and Garcia[12] in *Rhodnius prolixus* larvae and Baker[13] in *Attagenus megatoma* larvae reported a correlation between the increase in proteolytic activity and total midgut protein. Stimulation of protease and amylase activity in *Drosophila melanogaster* larvae[14] and protease and sucrase activity in *A. megatoma* larvae[15] were found to be substrate specific.

In contrast, there are number of reports which have shown the failure of substrate specific stimulation of digestive enzymes. Day and Powning[2] demonstrated that in *B. germanica,* the digestive enzymes increase irrespective of the diet, and a starchy meal did not result in a relative increase in amylase activity. It has already been reported that *Periplaneta* was unable to change its digestive enzymes from one diet to another[6]. Similarly in *Dytiscus,* fluctuations of amylase and protease occurred on water ingestion. Even in some insects in which a secretagogue mechanism has been shown to be operative, there are some apsects which can not be explained on the basis of substrate induction of enzymes and it has been suggested that protein affects digestive enzymes in general and not only the enzyme specifically acting on the protein substrate.

On the basis of the present results showing that the midgut protease activity changes are not directly related to the dietary and midgut protein (substrate) contents, a secretagogue mode of stimulation of digestive enzymes appears not to be involved, at least with regard to proteolysis. Evidence for the unlikeliness of neural involvement is already available[2,3] and therefore, a hormonal pathway is the only one which seems to be probable in the system. The direct demonstration of this 3rd regulatory mechanism needs further detailed experimentation, which is in progress.

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Simultaneous syntheses of cytoplasmic and chloroplastic ribosomal RNA during the cell cycle of *Dunaliella*

F. Marano and A.C. Dazy

Laboratoire de Biologie cellulaire végétale, Université Paris VII, CNRS, ERA No. 325, 2, Place Jussieu, F-75005 Paris (France), 16 January 1981

Summary. Chloroplastic and cytoplasmic ribosomal RNA syntheses were analyzed in synchronous cultures. All 4 rRNA species are simultaneously accumulated during the light period of the cell cycle. Cytoplasmic rRNA synthesis is repressed only during the later part of the S phase.

Our previous studies on the cell cycle of *Dunaliella,* a naturally wall-less volvocale, synchronized by light-dark periods, have shown that nuclear and chloroplastic DNA syntheses occur almost completely during the light period[1]. The replication of both DNAs is simultaneous, which is not the case in some other green algae, for example *Chlamydomonas*[2] and *Chlorella*[3]. In these algae, chloroplastic DNA is synthesized a long time before nuclear DNA. Our results suggested some relationship between the DNA synthesis in the *Dunaliella* nucleus and that in the plastid. In the present work, our intent was to determine the chronology of the ribosomal RNA transcription during the cell cycle in the nucleus and the chloroplast to point out other possible temporal relationships between metabolic processes in the two organelles.

Materials and methods. Dunaliella bioculata (Volvocales), a unicellular green alga, was grown on a mineral growth medium[4] at 24 °C, under a 8/16 light-dark cycle at 10 klx. Under these conditions, synchronous divisions occurred every 24 h during the first part of the dark period[4]. For radioactive labelling, the cells were incubated for 1 h with $Na_2\ ^{32}PO_4$ (0.3 μCi/ml in the culture medium) at different times during the cell cycle. The cells were then harvested by centrifugation at 5000 × g for 10 min at 4 °C. The pellet was washed twice in the culture medium and frozen for later use.

Ribosomal RNA extraction followed Laulhère and Rozier's technique[5]. The rRNA was analyzed by electrophoresis on 2.5% polyacrylamide gel according to Loening[6]. The radioactivity was detected by liquid scintillation counting in Aqualuma or in Lipoluma after eluting the gel slices in Lumasolve.

Results. The ribosomal ribonucleic acid from *Dunaliella bioculata,* separated by electrophoresis, showed 4 species (fig. 1) although Ralmsdorf et al.[7] showed only 3 species in *Dunaliella* sp.: 26 S, 23 S and 17.5 S. As compared with the results obtained from very similar algae such as *Chlamydomonas*[8], we called these 4 species 25 S and 18 S (cytoplasmic rRNA), 23 S and 16 S (chloroplastic rRNA). These designations are intended only for identification of the RNA components and do not imply accurately measured sedimentation coefficients or electrophoretic mobilities. It was difficult to obtain undegraded 23 S RNA (as with numerous green algae) and the absorbancy ratio between the peaks of 23 S and 16 S RNA was not the same for each extraction (1–1.8) even though the ratio between the peaks of 25 S and 18 S RNA was always close to the expected value (about 1.9). Galling[9] reported the same degradation in *Scenedesmus* and *Chlorella,* as did Dazy[10] with *Acetabularia.*

In a previous study[4], we pointed out that the total cellular rRNA is accumulated during the light period of the cell

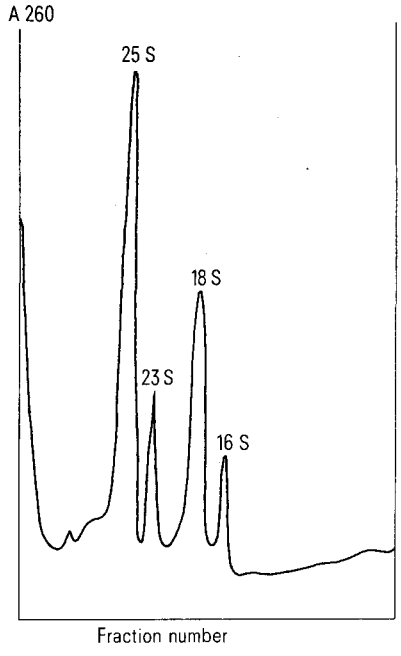

Figure 1. The polyacrylamide gel electrophoretic patterns of rRNA from *Dunaliella bioculata;* cytoplasmic rRNA 25 S and 18 S; chloroplastic rRNA 23 S and 16 S.

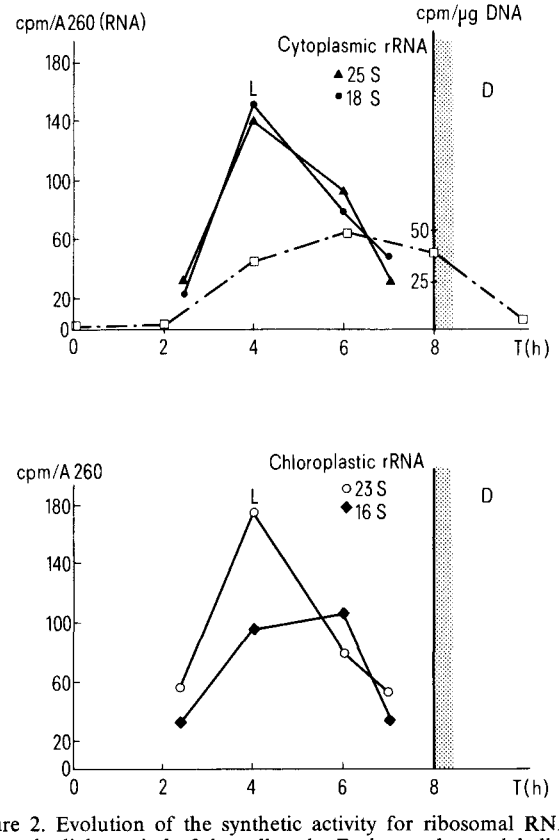

Figure 2. Evolution of the synthetic activity for ribosomal RNA during the light period of the cell cycle. Each sample was labelled for 1 h with $Na_2\ ^{32}PO_4$. Then the rRNA were extracted as described in 'materials and methods'. Specific activities were determined according to the height of the absorbancy peak of each species of rRNA and its labelling. The dotted line shows the S phase of the nuclear DNA[1].

cycle. It begins at the onset of the light and levels off during the latter part of the light period. No accumulation was detected during the dark period. To determine the rate and the sequence of the synthesis of chloroplastic and cytoplasmic rRNA, we performed a pulse-labelling experiment during the period of accumulation (light) as described in the 'materials and methods' section. First, we evaluated the uptake of ^{32}P into the cells after each pulse and we found that it was not a limiting factor for RNA labelling.

We can see in figure 2 that all 4 rRNA species were synthesized during the light period, and simultaneously. The highest specific activity of the chloroplastic and cytoplasmic rRNA was found at about the 4th hour of light which is the beginning of the S phase. Then the specific activity decreased for all the rRNA during the latter part of the S phase. The specific activities of 25 S and 18 S RNA were very close to each other throughout the period of synthesis but, for 23 S and 16 S RNA, a discrepancy was detected. Perhaps it could be the consequence of a lesser degradation, during the extraction, of the newly synthesized 23 S RNA, as has been seen in *Chlorella*[9].

Discussion. From our results, it is clear that the accumulation of both chloroplastic and cytoplasmic rRNA occurs during the light period which is in agreement with the data obtained for green algae. In contrast, the results obtained with protists[11,12] which are not dependent on the light show that the rRNAs are synthesized throughout the entire cell cycle. However, in *Chlamydomonas*[8] a significant turnover of rRNA was shown during the dark period and this point was also demonstrated in *Dunaliella* (unpublished results).

We have also found that the synthesis of the 4 species of rRNA are simultaneous and that their rates approximately coincide with each other during the entire period of accumulation. This temporal relation suggests that the transcription as well as the replication in the plast and the nucleus, may have some common regulation in *Dunaliella.* This hypothesis is supported by the fact that the nuclear and chloroplastic divisions are also synchronous.

In many eucaryotes, the transcription of cytoplasmic rRNA is strongly repressed during the replication of DNA, yet a few species show a continuous synthesis during all the S phase[11, 12]. Our results point out that cytoplasmic rRNA synthesis is high during the 1st part of the S phase, but it is very low during the 2nd part. Previously, we showed that the DNA replication during this 2nd period affected the A-T rich fraction[1]. These results suggest that the transcription of cytoplasmic rRNA is repressed only during the period of replication of the A-T rich fraction of DNA which is perhaps the ribosomal nucleus DNA as in *Chlorella*[13] or *Chlamydomonas*[14].

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