Control of Macromolecular Composition and Cell Division in the Blue-green Alga *Anacystis nidulans*

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SUMMARY

The cell volume and macromolecular composition, in terms of DNA, RNA and cell mass, were examined in *Anacystis nidulans* at different growth rates of the organism. Both DNA and RNA increased exponentially with increasing growth rate, as has been found in several heterotrophic bacteria. However, in this blue-green alga the ratio of DNA to RNA was independent of growth rate. Cell mass and volume also increased exponentially with growth rate though at a slower rate than RNA and DNA. These results also indicate a constant ratio of tRNA and rRNA to DNA in contrast to the situation in heterotrophic bacteria so far studied.

The variation of cell volume in this organism can be related to the control of cell division, and indicates that the commencement of DNA replication and the processes of cell division are associated with the achievement of a critical cell volume, as has been demonstrated for *Escherichia coli*.

INTRODUCTION

The ways in which macromolecular composition and cell size vary in response to the growth rate have been examined in several heterotrophic bacteria (Schaechter, Maaløe & Kjeldgaard, 1958; Neihardt & Magasanik, 1960; Rosset, Julien & Monier, 1966; Gray & Midgley, 1970; Skjold, Juarez & Hedgcoth, 1973) and much of the relevant data have been reviewed by Maaløe & Kjeldgaard (1966). The pattern observed was that DNA, RNA, protein and cell mass all increased exponentially with growth rate. Cell mass increased at a rate slightly greater than DNA/cell, which increased at the same rate as protein/cell. The most significant observation was the greatly increased levels of RNA relative to DNA at fast growth rates. The relative levels of rRNA and tRNA have also been examined as a function of growth rate. The results indicated an increase in the relative level of rRNA with increasing growth rate (Kjeldgaard & Kurland, 1963; Forchhammer, Kjeldgaard & Moldave, 1965; Rosset *et al.* 1966; Forchhammer & Lindahl, 1971; Gray & Midgley, 1970; Skjold *et al.* 1973).

It has been demonstrated in *Escherichia coli* by Cooper & Helmstetter (1968) that the time of initiation of rounds of DNA synthesis in the cell cycle varied in relation to the previous cell division, but that there was a constant interval of approximately 60 min between initiation of DNA replication and subsequent cell division. This period of 60 min comprised an initial 40 min during which DNA replication occurred and a further 20 min before the subsequent cell division. It follows that when the organism is growing at mean generation times shorter than 40 min, it becomes necessary to begin a second round of DNA replication before the previous one is completed, leading to a greater DNA content in faster growing cells.

The combination of these results with observations of Schaechter *et al.* (1958) that the average cell size was an exponential function of growth rate was shown by Donachie (1968) to indicate that the initiation of a round of DNA replication takes place when the ratio of cell mass to chromosome origins achieves a certain critical value. Thus the cell size at initiation will be an integral multiple of a particular cell mass, dependent on the number of chromosome origins. This accounts for the increasing size of cells with growth rate and indicates methods by which the cell can regulate initiation of DNA replication (Donachie, 1968).

We examined whether a dependence of macromolecular composition on growth rate exists in the obligately phototrophic micro-organism, *Anacystis nidulans*, and whether the nutritional mode of this organism is reflected in differences, in its control of macromolecular composition, from the pattern found in heterotrophic bacteria.

METHODS

Growth conditions. *Anacystis nidulans* (Indiana University Culture Collection strain 625) was grown in medium C (Kratz & Myers, 1955) supplemented with 0.1 % NaHCO_3 in 1 l reaction vessels at 34 °C. The cultures were gassed with 95 % air/ CO_2 at a rate of 100 ml/min and illuminated by 75 W reflector lamps (Cryselco) 12 cm from both sides of the culture. Under these conditions, mean generation times of approximately 3 h were achieved. Alterations of the incident light intensity were employed to produce differing mean generation times. Cell mass (as measured by E_{650}) and cell number were monitored to establish that the organism was in a steady state of growth. After at least 4 generations of balanced growth samples were withdrawn for estimation of cell volume, DNA/cell and RNA/cell. Under the conditions employed, exponential growth of the organism would continue until an E_{650} of approximately 0.8 was reached; experimental samples were always withdrawn while the E_{650} was between 0.4 and 0.6.

RNA estimation. Four samples each containing at least 10^9 cells were cooled in ice and harvested. The pellet was washed twice with 10 ml absolute alcohol and the organisms resuspended in 2.5 ml 0.2 M-NaCl and 0.1 ml 60 % perchloric acid. After incubation at 70 °C for 80 min the suspension was chilled in ice and centrifuged. The ribose in the supernatant was then estimated by a modification (Taggart, 1967) of the orcinol reaction (Schneider, 1957), in which 1 ml of the supernatant was heated to 100 °C for 30 min with 1.0 ml 0.05 % (w/v) FeCl_3 in concentrated HCl and 0.1 ml 6.0 % (w/v) ethanolic orcinol. The samples were then chilled in ice and the colour stabilized by the addition of 20 % (v/v) ethanol. The E_{650} of the samples was measured against a reagent blank and the results compared with D-ribose standards.

DNA estimation. Four samples each containing at least 4×10^9 cells were cooled in ice and perchloric acid added to a final concentration of 0.5 N. After 20 min the organisms were centrifuged, resuspended in 0.5 N-perchloric acid and incubated at 70 °C for 40 min. The extracts were then chilled in ice, centrifuged, and 1 ml samples of the supernatant assayed for DNA by the diphenylamine method of Burton (1956) and compared with D-deoxyribose standards.

Estimation of cell number. A suitable dilution of unstained organisms was placed in a Neubauer counting chamber and, after allowing 15 min for the cells to settle out, was counted. The electronic particle counter (see below) was also employed for this purpose.

Estimation of cell volume. Cell volume was estimated by means of an electronic particle counter (Model ZB, Coulter Electronics Ltd, Dunstable). A sample of the culture was

Fig. 1. Cell levels of DNA (■), RNA (●), $E_{650}/10^9$ cells (◐) and cell volume, V (○) as functions of growth rate of *A. nidulans*.

suitably diluted in Isoton (Coulter Electronics Ltd) and by using dual threshold settings the distribution of cell volumes between 0.37 and $4.57 \mu\text{m}^3$ was obtained. A mean cell volume was calculated from these results.

RESULTS AND DISCUSSION

Experimental growth was maintained under a variety of different light intensities and samples were analysed for DNA and RNA (see ordinate, Fig 1), mean cell volume $V (\mu\text{m}^3)$ and E_{650}/cell . The relationships of these parameters to growth rate are shown in semi-logarithmic plots (Fig. 1) and are expressed below as a series of equations, where μ is the growth rate and the constant is derived from the intercept on the ordinate axis at zero growth rate.

$$\log \text{DNA} = 3.327\mu + 0.627, \quad (1)$$

$$\log \text{RNA} = 3.243\mu + 0.393, \quad (2)$$

$$\log V = 0.962\mu + 0.071, \quad (3)$$

$$\log (E_{650}/10^9 \text{ cells}) = 0.900\mu + 0.522. \quad (4)$$

The relationships which existed in *Anacystis nidulans* between DNA/cell, RNA/cell and cell size with growth rate were qualitatively similar to those found in heterotrophic bacteria (see Introduction), in that there is an exponential increase of these parameters with increasing growth rate. Two important quantitative differences exist, however, in that cell mass increased more slowly than DNA/cell with growth rate and that the DNA/RNA ratio was independent of growth rate. The constant DNA/RNA ratio has also been observed in the filamentous blue-green alga *Anabaena variabilis* (Leach, Old & Carr, 1971). The significance of this observation is emphasized by the fact that the relative levels of tRNA and

Fig. 2. The increase in cell number of *A. nidulans* during a 'step up' induced by increased light intensity.

rRNA do not vary with growth rate (Mann & Carr, 1973) and the ratios of these two stable species to DNA are also independent of growth rate, whereas in several species of heterotrophic bacteria (see Introduction) a variation of the relative levels of tRNA and rRNA has been reported. It would seem that *A. nidulans* is unable to respond to an altered growth rate by an alteration in the relative frequency of transcription of the stable RNA genes. This is in accord with the suggestion (Carr, 1973) that the underlying feature of obligate phototrophy may be a lack of selective gene expression.

The response of bacterial cells taken from a nutritionally poor to a rich medium, a 'step up' transition, is to maintain the old rate of cell division for a characteristic length of time before the new rate of cell division is established (Kjeldgaard, 1961; Sud & Schaechter, 1964; Schleif, 1967; Kjeldgaard, 1967). This maintenance of the rate of cell division at the rate prior to step-up may be explained in terms of the *C* and *D* intervals, where *C* is the time taken for a replication fork to traverse the chromosome and *D* is the time between the completion of replication and subsequent division. After (*C* + *D*) min in the new medium the cells begin dividing at the new rate characteristic of the medium, as a consequence of the increased frequency of insertion of replication forks which can only be expressed in cell division after (*C* + *D*) min (Helmstetter, Cooper, Pierucci & Revelas, 1968).

The time taken for a culture of *A. nidulans* to achieve a new rate of cell division, the step-up time, was measured after an increase in the incident light intensity (Fig. 2). Four such experiments gave an average step-up time for cell division of 178 min.

It has been shown (Donachie, 1968) that the initiation of DNA replication is related to the achievement of a critical cell volume by the bacterial cell. By combining the estimate of (*C* + *D*) from the step-up experiments with the rate of change of cell volume with growth

Fig. 3. The growth of individual cells at four different growth rates, from the volume just after division (●) to the volume just before division (■). For the arbitrary units of cell volume, cell volume just after division is taken as unit volume, at an infinitely long mean generation time.

rate it becomes possible to demonstrate a similar kind of relationship in *A. nidulans*. Assuming that the volume of the cell just after division bears a constant relationship to the average volume of the cell at that particular growth rate, the growth of individual cells between divisions may be represented (Fig. 3). Growth is expressed logarithmically, because it has been shown by Bazin (see Williams, 1971) that in *A. nidulans* the growth of the individual cell approximates most closely to an exponential model. It is clear that at about 178 min ($C + D$) before division the cell achieves a certain critical volume, regardless of the rate at which the cell is growing and consequently regardless of the average volume of the cell population.

It has been shown (Donachie, 1968; Pritchard, Barth & Collins, 1969) that the mean cell volume can be related to the growth rate by the equation:

$$V = k \times 2^{(C+D)/g},$$

where k is a constant and g is the mean generation time (h).

If the ($C + D$) value of 178 min is introduced into this equation it predicts that a plot of $\log V$ versus growth rate ($= 1/g$) should have a slope of 0.90, which is in good agreement with the observed value of 0.96 h^{-1} and the value 0.90 h^{-1} (see eqns 3 and 4) calculated from the slopes for cell volume and $E_{650}/10^9$ cells in Fig. 1. The interpretation of the cell cycle of *Anacystis nidulans* in terms of the C and D intervals is supported by the measurement of DNA replication time and the ensuing interval before cell division in synchronous cultures of *A. nidulans* (Herdman, Faulkner & Carr, 1970) where C and D had values of 65 and 115 min respectively.

It is apparent that the relationship of cell volume to growth rate in *A. nidulans* and the behaviour of cells during a step-up can be satisfactorily described in terms of the analysis of the cell cycle as used by Cooper & Helmstetter (1968). However, efforts to describe DNA/cell or genome equivalents/cell in terms of (C and D) and the growth rate have failed. Before a culture of *A. nidulans* has reached a growth rate at which a second round of DNA replication begins before the cell divides, i.e. at mean generation times shorter than $C + D$,

the DNA content of the cell is already of the order of 16 times greater than the DNA content at zero growth rate obtained by extrapolation of the DNA/cell slope in Fig. 1. At present there is no satisfactory model to describe this increase in DNA content.

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