after the addition of arginine to the medium (Fig. 3). The strain used carried two mutant genes (*arg-1r3*, *arg-10r1*) that result in severely reduced activities for the arginine pathway enzymes argininosuccinate synthetase and argininosuccinase respectively (Barthelmess *et al.*, 1974). As a result this strain displays a decreased arginine pool and elevated activities for many amino acid biosynthetic enzymes,

Table 3. *Effect of casein hydrolysate on the activities of amino acid synthetic enzymes*
Wild-type cultures were grown on minimal medium or on minimal medium supplemented with 0.5% (w/v) casein hydrolysate. The values represent the means of three harvests taken between 17 and 22 h after inoculation (standard errors in parentheses).

Medium	Enzyme activity [milliunits (mg protein) ⁻¹ h ⁻¹]*					
	Acetylornithine aminotransferase	Glutamate acetyltransferase	Ornithine carbamoyltransferase	Argininosuccinase	Saccharopine dehydrogenase	Histidinol dehydrogenase
Minimal	14.1 (0.6)	32.2 (0.5)	417 (18)	12.5 (2.9)	16.1 (1.0)	4.3 (1.3)
Minimal + casein hydrolysate	8.9 (0.2)	18.3 (0.7)	356 (41)	11.3 (1.7)	14.8 (1.6)	4.2 (0.5)
					Glucose-6-phosphate dehydrogenase	40 (2) 38 (1)

* See Methods for definition of units.

Table 4. *Enzyme derepression in response to arginine limitation in an arginine auxotroph (arg-5)*

Cultures of the *arg-5* strain grown on high-arginine medium (2 mM) were harvested at times up to 28 h after inoculation, while still in the exponential phase of growth. Those grown on low-arginine medium (0.12 or 0.04 mM) were harvested after 40 h, when growth had virtually ceased due to exhaustion of the arginine provided. Each value is for a single harvest except for the 2 mM-arginine incubations (means of four harvests). Data for 3AT-treated wild-type cultures (based on the data shown in Table 2 for harvests taken 23 h after 3AT addition) are shown for comparison. The specific activity ratios show the enzyme activities relative to those for minimal-grown wild-type cultures (see Table 2).

Strain	Initial arginine concn in growth medium (mM)	Specific activity ratio						
		Acetylornithine aminotransferase	Glutamate acetyltransferase	Ornithine carbamoyltransferase	Argininosuccinase	Saccharopine dehydrogenase	Histidinol dehydrogenase	Glucose-6-phosphate dehydrogenase
<i>arg-5</i>	2	—	1.18	1.13	1.02	1.18	1.22	0.98
	0.12	—	3.8	6.4	6.2	3.3	3.7	0.82
	0.04	—	4.7	7.1	6.6	3.8	3.9	0.90
3AT-treated wild-type	0	4.0	3.3	5.3	5.6	2.7	2.0	0.84
	1.2	4.1	3.6	7.4	6.8	3.8	3.8	1.35

Fig. 3. Stability of ornithine carbamoyltransferase following repression by arginine in an arginine 'leaky' auxotroph. The decline in ornithine carbamoyltransferase activity (O) and the increase in the mycelial arginine pool (●) are shown for the arginine 'leaky' auxotrophic strain *arg-1r3 arg-10r1* following the addition of arginine to the medium (to a concentration of 1.2 mM). The dashed line indicates the activity observed in cultures grown on 1.2 mM arginine from the time of inoculation. Each point represents the mean of two replicate harvests; the values for individual replicates were within 5% of the mean value shown. The curve for the decline in enzyme activity represents the decline expected for a stable enzyme, based on the observed doubling time of 2.8 h. [For a stable enzyme $a_t = (a_0 - a_r) e^{-kt} + a_r$, where k is the exponential growth constant, a_t is the activity per unit protein at time t , a_0 is the activity at t_0 when arginine is added and a_r is the 'fully repressed' activity.]

Fig. 4. Interrupted derepression of ornithine carbamoyltransferase. Ornithine carbamoyltransferase activity (open symbols) and the mycelial histidine pool (solid symbols) are shown for wild-type cultures to which 3AT was added (final concentration 2.5 mM) after 17 h growth on minimal medium. Cultures either received no further addition ($\circ$, $\bullet$) or received histidine (6 mM) ($\square$, $\blacksquare$) or histidine (6 mM) plus cycloheximide ($12.5 \mu\text{g ml}^{-1}$) ($\triangle$) at the time indicated by the arrow. The dashed line shows the decline in enzyme activity expected if the repressed rate of enzyme synthesis became restored immediately following the addition of histidine (in the absence of cycloheximide) (see text). The histidine level in minimal-grown cultures is also indicated ($-\cdot-\cdot-$). Each point represents the mean of two replicate harvests; the values for the two replicates typically differed from the mean value by less than 2.5%, and never by more than 5%.

including OCTase, during growth on minimal medium. The decline in OCTase activity (Fig. 3) following addition of arginine is accounted for simply by dilution of existing enzyme due to growth, and it may therefore be concluded that degradation of the enzyme is negligible under these conditions. Similar types of experiments were used to confirm that OCTase is also stable following the addition of arginine to starved *arg-5* cultures, following the addition of histidine to 3AT-treated wild-type cultures and following the inhibition of protein synthesis by cycloheximide.

'Interrupted' derepression of ornithine carbamoyltransferase

No 'lag' in the onset of repression that might be ascribed to the decay of derepressed OCTase synthetic capacity (i.e. OCTase-specific mRNA) was detected in the experiments described in the preceding section. However, because of the extended sampling periods employed this merely implies that, if control is transcriptional, the half-life of OCTase synthetic capacity is a matter of minutes rather than hours. In an experiment of shorter duration (Fig. 4), wild-type cultures were grown for 90 min in the presence of 3AT alone, by which time OCTase activity had risen to twice the level found during growth on minimal medium, before receiving either histidine alone or histidine and cycloheximide. The addition of cycloheximide, which is a potent inhibitor of translation at the concentration used here (Flint, 1977), prevented any further increase in enzyme activity and also prevented any further growth. Because OCTase is stable (see Fig. 3) its activity remained substantially constant in the presence of cycloheximide. The absence of any detectable lag in the effect of cycloheximide on OCTase activity in this experiment confirms that the increase in this enzyme caused by 3AT is due to *de novo* enzyme synthesis rather than the activation of an enzyme precursor. Although

OCTase is a mitochondrial enzyme (Bernhardt & Davis, 1972) this experiment does not suggest the existence of any inactive cytoplasmic precursor. In cultures that received histidine, but no cycloheximide, OCTase activity continued to increase for about 30 min before declining, while growth was found to be greater than in cultures grown in the presence of 3AT only (see Fig. 1). Had the addition of histidine resulted in an immediate return to the repressed rate of OCTase formation typical of minimal-grown cultures, an immediate decline in OCTase activity would have been observed. The dashed line in Fig. 4 shows the decline predicted on this basis, taking into account the growth rate of the culture subsequent to histidine addition. Two explanations for this lag in histidine action were considered. First, histidine may enter the mycelium slowly (despite the high concentration used – 6 mM) and it may therefore take an appreciable time to attain mycelial histidine concentrations high enough to cause repression. Secondly, repression may be immediate and the lag due to the decay of derepressed OCTase synthetic capacity. The mycelial histidine content was found to respond rapidly to the presence of histidine in the medium (Fig. 4), reaching a level 10 times higher than that found in minimal-grown cultures 15 min after histidine addition. Since repressive conditions are therefore likely to have been restored within a few minutes of histidine addition, the explanation is favoured that the lag in histidine action represents the decay of OCTase synthetic capacity, present at high levels in the derepressed cultures. This would be consistent with control of OCTase formation exerted at the level of transcription or at the level of RNA processing or transport. A reliable estimate for the half-life of OCTase synthetic capacity cannot be obtained from these data. However, since the rate of increase in activity fell from about 5 milliunits min^{-1} to zero within 30 min of histidine addition (during which time the increase in total protein was less than 10%) the half-life must clearly be considerably less than 30 min (see Fig. 4). Similar experiments performed with *Saccharomyces cerevisiae* have yielded estimates of 5 to 7 min for the half-life of OCTase synthetic capacity in that organism (Messenguy & Cooper, 1977).

DISCUSSION

'Cross-pathway' regulation (i.e. the regulation of an enzyme by a metabolite belonging to a different pathway) has previously been observed for a number of enzymes involved in tryptophan, histidine and arginine synthesis in *Neurospora crassa*, under conditions of histidine or tryptophan limitation (Carsiotis *et al.*, 1970; Carsiotis & Jones, 1974; Carsiotis *et al.*, 1974; Spurgeon & Matchett, 1977). It has been taken to imply the existence of a 'general' control system governing enzymes in many different amino acid pathways and enabling them to respond to the levels of various amino acid end-products.

In the present study the six amino acid synthetic enzymes examined included four concerned with arginine synthesis, one with histidine synthesis and one with lysine synthesis. These enzymes were all found to display cross-pathway regulation, responding to reductions in histidine and in arginine. If a single general system of control governed the derepression of these enzymes in response to either amino acid, the relationship between the activities of any two enzymes (belonging to the same or different pathways) should be the same regardless of which amino acid is responsible for derepression. The evidence from this study (Fig. 2, Table 4) suggests that this is the case. Thus the response of the four arginine enzymes to conditions of arginine limitation can be accounted for solely through the action of the general control system. In yeast all eight enzymes involved in arginine synthesis are known to be subject to general control, but six of them (including ornithine carbamoyltransferase and acetyl-ornithine aminotransferase) show an additional 'pathway-specific' form of control, for which arginine is the signal (Delforge *et al.*, 1975; Messenguy, 1979). The latter system is responsible for the repression observed for most of the arginine enzymes in yeast when minimal-grown cultures are supplemented with arginine. The four enzymes examined in *N. crassa* in the present study do not appear to display such pathway-specific control, and are

not repressed in wild-type cultures by exogenously provided arginine. Indeed, only one arginine synthetic enzyme (carbamoyl-phosphate synthetase) responds to exogenously provided arginine in *N. crassa* (Cybis & Davis, 1975). This enzyme was not included in the present study, but has previously been shown to be subject to cross-pathway regulation (Carsiotis *et al.*, 1974; Spurgeon & Matchett, 1977). 'Dual' control by general and pathway-specific systems therefore remains a possibility for this enzyme.

The spectrum of amino acids capable of causing derepression of ornithine carbamoyl-transferase has been studied in *Aspergillus nidulans* (Piotrowska, 1980) where it was found that starvation of auxotrophic strains for histidine, arginine, proline or tryptophan led to derepression whereas starvation for lysine, leucine or phenylalanine did not. A broadly similar pattern of control is found in *N. crassa* (unpublished results). Any proposed mechanism for general control must take account of the fact that many, but not all, amino acids appear to be capable of eliciting derepression.

Another important feature of cross-pathway regulation in *N. crassa* is that derepression resulting from the limitation of one amino acid is not reversed by increases in other amino acids (Carsiotis *et al.*, 1970). In the present study the histidine-mediated derepression of arginine enzymes occurred despite being accompanied by increases in many other amino acid pools, and was not reduced by the presence of arginine in the medium. This is explained by the fact that enzyme levels are unresponsive to variation in any amino acid when this variation occurs at levels above those found in the minimal-grown wild-type. Thus supplementation of wild-type cultures with casein hydrolysate failed to produce any major repressive effect for the enzymes examined here. A detailed study of the relationship between mycelial arginine and the last three enzymes of arginine synthesis in arginine bradytrophic strains (Barthelmess *et al.*, 1974) has shown that enzyme levels are insensitive to arginine at concentrations ranging from sixfold greater to fourfold less than those found in the minimal-grown wild-type. Further reductions, however, caused a more than proportionate increase in enzyme. This sharp response may be a significant property of the mechanism involved in general control. If so, similar responses should be found at low levels of the other amino acids capable of eliciting derepression.

Finally, the more detailed investigations reported here into the regulation of ornithine carbamoyltransferase lead to several conclusions concerning the nature of general control. The stability of the enzyme under a variety of conditions (see Fig. 3) effectively rules out control at the level of enzyme degradation. The requirement of *de novo* synthesis for enzyme depression is suggested by the effect of cycloheximide when added to cultures undergoing derepression, while the consequences of reversing histidine-mediated derepression through histidine addition are consistent with control at a stage prior to translation (see Fig. 4). More decisive support for this latter conclusion would come if it were possible to demonstrate that enzyme synthetic capacity could accumulate in the presence of a translational inhibitor, as has been shown for some inducible enzymes (Subramanian & Sorger, 1972; Cybis & Weglenski, 1972). However, such an approach cannot be used to study derepression in response to amino acid limitation because the inhibition of protein synthesis prevents amino acids becoming limited. Thus, in the immediate absence of specific inhibitors of transcription, temperature-sensitive blocks in transcription, or means of estimating specific mRNA concentrations, experiments of the type reported here provide the best available approach.

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