CHLOROPLAST AND CYTOPLASMIC RIBOSOMES IN *EUGLENA GRACILIS*

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1. Introduction

Ribosomes from chloroplasts resemble in many respects ribosomes from procaryotic cells [1] while those from the cytoplasm of photosynthetic cells are similar to those in the cytoplasm of other eucaryotic cells. In general, chloroplast and cytoplasmic ribosomes have sedimentation coefficients of about 70 S and 80 S respectively, although exceptions to such a classification based on size are to be expected [1,2]. The ribosomes of *Euglena gracilis* constitute one apparent exception and there are several conflicting reports of the size and composition of both chloroplast and cytoplasmic ribosomes in this organism [3–12]. The earlier reports in particular have led to the conclusion that either *Euglena* ribosomes and their components are smaller than average, or that they are easily broken down during isolation.

Recently we showed that chloroplast ribosomes in *Euglena* contain 23.5 S and 16.5 S RNA in the molar ratio of 1:1 [10] suggesting that these ribosomes would be of the 70 S type. In this report we show that *Euglena* chloroplast ribosomes are 70 S with subunits of 50 S and 30 S containing the 23.5 S and 16.5 S RNA, respectively. The cytoplasmic ribosomes are 88 S with subunits of 67 S and 46 S. We have isolated 26 S, 24 S and 22 S RNA from the large cytoplasmic subunit and 22 S RNA from the small cytoplasmic subunit.

2. Materials and methods

Euglena gracilis strain Z was grown in the dark in the basal medium of Hutner et al. [13] supplemented with vitamin B_{12} (0.1 μg/l), or autotrophically in an atmosphere of 5% CO_2 in air in the light (600–800 foot candles) in the same medium from which all compounds containing carbon, except EDTA and vitamins, had been omitted.

Chloroplasts were isolated at 0–2° as previously described [14] using fresh material and ensuring that in all procedures the Mg^{2+} and K^+ concentrations, nominated in the results section, were maintained. The extent of breakage of the cells in the French Pressure Cell was monitored by light microscopy and the pressure (about 11,000 p.s.i.) was adjusted so that all the cells were broken, at the expense of some of the chloroplasts. This minimized contamination of the chloroplasts by the cytoplasm and nucleus. The overall yield of chlorophyll in the purified chloroplast fraction was 30 to 50%. To obtain chloroplast ribosomes the chloroplasts were lysed in 2 vol of the relevant buffer containing 0.2% deoxycholate, the lysate was centrifuged at 20,000 *g* for 10 min and the supernatant loaded on a sucrose gradient.

To obtain cytoplasmic ribosomes, dark-adapted heterotrophic cells were washed in buffer, resuspended in 3–4 vol of buffer, broken in a French Pressure Cell at 15,000 p.s.i., centrifuged at 20,000 *g* for 10 min and the supernatant loaded on a sucrose gradient.

The sedimentation values of *Euglena* cytoplasmic ribosomes were measured by analytical centrifugation in a Spinco model E ultracentrifuge [15]. *Euglena* cytoplasmic ribosomes and *Escherichia coli* ribosomes (100 S, 70 S, 50 S and 30 S [16]) were used to calibrate 12 to 24% [17] sucrose gradients. Samples of ribosomes (0.1 ml) were centrifuged

t through 5 ml gradients in the SW39 rotor of a Spinco $L_2$65B for 150 min at 35,000 rpm or 1 ml samples were centrifuged on 25 ml gradients in the SW25 rotor for 17 hr at 17,000 rpm. The gradients were analysed in a flow cell positioned in a Shimadzu MPS–50L recording spectrophotometer.

To extract RNA, ribosomal fractions were collected and dialysed for 2 hr against 0.1% sodium dodecyl sulphate, 10 mM tris pH 7.5 and 10 mM $MgCl_2$, and the RNA extracted from the ribosomes as previously described [10] except that bentonite was omitted. The S values of the RNA were determined by coelectrophoresis with *E. coli* ribosomal RNA in polyacrylamide gels as previously described [10] using the Mg^{2+} free buffer of Loening [18].

3. Results

3.1. *Chloroplast ribosomes*

Fig. 1a shows the sucrose gradient centrifugation of chloroplast ribosomes prepared in 10 mM tris-HCl pH 7.5, 10 mM $MgCl_2$ and 40 mM KCl. Most of the UV-absorbing material was in the 70 S ribosomes or in the 50 S and 30 S subunits. Examination of the RNA in the double peak to the left of the 70 S peak revealed mainly chloroplast RNA with a small amount of contamination from 88 S cytoplasmic ribosomes. Very few 70 S ribosomes were obtained if the ribosomes were sedimented prior to centrifuging on the gradient or if lower concentrations of $MgCl_2$ were used. Fig. 1b and fig. 1c show the sucrose gradient centrifugation of the 50 S and 30 S ribosomal subunits after recycling the appropriate peaks shown in fig. 1a through sucrose gradients containing 10 mM $MgCl_2$ and 40 mM KCl.

3.2. *Chloroplast ribosomal RNA*

RNA prepared from the 70 S ribosomes shown in fig. 1a was separated by polyacrylamide gel electrophoresis (fig. 1d) and 23.5 S and 16.5 S chloroplast ribosomal RNA were obtained. There was some evidence of minor breakdown or contamination at the bottom of the peaks. The RNA from the purified 50 S ribosome subunit (fig. 1b) showed a 23.5 S peak with 2 smaller peaks at 18 S and 16.5 S (fig. 1e), while the RNA from the 30 S subunit (fig. 1c) showed a 16.5 S component only (fig. 1f).

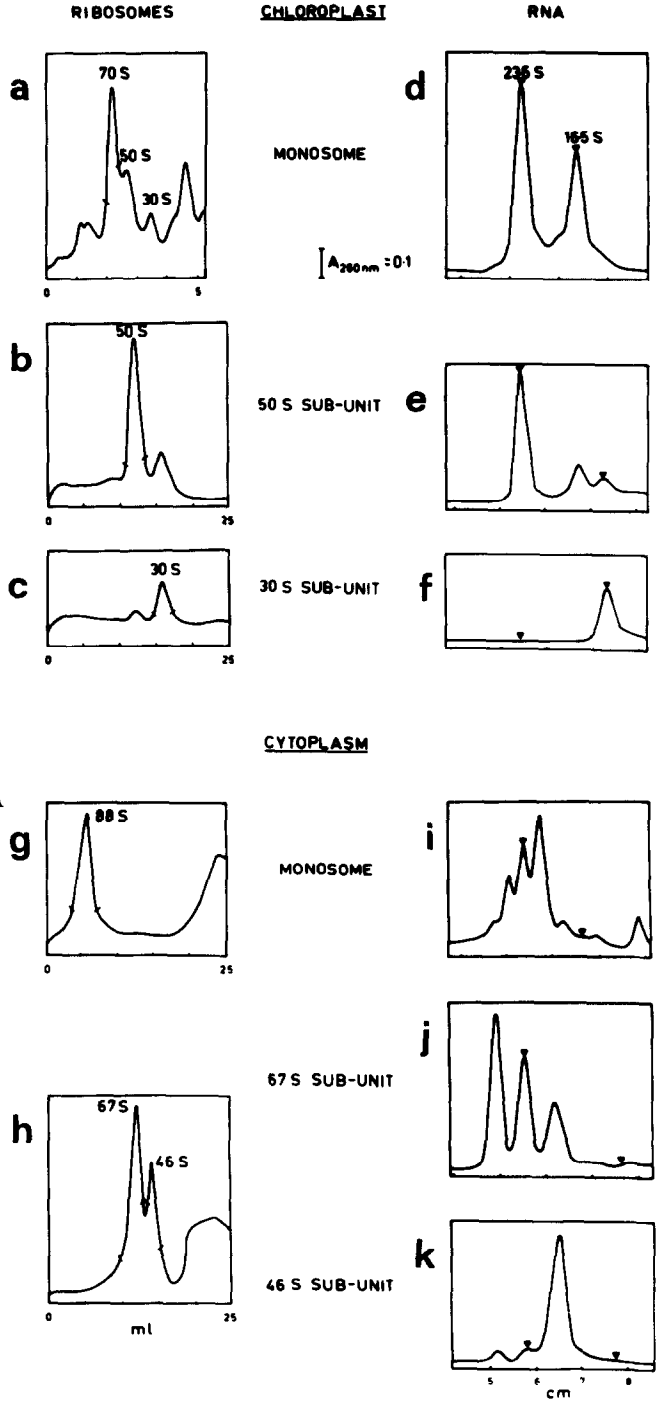

Fig.1. Characterization of *Euglena* ribosomes. The left-hand side of the figure shows the distribution of ribosomes and ribosomal subunits from *E. gracilis*, centrifuged on sucrose gradients as described in the text. The robosomes were collected between the points marked on the graphs and the RNA was extracted from them and characterized by gel electrophoresis as described in the text. The resulting electrophoretograms are shown on the right-hand side of the figure. Arrows indicate the positions for 23.5 S and 16.5 S RNA.

3.3. *Cytoplasmic ribosomes*

Fig. 1g shows the 88 S monomer peak of the cytoplasmic ribosome, isolated in 10 mM tris-HCl, pH 7.5 2 mM $MgCl_2$ and 40 mM KCl. When $MgCl_2$ was omitted from the buffer only the 67 S and 46 S subunits were obtained (fig. 1h). The values of 88 S and 67 S were obtained by analytical centrifugation and were confirmed on sucrose gradients calibrated with *E.coli* ribosomes. Similar S values were obtained for the cytoplasmic ribosomes of two bleached mutants of *Euglena*, ZUV-1 and BEN.

3.4. *Cytoplasmic ribosomal RNA*

The RNA prepared from the 88 S ribosome (fig. 1g) and its subunits (fig. 1h) was characterized on polyacrylamide gels. The 88 S ribosome yielded major peaks at 26 S, 24 S and 22 S with other minor peaks (fig. 1i). A somewhat similar pattern was obtained from the large subunit of the cytoplasmic ribosome (fig. 1j), but the small subunit yielded a single 22 S species of RNA (fig. 1k).

4. Discussion

The methods we have used to prepare chloroplast ribosomes from *Euglena* involve working rapidly with fresh material, careful maintenance of $MgCl_2$ concentrations and omission of steps involving precipitation of the ribosomes. They result in a high yield on sucrose gradients of the 70 S ribosome free of significant cytoplasmic contamination as evidenced by the absence of 22 S RNA in the gel patterns of chloroplast RNA shown in fig. 1d.

Many of the earlier conflicting results referred to in the Introduction can be explained on the basis of two observations. Firstly, after sedimentation of preparations containing 70 S ribosomes (crude or purified) no more than 10 to 20% of the original number of 70 S ribosomes were recovered on sucrose gradients and there were more subunits than ribosomes. Secondly, the ribosomes appeared to break down irreversibly and their S values changed if they were exposed to low Mg^{2+} concentrations. For instance, when chloroplasts were prepared and centrifuged in 4 mM $MgCl_2$, a single 40 S peak was obtained. If chloroplasts prepared in 4 mM $MgCl_2$ were lysed and centrifuged in 10 mM $MgCl_2$ peaks were found at 60 S and 28 S, while in 20 mM $MgCl_2$ peaks were found at 53 S and 35 S. Thus only when the chloroplasts were carefully isolated and lysed in 10 mM $MgCl_2$ was a 70 S, procaryotic type of ribosome possessing 50 S and 30 S subunits obtained. These latter S values agree with those reported by Rawson and Stutz [12].

Our results also show that the 16.5 S chloroplast ribosomal RNA comes from the 30 S subunit while the 23.5 S RNA comes from the 50 S subunit. In this instance the 23.5 S chloroplast ribosomal RNA is notable because of its apparent stability compared with the 23.5 S ribosomal RNA species of chloroplasts from other organisms [19].

In the cytoplasm of *Euglena* we found an 88 S ribosome with 67 S and 46 S subunits and these results confirm those reported recently by other groups [9, 12]. Thus it is clear that *Euglena* contains an unusually large cytoplasmic ribosome, and so one might expect to find an unusually large ribosomal RNA. This is true of the small 46 S ribosomal subunit from which we isolated a 22 S RNA species and Rawson and Stutz [11] a 20 S RNA. However, in the large 67 S subunit we found only 26 S, 24 S and 22 S RNA with the 26 S RNA predominating (fig. 1j) and similar results have been obtained using sucrose gradients. The 26 S and 22 S RNA probably correspond to the 24 S and 20 S RNA obtained from the large subunit by Rawson and Stutz [11].

In either case, the largest RNA species observed, 26 S (fig. 1j) or 24 S [11], seems small when compared with the size of the ribosomal subunit. In gel patterns of RNA isolated from the 88 S ribosomes we found a minor peak at 28 S (fig. 1i) and perhaps this is the native RNA (c.f. [8, 20]). The *Euglena* cytoplasmic ribosomal RNA appears to be easily degraded and extraction of "aged" ribosomes or their subunits yielded only 22 S RNA.

These results, together with those of Rawson and Stutz [12] resolve the confusion that has existed about the size of *Euglena* ribosomes. However, there is no clear indication of size of native undegraded RNA in the large subunit of the cytoplasmic ribosome.

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