POLYAMINE STIMULATION OF *IN VIVO* RATES OF MACROMOLECULAR SYNTHESIS IN A PUTRESCINE AUXOTROPH OF *ASPERGILLUS NIDULANS*

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1. Introduction

The naturally occurring polyamines putrescine (NH_2–$(CH_2)_4$–NH_2), spermidine (NH_2–$(CH_2)_3$–NH–$(CH_2)_4$–NH_2) and spermine (NH_2–$(CH_2)_3$–NH–$(CH_2)_4$–NH–$(CH_2)_3$–NH_2) are ubiquitous cations whose precise physiological roles remain unclear. It has been proposed that polyamines are involved in the synthesis of nucleic acids and proteins and/or maintaining membrane stability [1]. Investigations of *E. coli* polyamine auxotrophs have demonstrated a role for polyamines in protein synthesis that is independent of transcription [2,3]. However, since both the relative amounts of the different polyamines and the control mechanisms of macromolecular synthesis in bacteria are distinct from those of eukaryotes the role of polyamines may not be comparable in both groups of organisms.

Inhibitors of the polyamine biosynthetic pathway in eukaryotes can bring about decreases in polyamine and DNA synthesis while RNA synthesis continues unchanged [4–7], suggesting an essential role for polyamines in DNA synthesis. But starvation by using inhibitors is slow and the inhibitors themselves may have secondary effects. For these reasons, using a putrescine auxotroph of a lower eukaryote, we first deprived cultures of putrescine and then sought to determine which metabolic phenomena were stimulated during the resumption of growth when putrescine was added to the cultures. We report here that a putrescine auxotroph of the ascomycete *Aspergillus nidulans* has a separate requirement for polyamines for both protein synthesis and for RNA synthesis.

2. Materials and methods

2.1. *Chemicals*

Cycloheximide, 5-fluorouracil, cadaverine, putrescine, spermidine and spermine were from Sigma, St Louis, MO. Proflavine hemisulphate and 1,3-diaminopropane were from BDH Chemicals, Poole, England. L-[1-^{14}C]leucine and [8-^{3}H]adenine were from the Radiochemical Centre, Amersham, Bucks, England.

2.2. *Organism and growth conditions*

The polyamine auxotroph *Aspergillus nidulans* puA, [8] was maintained on malt extract agar supplemented with putrescine (50 μg/ml) and grown in submerged culture in minimal media as described [9]. The conidia, harvested and washed several times with Tween 80, 0.1% v/v, were inoculated into media without putrescine and incubated for 12 h after which time supplements and/or inhibitors were added. Further experimental details are given in the legends.

2.3. *Rates of macromolecular synthesis*

Conidial suspension (5 ml at a density of 10^7 conidia/ml) as incubated with (a) 1 ml 1.2×10^{-4} M DL-leucine containing 0.1 μCi [^{14}C]leucine for the measurement of incorporation into protein, or (b) 1 ml 6×10^{-6} M adenine containing 1.0 μCi [^{3}H]-adenine for incorporation into nucleic acids. Incubation was for 10 min at 37°C and stopped by the addition of 0.7 ml cold trichloroacetic acid (50%, w/v). After disruption of the conidia in an MSE ultrasonic disruptor the insoluble material was collected on

Whatman GF/C glass fibre filters and washed in 40 ml ice cold 5% trichloroacetic acid followed by 5 ml cold acetone. To measure separately the rates of RNA and DNA synthesis, precipitates were incubated with 0.5 M NaOH for 2 h at 37°C and the DNA reprecipitated. After air-drying the filters radioactivity was determined in 5 ml scintillation fluid (31.5 g PBD [5-biphenyl)-2-(4-*t*-butylphenyl)-1-oxa-3,4-diazole], 1–5 litres Triton, 500 ml methanol and 3 litres toluene).

3. Results and discussion

When *Aspergillus nidulans* puA_1 is grown in minimal medium supplemented with putrescine growth, as measured by germ-tube emergence and increase in total protein, is equivalent to that of the wild type BWB 272. In the absence of putrescine growth becomes severely limited 5–6 h after inoculation. This time corresponds to the onset of rapid accumulation of polyamines during germination of the wild type [10] and of the putrescine-supplemented auxotroph. After germ-tube emergence the rate of accumulation of polyamines in the wild type is considerably reduced and this together with the fact that polyamines probably turnover slowly in *A. nidulans* as they are found to do in a number of animal tissues [11,12], means that it then becomes very difficult to starve the mycelia of polyamines. Thus starvation of polyamines through use of auxotrophic mutants of metabolic inhibitors is a slow process making the identification of primary effects of the polyamines very difficult.

However, we have found that a very rapid change in intracellular polyamine levels can be brought about by adding polyamines to putrescine-starved cultures of *A. nidulans* puA_1. After growth of the mutant for 12 h in the absence of putrescine the polyamines still present in the conidia are no longer adequate to sustain active growth. The addition of putrescine at this time results in immediate increases in the rates of both protein and nucleic acid synthesis (fig.1). Both rates increase linearly over the 2 h period with the stimulation of protein synthesis being twice that for the nucleic acids. Both RNA and DNA synthetic rates are increased under these conditions with 10–12% of the [^{3}H]adenine being incorporated into

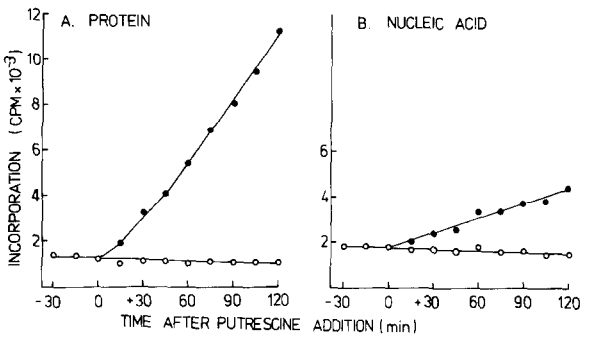

Fig.1. Time course of putrescine stimulation of protein and nucleic acid synthesis. The incorporation of [^{14}C]leucine (A) and [^{3}H]adenine (B) was measured at intervals in the absence of putrescine (○) and after the addition of putrescine at 100 μg/ml (●) to one part of the culture at the time shown.

DNA. Addition of putrescine (100 μg/ml) to the wild type *A. nidulans* has no effect on adenine or leucine incorporation rates.

The rapidity of the stimulation is consistent with the view that it is primary effects on macromolecular synthesis that are being observed. These results are similar to those obtained with a polyamine auxotroph of *E. coli* [3] where an immediate increase in the amount of protein was detected upon addition of putrescine to putrescine-depleted cultures while RNA and DNA did not increase for another hour. In the latter study the accumulation of radioactivity in nucleic acids and proteins was measured over an 8 h period after giving a single pulse of radioactive precursor whereas in our experiments samples were removed periodically and incubated with the radioisotope for 10 min. In the long incubations a progressive accumulation is measured whereas with the 10 min pulse changes in the rate are more readily detected.

In order to investigate the interrelationship between nucleic acid and protein synthesis stimulation a series of metabolic inhibitors was tested (fig.2). Cycloheximide at 20 μg/ml inhibited leucine incorporation but not adenine incorporation into trichloroacetic insoluble material. The transcriptional inhibitor proflavine [13] at 3 μg/ml had the opposite effect, preferentially inhibiting nucleic acid biosynthesis while the stimulation of leucine incorporation continued. 5-Fluorouracil had little effect on incorporation of leucine or adenine into trichloroacetic acid

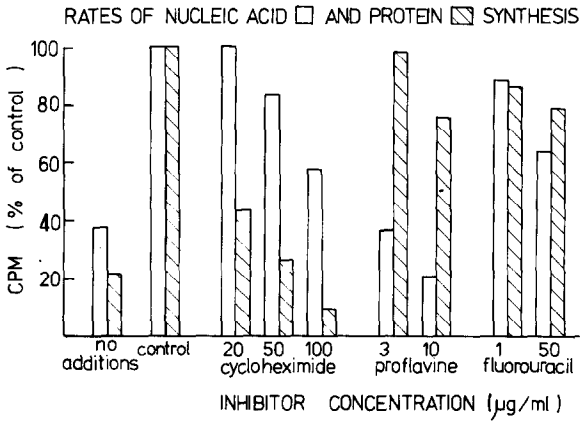

Fig.2. The effects of inhibitors on putrescine stimulation of nucleic acid and protein synthesis. The incorporation of [^{3}H]adenine and [^{14}C]leucine was measured 1 h after the addition of the inhibitors. Putrescine (100 μg/ml) was added to all the samples at the same time except for the 'no additions' samples. The control values for adenine and leucine incorporation were 2040 cpm and 6130 cpm, respectively.

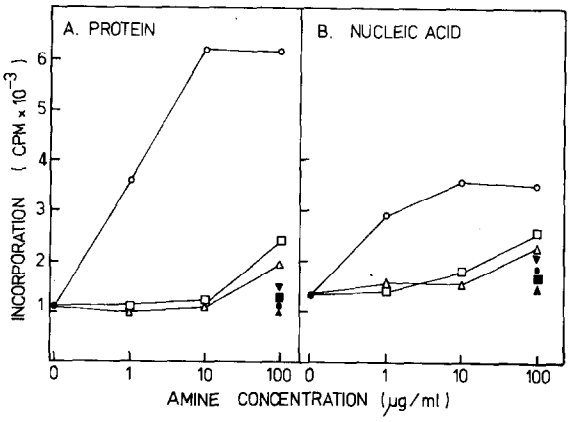

Fig.3. Stimulation of macromolecular synthesis by amines and amino acids. Incorporation of [^{14}C]leucine (A) and [^{3}H]adenine (B) was measured 1 h after the addition of the following compounds: putrescine (○), spermidine (△), spermine (□), cadaverine (■), 1,3-diaminopropane (▼), arginine (●) or ornithine (▲).

insoluble material at concentrations (1 μg/ml and 50 μg/ml) that decrease the rate of DNA synthesis by 48% and 63%, respectively. Thus the polyamine-stimulated increases in rates of synthesis of protein and RNA are largely independent of each other and of DNA synthesis.

The specificity of the stimulation of macromolecular synthesis by polyamines is shown in fig.3. Putrescine brings about the greatest stimulation of both nucleic acid and protein biosynthesis but spermidine and spermine are active in this respect as well. Cadaverine, 1,3-diaminopropane, ornithine and arginine had little effect on macromolecular synthesis. These differential abilities of the polyamines to stimulate synthesis correlate well with the observation that putrescine is more rapidly taken up by conidia than the other polyamines in *A. nidulans* [14]. Furthermore, we found that when grown with putrescine (10 μg/ml) for 1 h over 98% of the putrescine taken up is metabolized to spermidine and spermine so it is probable that putrescine serves only as a precursor of spermidine and spermine and that only the latter two amines are active in vivo.

The results presented here suggest that polyamines are likely to have multiplicity of functions within the cell rather than a single essential function. A similar hypothesis for polyamine functions in *E. coli* was suggested [15]. This is consistent with a range of effects on protein, RNA and DNA synthesis which have been demonstrated in vitro [1]. Polyamines bind to nucleic acids at physiological ionic strengths [16,17] and they stabilize nucleotide–nucleotide interactions in some circumstances to the extent of causing miscoding [18]. Polyamines are also known to exchange readily on and off nucleic acid-containing structures [19]. We therefore propose that the role of polyamines in the cell is primarily in transient stabilization of nucleotide–nucleotide interactions such as occur in the processes of protein, RNA and DNA synthesis. The involvement of polyamines in such transient stabilizations would be expected to lead to a rapid stimulation of protein and nucleic acid synthesis after putrescine starvation as observed here with the putrescine auxotroph of *A. nidulans*.

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