SHORT COMMUNICATION

Increasing Guanosine 3'-Diphosphate 5'-Diphosphate Concentration with Decreasing Growth Rate in *Anacystis nidulans*

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(Received 21 February 1979; revised 9 May 1979)

The concentration of guanosine 3'-diphosphate 5'-diphosphate increased exponentially with decreasing growth rate in *Anacystis nidulans* grown at different light intensities. This is inconsistent with the concept of guanosine 3'-diphosphate 5'-diphosphate as a regulator of transcription in *A. nidulans* since previous work has shown that in this organism the RNA/DNA ratio remains constant with increasing growth rate, implying a constant rate of transcription from individual cistrons.

INTRODUCTION

The cyanobacterium *Anacystis nidulans* possesses a regulatory mechanism capable of modulating RNA accumulation in response to a number of conditions which directly or indirectly reduce the size of the aminoacyl-tRNA pool (Smith & Carr, 1977; Smith, 1977; Mann *et al.*, 1975). The mechanism has a number of characteristics comparable with the RNA-control function of heterotrophic bacteria. In particular the organism accumulates guanosine 3'-diphosphate 5'-diphosphate (ppGpp) upon elicitation of the regulatory mechanism.

The nucleotides guanosine 3'-diphosphate 5'-triphosphate (pppGpp) and ppGpp are putative regulators of stable RNA accumulation, though at present their true function appears a matter of conjecture (see Van Ooyen *et al.*, 1976; Sokawa *et al.*, 1975; Gallant *et al.*, 1976; Hansen *et al.*, 1975). Several species of heterotrophic bacteria exhibit exponentially increasing DNA and RNA contents with increasing growth rate (Maaløe & Kjeldgaard, 1966). Any inhibitory regulator would be expected to vary inversely with growth rate and RNA content. An inverse proportionality of ppGpp concentration to RNA content has been demonstrated (Lazzarini *et al.*, 1971; Hansen *et al.*, 1975), but doubts have been expressed as to the value of this observation (Gallant *et al.*, 1976).

DNA and RNA content also increase exponentially with increasing growth rate in *A. nidulans* (Mann & Carr, 1974) in a manner akin to that described for *Escherichia coli*. However, the situations differ since the RNA/DNA ratio remains constant and independent of growth rate in *A. nidulans*. A constant RNA/DNA ratio has also been described in the cyanobacterium *Anabaena variabilis* (Leach *et al.*, 1971). This communication reports the variation in GTP, ppGpp and pppGpp content with respect to growth rate in *A. nidulans* cultures.

METHODS

Anacystis nidulans (strain 625; Culture Collection of Algae, University of Texas, previously University of Indiana) was cultured as described previously (Smith & Carr, 1977). Growth rate was varied by altering the intensity of incident light which was regulated through the use of a variety of neutral density filters:

Fig. 1. Concentrations of GTP (●), ppGpp (○) and pppGpp (□) as functions of growth rate in *Anacystis nidulans* grown at different light intensities. *Anacystis nidulans* cultures were monitored over three generations to ensure steady state growth, incubated with [³²P]P_i for at least one generation and then sampled in triplicate between turbidities (A_{750}) of 0.4 and 0.5.

(i) slotted cardboard filters; (ii) white-painted flasks; (iii) flasks covered in aluminium foil. Each method was capable of producing a range of mean doubling times between 6 and 25 h.

[³²P]P_i (The Radiochemical Centre, Amersham) was added to the medium and labelled nucleotides were isolated by formic acid extraction as described previously (Smith & Carr, 1977). The nucleotides were fractionated on polyethyleneimine cellulose plastic-backed thin-layer plates (cat. no. G-1440 PEI, 20 × 20 cm; Schleicher & Schuell, Dassel, West Germany) according to the single-dimension technique of Cashel (1969), the two-dimensional techniques of Randerath & Randerath (1964) and method 2 of Gallant *et al.* (1976). Variation in measurements of ppGpp and GTP concentrations did not exceed 10% of the mean. Estimates of pppGpp concentration had greater variation but all values were within 18% of the mean.

RESULTS AND DISCUSSION

The concentration of ppGpp in *A. nidulans* increased exponentially with decreasing growth rate (Fig. 1). The absolute concentrations and the extent of variation were comparable with those reported for heterotrophic bacteria. A threefold increase [from 0.07 to 0.21 nmol (A_{750} unit)⁻¹] in the concentration of ppGpp occurred between mean doubling times of 6 and 25 h. Amino acid deprivation, reducing the RNA accumulation rate to 10% of the rate observed during non-inhibited growth, promoted ppGpp accumulation to concentrations exceeding 1.0 nmol (A_{750} unit)⁻¹. The concentration of pppGpp did not appear to increase in proportion with ppGpp. The relatively low incorporation of [³²P]P_i into pppGpp, in comparison with background values on polyethyleneimine cellulose plates, made assessment of pppGpp concentrations difficult. The GTP concentration declined exponentially from 1.20 to 0.60 nmol (A_{750} unit)⁻¹ with decreasing growth rate. A similar decrease in GTP concentrations occurred in strains CP78 and CP79 of *E. coli* (Sokawa *et al.*, 1975). ATP concentrations in *A. nidulans* remained constant, within experimental limits, at 3.7 to 4.0 nmol (A_{750} unit)⁻¹. Concentrations of both pyrimidine ribonucleotides increased by a factor of 2 with decreasing growth rate. An inverse proportionality between RNA and ppGpp content may be demonstrated using the results of Mann & Carr (1974).

Although the role of ppGpp in prokaryotes is at present uncertain, the inverse proportionality between ppGpp and RNA content with respect to growth rate has lent some support to the concept of ppGpp as a regulator of RNA accumulation. The RNA content of those heterotrophic bacteria and cyanobacteria which have been investigated increases exponentially with increasing growth rate. The available evidence indicates a significant difference between these two prokaryotic groups. In heterotrophic bacteria the RNA/DNA ratio increases with increasing growth rate whereas in *A. nidulans* the ratio remains constant

(Mann & Carr, 1974). Whilst a higher RNA/DNA ratio at faster growth rates suggests an increasing rate of transcription per stable RNA cistron, the constant RNA/DNA ratio in *A. nidulans* indicates a constant rate of transcription per cistron. Thus, the rate of initiation of RNA polymerase on promoter sites at stable RNA cistrons would be expected to remain constant irrespective of growth rate and no modulation of this process would be required. The significance of the relationship between ppGpp concentration and RNA content is thus reduced to the level of coincidence and detracts from the concept of ppGpp as a regulator of stable RNA accumulation.

I thank the Science Research Council for generous financial support and Mrs C. M. Taylor for excellent technical assistance.

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