

# Distribution of Ribosomes among Chloroplasts of *Euglena gracilis*<sup>1</sup>

By

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Although there is now complete unanimity that the chloroplasts of *Euglena gracilis*, along with chloroplasts generally, contain ribosomes of approximately 70 S and ribosomal subunits of approximately 50 S and 30 S (MENDIOLA et al., 1969, 1970; RAWSON and STUTZ, 1969; SCOTT and SMILLIE, 1970; LYMAN, 1971), the relative proportion of the three kinds of particles reported has appeared to vary from laboratory to laboratory. We succeeded recently in isolating chloroplasts by zonal centrifugation from *Euglena* in a reasonable state of purity and integrity (VASCONCELOS et al., 1971). We have thought therefore to examine the state of ribosomes in the isolated organelles. Since intact chloroplasts are distributed in more than one zone in the density gradient, we thought particularly to look for possible heterogeneity in the content of ribosomal particles among chloroplasts with different sedimentation properties.

The methods and materials were identical with those described by VASCONCELOS et al. (1971). The conditions of zonal centrifugation are repeated here:

**Zonal Centrifugation.** The general techniques of zonal centrifugation were those described by PRICE (1971). Forty milliliters of the clarified brei were loaded into a B-30 A rotor (International Equipment Co.) over 300 ml of gradient that was 0 to 10% (w/w) in Ficoll (Pharmacia), linear with volume, and contained 10% (w/w) sorbitol and 10% (w/w) sucrose and 5 mM HEPES throughout. The gradient was supported by 130 ml of a cushion or underlay of 18% (w/w) Ficoll, 10% (w/w) sorbitol, 10% (w/w) sucrose, and 5 mM HEPES, pH 7.6. The sample was followed by an overlay of 60 ml of the 3 : 5 : 1 solution. The gradient and cushion were delivered from an IEC two-cylinder gradient pump. All solutions contained 2  $\mu$ g of polyvinyl sulfate per ml.

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The sample was centrifuged at a maximal speed of 7000 rpm for  $5 \times 10^8 \text{ rad}^2 \cdot \text{sec}^{-1}$  as indicated on an  $\int \omega^2 dt$  meter; this was equivalent to about 17 min.

The gradient was unloaded from the center with the use of 55% (w/w) sucrose at 30 ml/min, and 30 ml fractions were collected. The absorbance at 260 nm and the refractive index of the gradient were monitored and recorded continuously. The recovered fractions were diluted immediately with equal volumes of 3:5:1 containing 30 mM  $\text{MgCl}_2$ .

### Results and Discussion

When a clarified brei of photoheterotrophic *Euglena* cells is centrifuged into an isosmotic gradient of Ficoll, a sedimentation profile is obtained as shown in Fig. 1. Chloroplasts are recovered mostly in a zone just above the Ficoll cushion (fractions 18–20 in the figure), but smaller amounts of chloroplasts appear higher in the gradient, often in one or more distinct zones.

In this experiment we recovered fractions 12, 15, 18, and 20 (hatched bars) by diluting portions of the gradient immediately into equal volumes of buffered 10% w/w

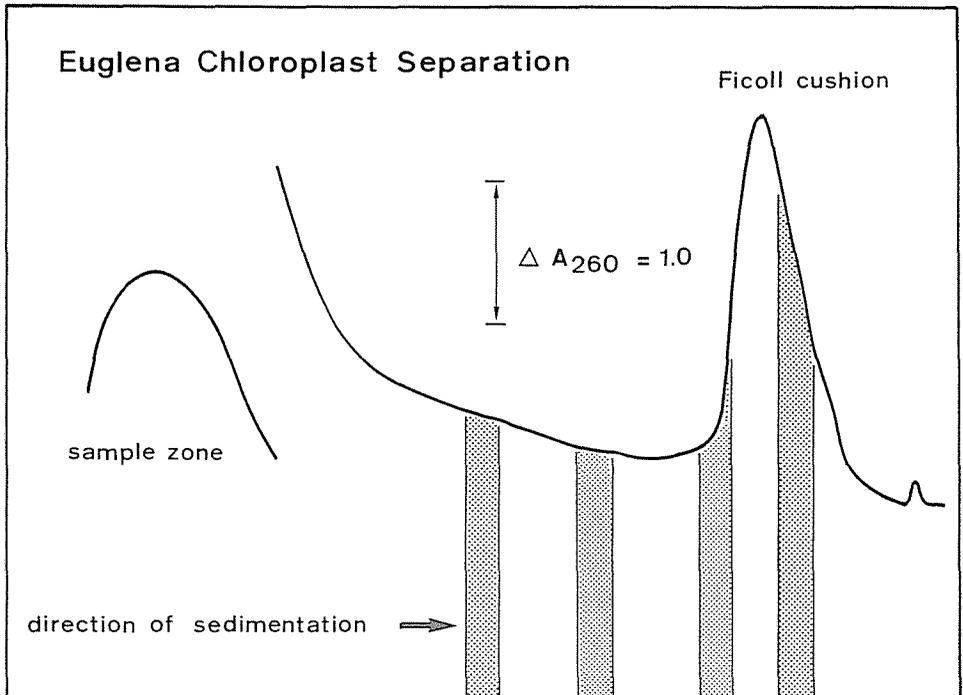


Fig. 1. Separation of chloroplasts by zonal centrifugation of a *Euglena* cell brei. Sedimentation was in a B-30A zonal rotor as described by VASCONCELOS et al. (1971). Solid trace is the optical absorption at 260 nm. Hatched vertical bars show fractions that were selected for further analysis of ribosomes.

sucrose-10% w/w sorbitol containing 30 mM MgCl<sub>2</sub> and centrifuging. The resulting pellets of chloroplasts were then extracted with 0.25% deoxycholate, a solution containing 15 mM MgCl<sub>2</sub>, 100 mM KCl, 1 μg polyvinylsulfate per ml, 10 mM TRIS-HCl pH 7.5, and 3.5 μM β-mercaptoethanol. The mixture was made 0.25% in deoxycholate and clarified by centrifugation. The resulting extracts were layered over 12-cc isokinetic gradients in swinging buckets and spun at 40,000 rpm for 3 × 10<sup>11</sup> radians<sup>2</sup>·seconds<sup>-1</sup>.

The resulting sedimentation profiles are shown in Fig. 2. We see that the principal chloroplast fraction (18 and 20) contains 30 S and 50 S subunits exclusively. The more slowly sedimenting particles (fraction 12) have appreciable amounts of 70 S particles, but they do not migrate in as narrow a band as one would expect of monosomes.

We naturally wondered whether the 70 S zone in fact contained 70 S ribosomes, and turned to the analysis of the constituent RNA's for evidence. Acrylamide gel electrophoresis (PEACOCK and DINGMAN, 1968) was performed on extracts of frac-

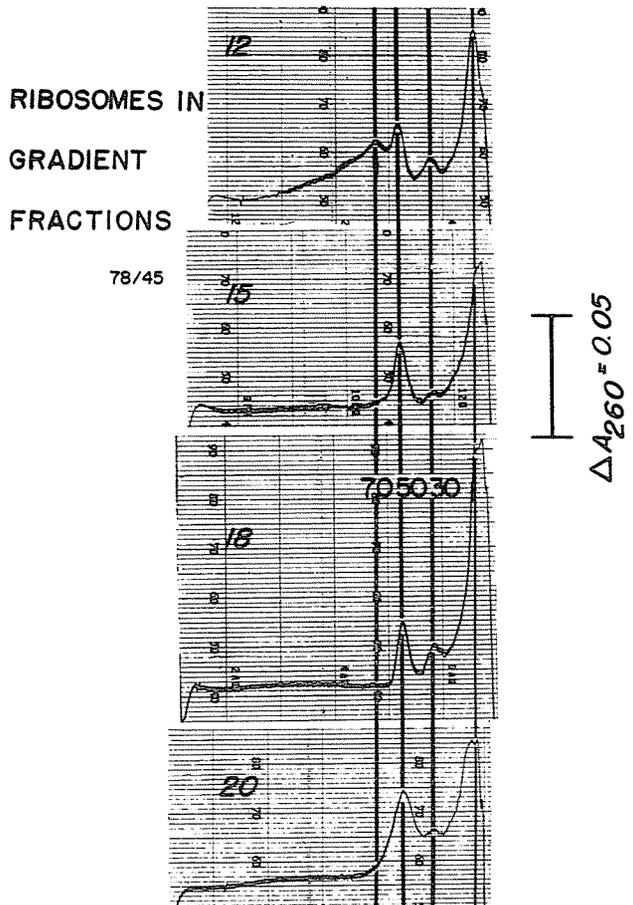


Fig. 2. Sedimentation of ribosomes from chloroplast fractions. Ribosomes were sedimented in isokinetic gradients from right to left. Numbers at left refer to gradient fractions; heavy vertical lines show positions of 30, 50, and 70 S particles. Fractions are same as those shown in Fig. 1.

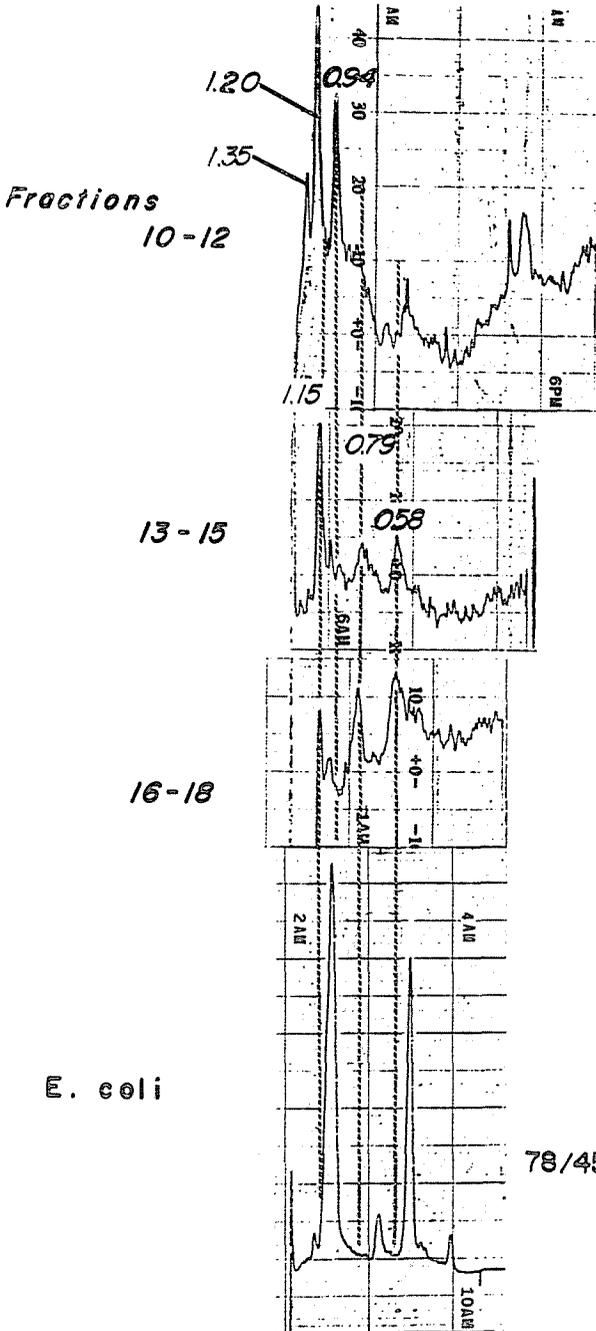


Fig. 3. Electrophoresis of RNA's of chloroplast fractions. Continuous line is densitometer trace of stained RNA species. Vertical dashed lines show positions of RNA species with particle weights indicated by italicized numbers in millions of daltons. Fractions were same as those shown in Fig. 1.

tions from the Ficoll gradient, stained with methylene blue, and the pattern recorded by densitometry. The profiles were compared with *E. coli* ribosomal RNA run simultaneously. The results are shown in Fig. 3. Fractions from 13 on contained RNA species of  $1.15$  and  $0.58 \times 10^6$  daltons and their breakdown products, all of which are characteristic of *Euglena* chloroplasts, but negligible traces of cytoplasmic ribosomal RNA. Fractions 10–12, in addition, contained substantial amounts of the  $1.35$  and  $0.94 \times 10^6$  daltons cytoplasmic RNA.

It is curious that cytoplasmic ribosomal RNA should appear in fractions 10–12, when no trace of cytoplasmic ribosomes was seen in fraction 12.

The more slowly sedimenting chloroplast fractions are clearly heterogeneous; in addition to the above evidence that they contain cytoplasmic RNA, electron photomicrographs show contaminating mitochondria and other particles. Nonetheless, we think that the 70 S zone in the swinging bucket gradients does contain chloroplast ribosomes. Our evidence is 1. that extracts of apochlorotic strains of *Euglena* do not contain such 70 S particles, and 2. direct analysis of the RNA in large amounts of 70 S particles isolated by zonal centrifugation shows the expected mixture of RNA of  $0.6$  and  $1.15 \times 10^6$  million daltons. These latter lines of evidence will be presented in a more detailed report to be published elsewhere (POLLACK, FUNKHOUSER, JO, and PRICE, in preparation).

### References

- MENDIOLA, L. R., A. P. HIRVONEN, A. KOVACS, and C. A. PRICE, 1969. Zonal sedimentation of chloroplast ribosomes from *Euglena gracilis*. Fed. Proc. 28: 879.
- MENDIOLA, L. R., A. KOVACS, and C. A. PRICE, 1970. Separation and partial characterization of chloroplast and cytoplasmic ribosomes from *E. gracilis*. Plant Cell Physiol. 11: 335–344.
- PEACOCK, A. C., and C. W. DINGMAN, 1968. Molecular weight estimation and separation of ribonucleic acid by electrophoresis in agarose-acrylamide composite gels. Biochemistry 7: 668–674.
- PRICE, C. A., 1971. Zonal centrifugation. In: W. W. UMBREIT et al., eds., Manometric Techniques, Ed. 5. Burgess Publishing Co., Minneapolis. In press.
- RAWSON, J. R., and E. STUTZ, 1969. Isolation and characterization of *Euglena gracilis* cytoplasmic and chloroplast ribosomes and their ribosomal RNA components. Biochim. Biophys. Acta 190: 368–380.
- SCOTT, N. S., R. MUNNS, and R. M. SMILLIE, 1970. Chloroplast and cytoplasmic ribosomes in *Euglena gracilis*. FEBS Letters 10: 149–152.
- VASCONCELOS, A., M. POLLACK, L. R. MENDIOLA, H.-P. HOFFMANN, D. H. BROWN, and C. A. PRICE, 1971. Isolation of intact chloroplasts from *Euglena gracilis* by zonal centrifugation. Plant Physiol. 47: 217–221.