MATURATION OF CHLOROPLAST RRNA IN *EUGLENA GRACILIS*

Philippe HEIZMANN

Section de Biologie générale et appliquée
Laboratoire associé au CNRS
Université de Lyon I
69621 VILLEURBANNE - France

Received November 8, 1973

SUMMARY

The incorporation of radioactive phosphorus into RNA of greening *Euglena* labels 21 S and 25 S cytoplasmic rRNA, 16 S and 23 S chloroplast rRNA (0.55 and 1.1 × 10^6 dalton), and two minor "p 16" and "p 23" chloroplast species, slightly larger than 16 S and 23 S RNA (0.62 and 1.2 × 10^6 dalton). Their role of precursors for 16 S and 23 S RNA has been demonstrated by quantitative conversion of "p 16" to 16 S RNA.

INTRODUCTION

In eucaryotes as in procaryotes, both ribosomal high molecular weight RNAs seem to result from the processing of a single large precursor molecule. This precursor contains, besides the sequences of each of the rRNA, some nonconserved excess RNA.

In mammalian cells the precursor, which appears in the nucleus as a 45 S molecule associated with proteins, is cleaved in the nucleolus and nucleoplasm by well documented steps. Mature rRNAs are then transported to the cytoplasm.

In bacteria, precursors to 16 S and 23 S RNA appear as slightly larger "p 16" and "p 23" species (1). The occurence of a common precursor for both rRNAs was suggested by different authors (2)(3).

In chloroplasts, Chiang and Wilson (4) in *Chlamydomonas*, and Brown and Haselkorn (5) in *Euglena* showed incorporation of radioactive phosphorus in mature rRNA without observation of precursor and maturation steps. Recently, however, Munsche and Wollgiehn (6) in *Nicotiana*, and Hartley and Ellis (7) in spinach, indicated that 1.1 × 10^6 and 0.56 × 10^6 dalton chloroplast rRNA possess larger precursors, with molecular weights of respectively 1.2-1.3 × 10^6 and 0.65-0.70 × 10^6 dalton. In spinach, a species migrating at 2.7 × 10^6 dalton level, could be a possible common precursor.

In this paper we show that chloroplast RNAs in *Euglena* result from the maturation of larger precursors moving as 0.62×10^6 and 1.2×10^6 dalton RNA molecules. These precursors are located in the chloroplasts. A precursor-product relationship between the 0.62×10^6 dalton RNA and the 0.55×10^6 dalton RNA has been demonstrated.

As this work was in preparation for publication, Carritt and Eisenstadt (8) described in *Euglena* chloroplasts incubated *in vitro*, newly synthesized rRNA as molecules slightly larger than mature RNAs.

MATERIALS AND METHODS

Euglena gracilis, strain Z, were grown in the dark, on a phosphate limited medium [PC medium (9)]. At the beginning of the stationary phase, the cultures were centrifuged in sterile conditions under green dim illumination and resuspended in a 3×10^{-6} M KCl solution at a concentration of 8×10^6 cells/ml. After starvation in the dark for 1 to 5 days, they were illuminated under white fluorescent light (3000 lux at the level of the cultures).

$H_3{}^{32}PO_4$ (carrier free, purchased from CEA, France) was added at a concentration of 30-50 mCi/l. Aliquots were rapidly sedimented in a SS-1 Sorvall centrifuge, and the pellets frozen in acetone-dry ice.

RNA was extracted by the phenol-m-cresol method (10), with an aqueous phase containing : Tris-HCl 0.1 M, pH 7.3 ;NaCl 0.1 M ; $MgCl_2$ 5.10^{-3} M ; sodium thioglycolate 0.05 M ; triisopropylnaphtalene sulfonate 1 %. The product was treated with DNase (RNase free, Worthington) at 50 µg/ml for 2 hours at 0°C. The RNA was analyzed by polyacrylamide gel electrophoresis on 2.4-2.8 % gels (11).

The gels were scanned in UV with a Joyce Loebl Chromoscan, and dried on glass paper sheets. Autoradiographs were obtained by contact of Kodak Regulix HS films with these dry gels, and scanned with the Chromoscan.

Quantitative estimations of radioactivity of the different RNA species were obtained with sliced gels counted by Cerenkov effect in a Packard 3375 Scintillation Spectrometer ; the counts were normalized with respect to the 16 S absorbance peaks on UV scans expressed in cm^2 (1 $cm^2 \sim$ 1.5 OD units). Surface estimations were obtained with a D-MAC pencil follower and a CII 500 computer.

RESULTS

Fig. 1 describes the results of an experiment in which *Euglena* were illuminated after two days of starvation in the dark. Radioactive phosphate was added during the phase of rapid synthesis of chloroplast rRNA (3-20 hours after the beginning of illumination) (12). Chloroplast rRNA is then rapidly and preferentially labeled.

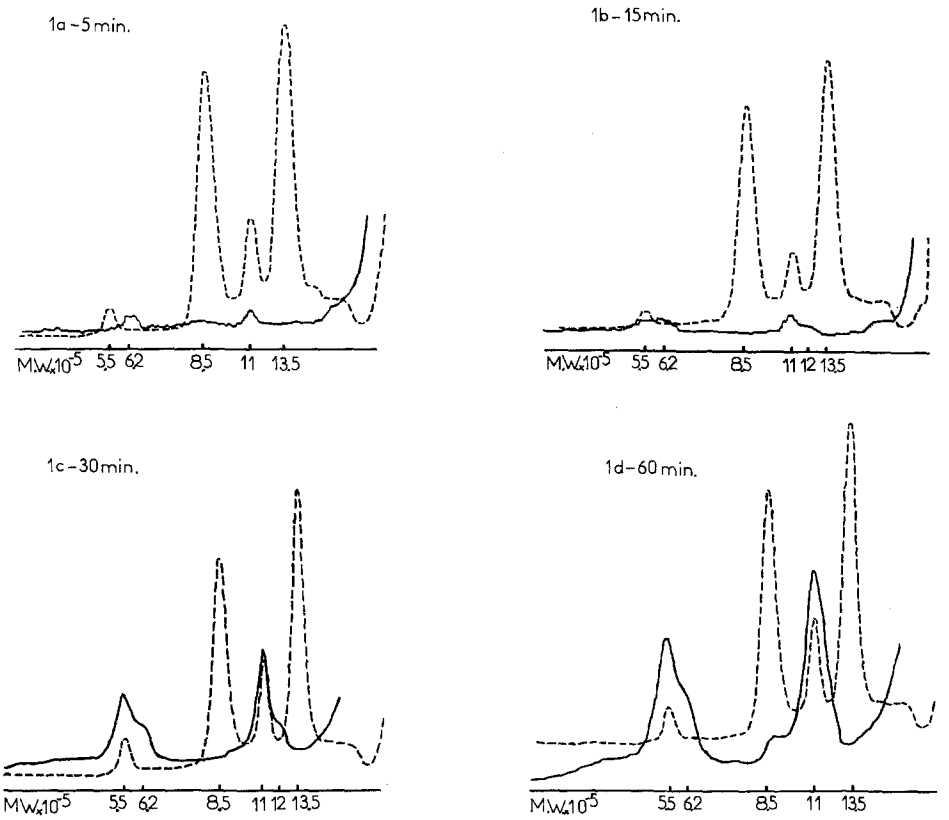

Fig. 1 - 2,8 % polyacrylamide gel electrophoresis of total RNA from *Euglena*. Etiolated cells were starved in the dark for 48 hours after harvesting, then illuminated for 5 hours ; they received ^{32}P (50 µCi/ml) for 5, 15, 30 or 60 minutes. ▬▬ UV scan of the gel ; ——— densitometric scan of the autoradiograph obtained with the same dry gel.

In the 16 S region of the gel, the radioactivity appears first, without lag (fig. 1 a), in a species similar to the "p 16" of bacteria (1) and slightly larger (0.62×10^6 dalton) than the

mature 16 S (0.55×10^6 dalton). This species rapidly reaches a constant level of labeling. The mature 16 S species is labeled after some lag, longer than 5 minutes in the conditions of this experiment (fig. 1 b). This stable product accumulates radioactivity.

In the 23 S region of the gel, the radioactivity enters the mature 23 S RNA (1.1×10^6 dalton) with a lag shorter than 5 minutes ; a small shoulder appears on the heavy side of this peak, similar to the "p 23" of bacteria. It is present in small amounts, and moves with an apparent molecular weight of 1.2×10^6 dalton.

The localization of 0.62×10^6 and 1.2×10^6 dalton RNAs in the chloroplast was showed with green cells furnished with $^{32}PO_4^{---}$. Their chloroplasts were isolated and purified by flottation on 50 % sucrose (13). This methods provides greatly altered chloroplasts, but essentially free of cytoplasmic ribosomal material (fig. 2) : only chloroplast RNA remains attached to plastidial membranes, and the satellite species are part of these chloroplast RNAs. No very large RNA, possible common precursor to the two rRNAs, can be detected on the gel between DNA and the 1.2×10^6 dalton peak.

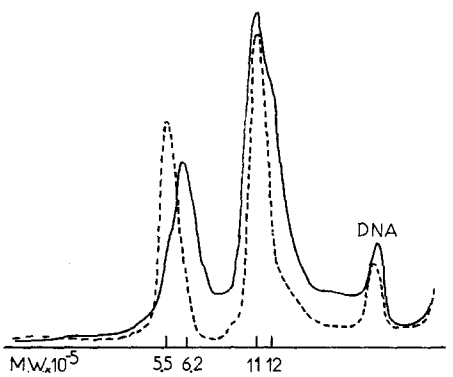

Fig. 2 - 2,4 % polyacrylamide gel electrophoresis of RNA from purified chloroplasts. Green cells (48 hours starvation in the dark plus 48 hours illumination) received ^{32}P (50 µCi/ml) for 30 minutes. The chloroplasts were purified by the flottation method of Brawerman (13), and their RNA extracted as described in *Methods* ; the label in the DNA peak disappeared after DNase treatment. — — — UV scan of the gel ; ——— densitometric scan of the correspondant autoradiograph.

The stoichiometric transformation of 0.62×10^6 dalton RNA into 0.55×10^6 dalton RNA has been demonstrated by blocking the incorporation of radioactive phosphate in the precursor species after a pulse labeling, and by following the transformation of precursor to product.

Brown and al. (14) showed that transcription of chloroplast RNA could be inhibited by rifampicin in *Euglena*, provided the drug was added in the presence of dimethylsulfoxide, to allow its penetration. We tried to use this inhibitor to stop the labeling of chloroplast RNA in *Euglena* cultures starved for 4 days on KCl medium. With such prolonged starvation, the maturation rate was considerably slower than with conditions used in the first experiment (fig. 3 a). Despite these precautions, complete inhibition of transcription during the course of the experiment could not be obtained (fig. 3 b).

Chase with cold phosphate, however, caused extensive dilution of the radioactive tracer after some lag and disappearance of the radioactivity in the 0.62×10^6 dalton RNA with concomitant appearance of radioactivity in 0.55×10^6 dalton RNA could be measured. This clearly demonstrated stoichiometric conversion of the larger species into the smaller one, characteristic of a precursor-product relationship (fig. 3 c).

DISCUSSION

Our results show a perfect analogy between maturation steps of bacterial and *Euglena* chloroplast rRNA. In both cases the precursor for 16 S RNA is easily detected, while the precursor for 23 S RNA seems more transient because of its rapid maturation rate. No evidence of a possible common precursor for both heavy rRNAs has been found under our conditions of labeling *in vivo*.

The maturation rate of 16 S chloroplast RNA is extremely dependent on the physiological state of the cultures. It is rapid after a short starvation or if the cells are illuminated during exponential growth. It becomes slower as starvation increases, without strongly affecting either chlorophyll synthesis or chloroplast rRNA accumulation rates.

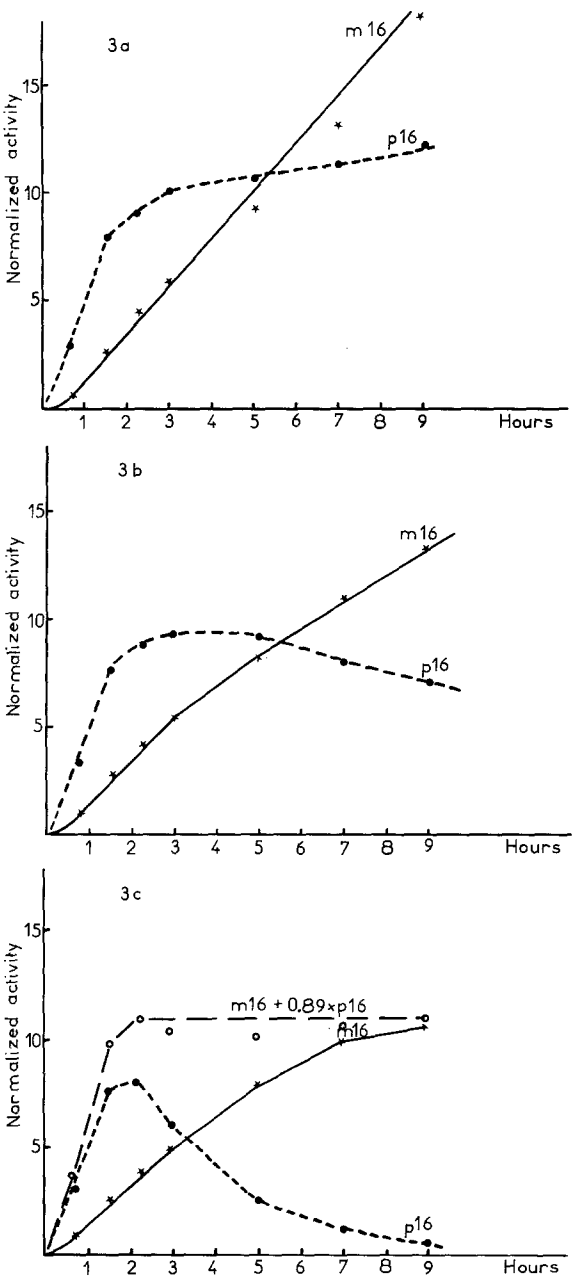

Fig. 3 - Kinetics of labeling "p 16" and "m 16" RNA. Etiolated cells starved for 96 hours in the dark after harvesting and illuminated for 5 hours, received 50 µCi/ml $^{32}PO_4$. The amounts of radioactivity were normalized with respect to the 16 S absorbance peak (see *Methods*).
—— "m 16" ; - - - "p 16" ; —— —— "m 16" + 0,89 "p 16" (89 % of "p 16" is conserved). a. Control ; b. Cultures added with rifampicin (10 µg/ml) and dimethylsulfoxide (1 %) 45 minutes after the addition of ^{32}P ; c. Cultures added with cold phosphate (0,3 mM) 45 minutes after the addition of ^{32}P.

Preliminary results with protein synthesis inhibitors indicate that chloroplast rRNA synthesis is strictly dependent on protein synthesis.

ACKNOWLEDGMENTS

I am grateful to Drs H. Duranton, F. Gros, R. Monier, V. Nigon and J.P. Weil for their kind criticism. This work was partly supported by a grant from the Commissariat à l'Energie Atomique (CEA).

BIBLIOGRAPHY

1. Adesnik, M. and Levinthal, C. *J. Mol. Biol.*, **46**, 281-303 (1969).
2. Miller Jr, O.L. and Hamkalo, B.A. *Intern. Rev. Cytol.*, **33**, 1-25 (1972).
3. Kossman, C.R., Stamato, T.D. and Pettijohn, D.E. *Nature* (London) *New Biol.* **234**, 102-104 (1971).
4. Wilson, R. and Chiang, K.S. *J. Cell. Biol.*, **55**, 285 a (1972).
5. Brown, R.D. and Haselkorn, R. *J. Mol. Biol.*, **59**, 491-503 (1971).
6. Munsche, D. and Wollgiehn, R. *Biochim. Biophys. Acta*, **294**, 106-117 (1973).
7. Hartley, M.R. and Ellis, R.J. *Biochem. J.*, **134**, 249-262 (1973).
8. Carritt, B. and Eisenstadt, J.M. *FEBS Letters*, **36**, 116-120 (1973).
9. Freyssinet, G., Heizmann, P., Verdier, G., Trabuchet, G. and Nigon, V. *Physiol. Végét.*, **10**, 421-442 (1972).
10. Parish, J.H. and Kirby, K.S. *Biochim. Biophys. Acta*, **129**, 554-562 (1966).
11. Loening, U.E.L. *Biochem J.*, **102**, 251-257 (1967).
12. Heizmann, P. *Biochim. Biophys. Acta*, **224**, 144-154 (1970).
13. Brawerman, G. *Biochim. Biophys. Acta*, **72**, 317-331 (1963).
14. Brown, R.D., Bastia, D. and Haselkorn, R. (1970). *In* RNA Polymerase and Transcription (Silvestre, L. ed), pp. 309-328, North-Holland, Amsterdam.