Maturation of Ribosomal RNA in Blue-Green Algae in Light and Darkness

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Abstract. The blue-green alga Plectonema boryanum was chosen for a study of the properties of rRNA of prokaryotic organisms. The process of rRNA species maturation was studied by labelling RNA with the isotope $^{32}$P. 23 S and 16 S rRNA molecules each have their own precursors. Molecules with molecular weights of $1.65 \times 10^6$ and $1.24 \times 10^6$ were identified as precursors of 23 S rRNA, while the molecules $0.87 \times 10^6$, $0.78 \times 10^6$ and $0.68 \times 10^6$ were found to be precursors of the 16 S rRNA. No common polycistronic precursor could be found.

Experiments done with algae cultivated in light and in the dark confirmed the fact, that the rate of rRNA synthesis is reduced in the dark, without however stopping completely.

Mitochondria and chloroplasts are unique in their significance for energy production in cells. These cellular particles are very similar to prokaryotic cells -- bacteria and blue-green algae (Carr and Craig 1970).

Differing from the multitude of publications dealing with RNA of bacteria, mitochondria and chloroplasts, studies of rRNA maturation in blue-green algae began to appear in the literature only in recent years (Doolittle 1972, Szalay et al. 1972, Gierson and Smith 1973). Data on the algae Anacystis nidulans and Tolypothrix distorta fully correspond to the diagram of rRNA maturation in bacteria, i.e. the functional molecules 23 S (m. w. $1.07 \times 10^6$) and 16 S (m. w. $0.56 \times 10^6$) are derived from their own precursors p23 S and p16 S (Szalay et al. 1972). Similarly, in algae stability of the 23 S molecule strongly depends on the Mg$^{2+}$ ions concentration, while 16 S is very stable irrespective of the Mg$^{2+}$ concentration.

Studies published up to now indicate that the 23 S molecules undergo specific endonucleolytic cleavage in vivo and produce fragments of m. w. roughly $0.9 \times 10^6$ and $0.2 \times 10^6$. Howland and Ramus (1971) found the molecular weight of the heavier fragment in the alga Phormidium persiciinum to be $0.80 \times 10^6$, Szalay et al. (1972) in Anacystis nidulans 0.90 $\times 10^6$ and $0.2 \times 10^6$, Doolittle (1973) in Anacystis nidulans $0.88 \times 10^6$ and $0.17 \times 10^6$, Gierson and Smith (1973) in Anacystis cylindrica $0.9 \times 10^6$ and in Tolypothrix distorta 0.93 $\times 10^6$.

Doolittle (1973) furthermore investigated the influence of the culture being grown in the dark or light, on the rate of cleavage of 23 S rRNA. While

Received April 8, 1977, accepted May 10, 1977
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the cleavage half-time was 5 h for the illuminated culture, cleavage of 23 S rRNA is slowed down in the dark and proceeds at roughly one-half this rate.

The aim of the present paper was to study the properties, particularly stability, biosynthesis and maturation of rRNA from the blue-green alga _Plectonema boryanum._

**Material and Methods**

The blue-green alga _Plectonema boryanum_ (GomOT) strain 594 from the University of Indiana Culture Collection was grown under conditions described in the authors' previous paper (SOFROVÁ et al. 1974).

**Extraction of RNA from Algae**

A suspension of blue-green algae, thickened by centrifuging, was frozen with solid CO₂ and extracted by a procedure described by LöNING (1965). To increase the yield, blue-green alga protoplasts were frequently used for extraction (SOFROVÁ et al. 1974). Purity of the RNA preparation was measured by spectrophotometric means in the UV region; furthermore, proteins were determined by Lowry's method (LOWRY et al. 1951) and neutral sugars were determined by the method reported by Dubois et al. (1956), with negative results.

Electrophoresis of rRNA on 2.4% polyacrylamide gel was performed in tubes of plexiglass, with a gel column 80 mm long and 7 mm in diameter, in 30 mM Tris-HCl buffer solution, pH 8.1, using Loening’s method (LOENING 1969). After 20 min pre-electrophoresis, a sample of 25 to 50 μg RNA was applied in electrophoretic buffer solution with 10% sucrose. Electrophoresis was done at room temperature for 1.5 to 2 h at a potential gradient of 8 V. cm⁻¹, 6 to 8 mA per tube. RNA on the gel was dyed by the Peacock and Dingman method (PEACOCK and DINGMAN 1967).

The relative molecular weight was determined by comparison with standard rRNA from rat liver (LOENING 1969).

**Incorporation of ³²PΟ₄⁻ into RNA of Algae**

Nucleic acids of algae were labelled after 24 h of cultivation in a phosphate-free medium (phosphate starvation) in a culture thickened by centrifuging 10 times compared with the logarithmic phase of growth, labelling being done by addition of KH₂³²PO₄ (activity 50 μCi ml⁻¹). Incorporation was performed with algae grown continuously in light as well as with a culture which had been incubated in the dark for 60 min before the experiment and was likewise not illuminated during incorporation.

In one part of the samples, incorporation of ³²PΟ₄⁻ was stopped after 5, 10, 20, 30 and 60 min; a second part was used for a so-called “pulse-chase” experiment: incorporation was followed by 40 min incubation in a cultivation medium with non-radioactive KH₂PO₄, after washing KH₂³²PO₄ out of the solution by several centrifugations.

After electrophoresis of nucleic acids on 2.4% polyacrylamide gel each gel column was cut into 50 slices 1.6 mm thick, and each slice was dissolved in 6 ml TSC scintillation cocktail with xylene (2 : 1). Radioactivity measurements were made with an ISOCAPÉ 300 instrument by Nuclear Chicago, USA (channel B; measured values were corrected according to the extinction curve).
Results

Biosynthesis of the rRNA of algae in light and darkness: The distribution of radiolabelled rRNA species on the gel varies in dependence on the duration of incubation with $^{32}$PO$_4^-$ and the "pulse-chase" experiment. When the relative amounts of individual rRNA species of algae grown in light are plotted in a graph (Fig. 1), the dynamics of biosynthesis of the two types functional rRNA (23 S and 16 S) can be determined.

![Graph showing kinetics of rRNA biosynthesis in light and dark.](image)

**Fig. 1.** Kinetics of rRNA biosynthesis in light. (a) synthesis of the 23 S rRNA; (b) synthesis of the 16 S rRNA; (c) synthesis of low-molecular RNA. The percentage of total radioactivity for individual fragments was calculated by adding the percentages for individual gel slices and corrected by subtracting the heterogeneous background.

**Fig. 3.** Kinetics of rRNA biosynthesis in the dark. (a) synthesis of the 23 S rRNA; (b) synthesis of the 16 S rRNA; (c) synthesis of low-molecular RNA. For other details see Fig. 1.

The decrease of the relative amounts of rRNA molecules with a relative molecular weight of $1.65 \times 10^6$ (denoted 1.65 rRNA) coincides with the rising amount of $1.24 \times 10^6$ rRNA molecules. At first, the relative amount of 1.1 rRNA (23 S) is very small, and does not increase. The amount of these molecules begins to rise only after 20 to 30 min incubation, while the relative amount of 1.24 rRNA decreases at the same time. The amount of 23 S molecules exhibits an increasing trend in the time interval of 5 to 60 min incubation only. Therefore, this molecule is stable in the time interval mentioned and is not subjected to any further marked cleavage.

The monotonic sinking trend of the 0.87 rRNA and 0.78 rRNA curves indicates that these rRNAs continue to decompose, the rate of cleavage of 0.78 rRNA molecules being roughly double that of 0.87 rRNA molecules (Fig. 1b). Cleavage of these two molecules coincides in the first 20 min of incubation with the rising relative amount of 0.68 rRNA. When this curve achieves a maximum (after 30 min of incubation), the relative amount of
0.68 rRNA begins to decrease while the number of 0.56 rRNA molecules (16 S) rises at the same time. The course of the curve of this rRNA species indicates that the 16 S molecule is likewise stable and does not undergo any further conversion. The molar ratio 16 S : 23 S achieves a value of 0.76 after 60 min incubation. It is clear from the course of this curve that this ratio will continue to rise and that it can achieve the value of 1.0, which was obtained for completely maturated 23 S and 16 S molecules (Grierson and Smith 1973).

Fig. 2. Electrophoretic distribution of RNA in algae cultivated in light. a — after 10 min of incubation with $^{32}$PO$_4$ (50 µCi ml$^{-1}$, 25°C); b — after “pulse-chase” experiment (followed by 40 min incubation in non-radioactive medium). The molecular weight of rRNA species is expressed in $10^8$.

Fig. 4. Electrophoretic distribution of RNA in algae cultivated in the dark. a — after 10 min of incubation with $^{32}$PO$_4$ (50 µCi ml$^{-1}$, 25°C); b — after “pulse-chase” experiment (followed by 40 min incubation in non-radioactive medium). The molecular weight of rRNA species is expressed in $10^8$.

Fig. 1c shows the variation of the amount of low-molecular rRNA species of molecular weight $0.45 \times 10^8$ and $0.20 \times 10^8$. The relative amounts of these rRNA species stabilise, after an initial decrease or increase resp., at values which approximately correspond to equimolar amounts. The trend of these two curves, however, does not correspond to the course of the curves shown in Figs. 1a and 1b, and thus the curves in Fig. 1c cannot be unequivocally included in the diagram of rRNA biosynthesis.

Our data indicate that the precursors of the 23 S molecule are $1.65 \times 10^6$ and $1.24 \times 10^6$, those of the 16 S molecule having molecular weights of $0.87 \times 10^6$, $0.78 \times 10^6$ and $0.68 \times 10^6$. The accuracy of these results is confirmed by the “pulse-chase” experiment (Fig. 2), which differentiates precursors from maturated functional molecules. The “pulse-chase” experiment, as well as the kinetic curves of rRNA biosynthesis (Fig. 1) indicate that the rate of maturation of 23 S molecules is higher than that of 16 S rRNA molecules.
The maturation of 23 S and 16 S molecules is the same for cultures grown in the dark and in light (Fig. 3). There is, however, a substantial difference in the rate of maturation, which is low in the dark. The rate of conversion of all precursors decreases, particularly that of 1.24 and 0.68 rRNA, which begin to accumulate in the dark. This is again confirmed by the result of the "pulse-chase" experiment (Fig. 4).

Discussion

Differences in the biosynthesis of the nucleic acids of the blue-green alga *Plectonema boryanum* were studied by means of $^{32}$P incorporation, the algae being cultivated under different conditions of light and dark. The following scheme of maturation is suggested on the basis of kinetic measurements of rRNA synthesis. For the 23 S molecule:

\[
1.65 \times 10^6 \rightarrow 1.24 \times 10^6 \rightarrow 1.1 \times 10^6
\]

The rate of cleavage of the two precursors, $1.65 \times 10^6$ and $1.24 \times 10^6$ is comparable in the 30 to 60 min interval. For the 16 S molecule:

\[
0.87 \times 10^6 \rightarrow 0.78 \times 10^6 \rightarrow 0.68 \times 10^6 \rightarrow 0.56 \times 10^6
\]

The rate of cleavage of 0.87 and 0.68 rRNA is comparable, while that of 0.78 rRNA is about twice as fast.

The maturation diagram of 23 S and 16 S molecules is the same in the dark and in light. There is, however, a considerable difference in the rate of maturation, which is low in the dark. The rate of cleavage of all precursors decreases, particularly that of the last precursors in both diagrams, i.e. the molecules $1.24 \times 10^6$ and $0.68 \times 10^6$ rRNA. Moreover, the divergent course of the curves in Fig. 3b indicates that in the dark, cleavage $0.68 \times 10^6 \rightarrow 0.56 \times 10^6$ is more strongly inhibited, i.e. is slower than the conversion $1.24 \times 10^6 \rightarrow 1.1 \times 10^6$ (see the convergent course of the curves in Fig. 3a). This probably means that blue-green algae have a very small pool of enzymes catalysing the maturation of the last precursor, and that synthesis of this enzyme is possibly dependent on light.

The scheme of rRNA synthesis suggested by the authors can be compared with that proposed by Grierson and Smith (1973) for the alga *Tolyphothrix distorta*, who observed precursors of the molecule 23 S rRNA with a molecular weight of $1.22 \times 10^6$ and precursors of 16 S rRNA with molecular weight of $0.76 \times 10^6$ and $0.68 \times 10^6$. They observed an rRNA molecule of molecular weight $1.66 \times 10^6$ at high RNA concentrations only and considered it to be an association product of smaller RNA molecules. We identified this molecule on gels each time, although a very dilute RNA solution (conc. less than 0.25 mg ml$^{-1}$) was used. Deviations in the results may perhaps be due to different arrangements of the experiment, e.g. Grierson and Smith (1973) incubated for one hour in the "pulse-chase" experiment.

The species $0.45 \times 10^6$ and $0.20 \times 10^6$ are not included in the maturation diagrams. No explanation can be given for these rRNA species, as these were not studied in the present work. It is interesting, however, that the $0.20 \times 10^6$ fragment was observed by Howland and Ramus (1971), Szalay et
al. (1972) and possibly also by Doolittle (1973), who describe degradation of rRNA in vivo, as well as in our maturation study.

More exact determination of the origin and function of these rRNA species requires further study.

References


