photosynthetic systems. It seems as if the blue-green bacteria are a key intermediate in the development of photosynthesis and also in the development of the 2Fe ferredoxins, possibly from ferredoxins containing a 4Fe–4S centre. This last point is also interesting in that it has now been shown that the 2Fe–2S centre is formally derivable from the 4Fe–4S centre in analogue compounds that have been synthesized by Holm's group (Mayerle et al., 1973). There are suggestions that the 4Fe–4S centre is a much more stable complex, whereas the 2Fe–2S centre will naturally devolve into a 4Fe–4S centre. One can speculate that the 2Fe ferredoxins need to be twice the size of a 4Fe ferredoxin or an 8Fe ferredoxin because it is much more difficult to maintain the 2Fe–2S centre in a given configuration than it is to maintain a 4Fe–4S centre in its normal configuration. This begs the question as to why blue-green bacteria did not use the 4Fe–4S centre; it may have to do with the incorporation of both soluble and membrane-bound ferredoxins into an association with membranes that are required for the photosynthetic systems characteristic of O₂-evolving organisms.


Nucleic Acid Synthesis and Regulation in Blue–Green Algae
NOEL G. CARR and NICHOLAS MANN*
Department of Biochemistry, University of Liverpool, P.O. Box 147, Liverpool L69 3BX, U.K.

Sedimentational properties of ribosomes and their subunits, and the physical form and dimensions of DNA, have been studied in blue–green algae for some years. The characterization of these macromolecules, which did not present any important difference from bacteria, contributed to the confirmation of the prokaryotic nature of blue–green algae. In this brief review we examine the control of nucleic acid synthesis and the ways in which

* Present address: Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, U.K.

1975
this process is integrated into the control of cell division and the establishment of macromolecule composition at different growth rates. In these aspects *Anacystis nidulans*, on which most work has been done, offers both similarities to and contrasts with heterotrophic bacteria.

**Steady states of growth**

In recent years considerable information on the control of macromolecule composition of bacterial cells has been derived from nutritional shifts imposed on otherwise steady-state cultures. The application of such an approach to *Anacystis nidulans* is facilitated by the ease and rapidity by which light-intensity, the sole energy source, may be manipulated. It is necessary to know the variation of macromolecule content of the organism, as a function of growth rate, before a meaningful analysis of shifts can be carried out. The composition of *Anacystis nidulans* cultures with doubling-times between 3 and 24h is shown in Fig. 1. Cell size and DNA and RNA contents per cell all exhibited exponential dependence on growth rate, in a manner analogous to that observed in *Salmonella typhimurium* (Schaechter et al., 1958). There are, however, important differences in the relative pattern of variation of these parameters of cell composition with growth rate. The ratio of RNA to DNA remains constant at all growth rates, whereas in bacteria this ratio increased with growth rate, i.e. the cell contained relatively more RNA at fast growth rates. The other unusual aspect found in *Anacystis nidulans* was that the amount of DNA relative to cell mass increased with growth rate, in contrast with the observation in heterotrophic bacteria that, whereas the total amount of DNA in the cell increased, the DNA to cell mass ratio decreased. A satisfactory model to explain the rate of DNA increase in *Anacystis nidulans* is not available from studies on heterotropic bacteria. The variation of cell mass (volume) with growth rate agrees with the equation (Pritchard et al., 1969) derived from the model proposed by Cooper & Helmstetter (1968):

$$\bar{M} = k \cdot 2^{(C + D)/\sigma}$$

In this $\bar{M}$ is the mean cell mass, $C$ is the chromosome replication time, $D$ is the time

Fig. 1. *Effects of growth rate on the macromolecular composition of Anacystis nidulans*

■, Cell content of DNA; •, cell content of RNA; ◌, $E_{650}/10^9$ cells; ○, cell volume. The data are taken from Mann & Carr (1974).
elapsed between termination and division and $g$ is the mean generation time. The values of $C$ and $D$ were derived from studies on synchronous cultures (Herdman et al., 1970).

**RNA synthesis during shift-up and shift-down**

When the illumination supplied to a culture of *Anacystis nidulans* in steady-state growth was increased there was an immediate increase in the accumulation of RNA (measured by the orcinol reaction). The initial rate of RNA accumulation after the shift-up was considerably greater than the eventual new rate of RNA accumulation that was observed when the new, faster, growth rate became established. At the time at which cell mass increase and cell number increase became balanced (i.e. the new steady state) the RNA content of the cells had increased to that characteristic of the new growth rate. The rapidity of the response of RNA accumulation after the shift-up argued against an increased synthesis of RNA polymerase being responsible for the increased rate of RNA accumulation. This increased rate could be explained by (a) the activation of a previously formed reserve of RNA polymerase or (b) the transfer of RNA polymerase from genes specifying mRNA to those of stable RNA. Although both of these suggestions are compatible with current ideas of the control of RNA synthesis in bacteria, there is no known mechanism by which alteration in light-intensity could mediate either type of control. In contrast with the results based on the chemical estimation of RNA accumulation, the rate of $[^{14}C]$uracil incorporation into RNA (cold-trichloroacetic acid-insoluble material) after shift-up gradually alters to a decreased rate. This anomalous result is discussed below in connexion with shift-down experiments.

The decrease in light-intensity supplied to a steady-state culture of *Anacystis nidulans* causes an immediate decrease in the rate of cell mass accumulation, whereas, as would be expected from the Cooper–Helmstetter model, the rate of cell division remains unaltered for 180 min, i.e. the $C$ plus $D$ time. In such a culture RNA accumulation measured chemically ceases immediately; there was a transient decrease (5%) in the total cellular RNA followed by a period of 130 min of very low accumulation, and then at the end of this period RNA accumulation recommenced at a rate characteristic of the new, slower, growth rate. By this time the RNA content had adjusted to that found in cultures of the new growth rate. The rate of $[^{14}C]$uracil incorporation after shift-down was initially lowered to zero, for a time period that correlated with the period of time during which there was a loss of RNA as measured chemically. This was followed by incorporation at an enhanced rate, relative to pre-shift-down. An explanation for these apparently curious $[^{14}C]$uracil-uptake results may lie in a greater dilution of radioisotope after shift-up consequent upon the increased endogenous rate of uracil synthesis coupled with an absence of alteration in the rate of $[^{14}C]$uracil uptake. It is thought that uracil entry into *Anacystis nidulans* is a non-active process (Pigott & Carr, 1971). The converse of this argument would apply to the increased rate of $[^{14}C]$uracil incorporation that follows shift-down.

**Guanine nucleotide concentrations**

In view of some current ideas concerning the role of guanine nucleotides (ppGpp and pppGpp) as putative regulators of the accumulation of stable RNA in bacteria (Cashel, 1969; Travers et al., 1970), such compounds were examined in an *Anacystis nidulans* culture undergoing a shift-down. The concentrations detected before shift-down were comparable with those reported for *Escherichia coli*. There was an inverse linear relationship between the concentration of ppGpp and growth rate of *Anacystis nidulans*, and, consequently, with the RNA content of the organism (R. J. Smith & N. G. Carr, unpublished work). After shift-down there were severalfold increases in the concentrations of ppGpp (Fig. 2) and pppGpp, reaching a maximum approx. 12 min after the shift. The concentrations then declined to reach a new steady value. The period during which there were elevated concentrations of these nucleotides coincided with the decrease in absolute amount of RNA measured. However, RNA accumulation was restricted for a much greater period of time after ppGpp concentration had declined.
indicating the operation of another mechanism of constraint on RNA accumulation after the shift-down. The observation that uracil incorporation continued throughout this period of constraint suggests that a degradative control may be present. The marked short-term increase in GTP is noteworthy.

**Stability of RNA species**

Recently some advances have been made in understanding the synthesis, maturation and stability of rRNA species in blue-green algae. Doolittle (1972) observed that in *Anacystis nidulans* the synthesis of both 16S and 23S rRNA was, at least in part, dependent on concurrent photosynthesis and was inhibited by the antibiotic streptolydigin. Formation of both rRNA species was also inhibited by chloramphenicol, with the accumulation of the higher-molecular-weight RNA species believed to be precursors. Pulse-labelling experiments with *Anacystis nidulans* (Szalay et al., 1972) indicated the existence of precursor molecules for both the 16S and 23S rRNA species and that the maturation process was dependent on Mg²⁺. Grierson & Smith (1973) also reported the existence of 16S and 23S rRNA precursor molecules in *Tolypothrix distorta, Anabaena cylindrica* and *Nostoc muscorum*, but found no evidence for a single polycistronic precursor. Seitz & Seitz (1973) found that the 16S rRNA in *Anacystis nidulans* was methylated and that this methylation took place after maturation; however, they found no evidence for a 23S precursor. Doolittle (1973) reported that the 23S rRNA in *Anacystis nidulans* was labile and underwent endonucleolytic cleavage in vivo to produce species of molecular weights 0.88 × 10⁶ and 0.17 × 10⁶. Cleavage was stimulated by light, and the half-life of 23S rRNA was about 5h in illuminated cultures and about 10h in unilluminated cultures.

In the filamentous species *Anabaena variabilis* the stability of RNA has been examined by following the loss of radioactivity in the cold-trichloroacetic acid-insoluble fraction after proflavin addition to a [¹⁴C]uracil-labelled culture. The stability of mRNA was more directly measured by the ability of RNA extracts to direct protein synthesis in vitro (Leach & Carr, 1974). The results indicated that the half-life of mRNA was approx. 10min, which was 3% of the mean generation time of the culture from which it was derived. These experiments indicated that 17.5% of the total RNA was unstable.
Fig. 3. Relation between growth rate and cell volume in Anacystis nidulans

The growth of individual cells at four different growth rates is shown from the volume just after division (○) to the volume just before division (■). For the arbitrary units of cell volume, the volume just after division at an infinitely long mean generation time was taken as unit volume. The data are taken from Mann & Carr (1974).

decay recorded presumably represents the breakdown of mRNA, nascent tRNA and nascent rRNA. This considerably higher proportion of unstable RNA in Anacystis variabilis may be another manifestation of the control of content of the organism by degradative mechanisms.

Control by DNA synthesis

Information about the control of DNA replication and cell division may be drawn from the rate of change of cell size with growth rate. Donachie (1968) has shown that the initiation of DNA replication in bacteria may be related to the achievement of a critical cell volume. A similar kind of relationship has been found in Anacystis nidulans by combining estimates of $C$ and $D$ times with the data on the rate of change of cell size with growth rate. It is taken that the volume of the cell just after division bears a constant relationship to the average cell volume at that particular growth rate. In Fig. 3 the growth of individual cells is represented in an exponential fashion. It is evident that approx. 3h before division cells, regardless of their growth rate and hence average size, are the same volume. The time between the attainment of this critical volume and the subsequent division is very close to the estimate of the $C+D$ times, and thus in Anacystis nidulans the initiation of DNA replication and the commitment to cell division occur at approximately the same time in the cell cycle, as a particular cell mass is achieved (Mann & Carr, 1974).

Comment

The data discussed indicate that the control of nucleic acid synthesis in Anacystis nidulans is in many respects similar to that in the more extensively studied heterotrophic bacteria. There are, however, several features that encourage us to believe that further study with blue–green algae will be productive in the development of an understanding of the relationship of the control of nucleic acid synthesis to the nutrition characteristics of the organism. Equally valuable will be the extension of such studies to those species of blue–green algae that form specialized cell types and exhibit, certainly in prokaryotic terms, complex life-cycles.
Dark–Light Transitions with a Heterotrophic Culture of a Blue–Green Alga

E. HILARY EVANS and NOEL G. CARR

Department of Biochemistry, University of Liverpool, P.O. Box 147, Liverpool L69 3BX, U.K.

Although a significant number of blue–green algae are capable of heterotrophic growth in the dark (Khoja & Whitton, 1971), our knowledge of such metabolism is limited, and in particular very little is known of the mechanism by which phototrophic growth is re-established. *Chlorogloea fritschii* Mitra, probably incorrectly named (Bourrelly, 1970), can grow heterotropically on sugars, with sucrose as the favoured substrate (Fay, 1965). Dark growth led to substantial loss of the accessory photosynthetic pigment phycocyanin (Fay, 1965; Lex *et al.*, 1974) and the capacity for light-induced O₂ evolution was lost (Lex *et al.*, 1974; Evans & Carr, 1974). On re-introduction into light the ability to evolve O₂ was regained rapidly with a half-recovery time of about 1½ h (Evans & Carr, 1974). There was a 2–3 h lag before any increase was seen in the ratio of chlorophyll to protein.

Sucrose was assimilated by both dark- and light-grown *Chlorogloea fritschii* but at a much greater rate in the light (Fay, 1965). Since sugar uptake has been suggested to be a measure of photophosphorylation in algal cells (see Simonis & Urbach, 1973), this result may reflect the increased capacity for ATP production by *Chlorogloea fritschii* on transference into light. There was a 2 h lag before the sucrose uptake, after transfer from dark to light, began to recover. The present paper investigates the effect of the protein-synthesis inhibitor chloramphenicol on the re-acquisition of light-induced O₂ evolution and the rate of sucrose uptake.

**Methods**

*Chlorogloea fritschii* was grown at 32–34°C in medium C described by Kratz & Myers (1955), supplemented with 10 mm-sucrose, in total darkness, for 5–6 weeks. The culture employed had been maintained heterotrophically in the dark for over 3 years. Dark-to-light transitions involved transfer of dark-grown cultures to an illuminated shaker with fluorescent lights (Grolux type) and maintained at 34°C. [U-¹⁴C]Sucrose uptake was measured as trichloroacetic acid-precipitable material. O₂ evolution was measured with a Rank electrode, and chlorophyll was estimated at $E_{680}$ after extraction of a ultrasonically broken suspension.