Plant Physiol. (1974) 53, 543–554

The Chloroplast and Cytoplasmic Ribosomes of *Euglena*

II. CHARACTERIZATION OF RIBOSOMAL PROTEINS[1]

Received for publication July 27, 1973 and in revised form November 8, 1973

GEORGES FREYSSINET[2] AND JEROME A. SCHIFF
Department of Biology, Brandeis University, Waltham, Massachusetts 02154

ABSTRACT

Cytoplasmic and chloroplast ribosomal proteins were isolated from *Euglena gracilis* and analyzed on polyacrylamide gels. Cytoplasmic ribosomes appear to contain 75 to 100 proteins ranging in molecular weight from 10,200 to 104,000, while chloroplast ribosomes appear to contain 35 to 42 proteins with molecular weights ranging from 9,700 to 57,900. This indicates that the cytoplasmic ribosomes are similar in composition to other eucaryotic ribosomes, while chloroplast ribosomes have a protein composition similar to the 70S procaryotic ribosome. The kinetics of light-induced labeling of cytoplasmic ribosomal proteins during chloroplast development has been determined, and the results are compared with the kinetics of ribosomal RNA synthesis.

Euglena gracilis contains three types of ribosomes which can be attributed to the cytoplasm (89S), plastid (68S), and mitochondrion (71S), respectively. The ribosomal RNAs have also been characterized. The cytoplasmic ribosomes yield 25S and 20S RNA, those of the plastid yield 23S and 16S RNA, and the mitochondrial ribosomes have 21S and 16S RNA (ref. 1, and papers cited therein). The ribosomal proteins, however, have not been as well characterized (2, 6, 19). The present paper reports studies of the ribosomal proteins from cytoplasmic and plastid ribosomes which include a determination of their properties and, in the case of the cytoplasmic ribosomal proteins, a study of their light-induced labeling during chloroplast development.

MATERIALS AND METHODS

Growth Conditions. *Euglena gracilis* Klebs var. *bacillaris* Pringsheim, mutants of this strain, W_3BUL and Sm^r_1BNgL, and *Euglena gracilis* Klebs strain Z Pringsheim were the organisms used in this study. Cultures were grown aseptically in 20-liter carboys containing 14 liters of Hutner's pH 3.5 medium (10) bubbled with air. The medium had been previously autoclaved for 3 hr. The cultures were incubated at 26 C in light or darkness and harvested at the beginning of the stationary phase. Freshly harvested cells were used immediately.

For studies of light-induced chloroplast development, dark-grown cells were grown in 2-liter flasks containing 1 liter of Hutner's 3.5 medium. A culture in the log phase of growth was harvested aseptically by centrifugation at 500*g* for 2 min at room temperature, and the cells were resuspended in pH 4.5 resting medium which contains in g/l: mannitol, 10; $MgCl_2 \cdot 6H_2O$, 2; KH_2PO_4, 0.018; cyclo acid (1,2,3,4-cyclopentanetetracarboxylic acid), 2.5 (4). After shaking for 3 days in the dark at 26 C, development was initiated by exposing the cultures to light as previously described (4).

Buffers. Buffer I contains: tris-HCl, 100 mM, pH 7.6; KCl, 30 mM; magnesium acetate, 2 mM. Buffer II contains: tris-HCl, 10 mM, pH 7.6; KCl, 30 mM; magnesium acetate, 2 mM. Buffer III contains: tris-HCl, 10 mM, pH 7.6; NH_4Cl, 100 mM; magnesium acetate, 2 mM. Buffer IV contains: tris-HCl, 10 mM, pH 7.6; magnesium acetate, 0.1 mM; mercaptoethanol, 20 mM. Buffer V contains: tris-HCl, 10 mM, pH 7.6; magnesium acetate, 0.5 mM. Buffer VI contains: tris-HCl, 10 mM, pH 7.6; magnesium acetate, 200 mM. Buffer VII contains: urea, 6 M; sodium acetate, 5 mM (adjusted to pH 5.6 with acetic acid). Buffer VIII contains: urea, 6 M; sodium acetate, 5 mM; disodium EDTA 5 mM (adjusted to pH 5.6 with acetic acid).

Isolation of Ribosomes and their Subunits. All operations were performed at 0 to 4 C. For cytoplasmic ribosomes, freshly harvested cells were washed with a sucrose solution (27% [w/w] prepared in buffer I) and resuspended in the same solution (1.0 ml/g of cells). The suspension was passed through a French press at 1500 to 2000 psi, and the homogenate was immediately diluted with 1 volume of 27% sucrose in buffer I. The diluted homogenate was centrifuged at 3,000*g* for 5 min, and the supernatant was recentrifuged at 15,000*g* for 10 min. The 15,000*g* ribosomal supernatant was layered over 3 ml of 40% (w/w) sucrose prepared in buffer III and was centrifuged in the Beckmann SW-27 rotor (38-ml tubes) for 5 hr at 25,000 rpm; the 27% sucrose layer was removed with suction and the ribosome pellet was resuspended. The 40% sucrose cushion was mixed with 20 ml of buffer III. The suspension was kept at 0 C for 24 hr and was then centrifuged for 10 min at 15,000*g*. The 15,000*g* supernatant was centrifuged for 2.5 hr at 130,000*g*. This pellet was resuspended in buffer II and centrifuged for 10 min at 15,000*g*. This supernatant was layered onto a 2.0 ml cushion of 40% (w/w) sucrose prepared in buffer II and was centrifuged for 16 hr at 25,000 rpm in the Beckman SW-27 rotor using 17-ml tubes. This last pellet, representing purified cytoplasmic ribosomes, was clear and transparent; it was washed with buffer II and was used for all further analysis.

Cytoplasmic ribosomal subunits are prepared by resuspending the purified ribosome pellet in buffer II and dialyzing this suspension against buffer IV for 5 hr at 2 C. The dialyzed suspension was layered onto a linear 5 to 20% (w/w) sucrose

[1] This work was supported by Grant GM 14595 from the National Institutes of Health.

[2] Postdoctoral research associate, Brandeis University; supported during a portion of this work by a fellowship from North Atlantic Treaty Organization. Present address: Section Biologie Générale et Appliquée, Université Lyon I, 69 Villeurbanne, France.

gradient prepared in buffer V and centrifuged for 5 hr at 20,000 rpm in a Beckman SW-27 rotor using 17-ml tubes. Fractions containing the separated subunits were collected and the ribosomal subunits were recovered by overnight centrifugation at 25,000 rpm.

Crude chloroplast ribosomes were prepared as described previously (23, ribosome pellet). The 68S chloroplast ribosomes were purified by centrifuging the chloroplast ribosome pellet through a 5 to 20% (w/w) sucrose gradient for 5 hr at 25,000 rpm and the 68S fraction was recovered and pelleted at 25,000 rpm for 18 hr. The stabilizing buffer (tris-HCl, 10 mM; KCl, 60 mM; magnesium acetate, 12 mM; spermidine, 0.5 mM; β-mercaptoethanol, 14 mM) previously described (23) was used for this purification.

Where indicated in the text, figure, and table legends, linear sucrose gradients (12.5 ml) were prepared over a 2- or 3-ml cushion of 40 or 50% (w/w) sucrose. The gradients were centrifuged at 2 C in the SW-27.1 rotor of a Spinco Model L2 ultracentrifuge. Gradients were analyzed as described previously (23).

Extraction of Ribosomal Proteins. Ribosomal proteins were extracted with acetic acid or by RNAase treatment (13). Using the acetic acid method, 2 volumes of glacial acetic acid were added to 1 volume of buffer VI containing 300 to 500 A_{260}/ml of ribosomes or ribosomal subunits. The suspension was stirred for 30 min at 2 C and then centrifuged for 10 min at 10,000g in order to remove the precipitated RNA. The supernatant was dialyzed against distilled water for 4 hr at 2 C, and the con-

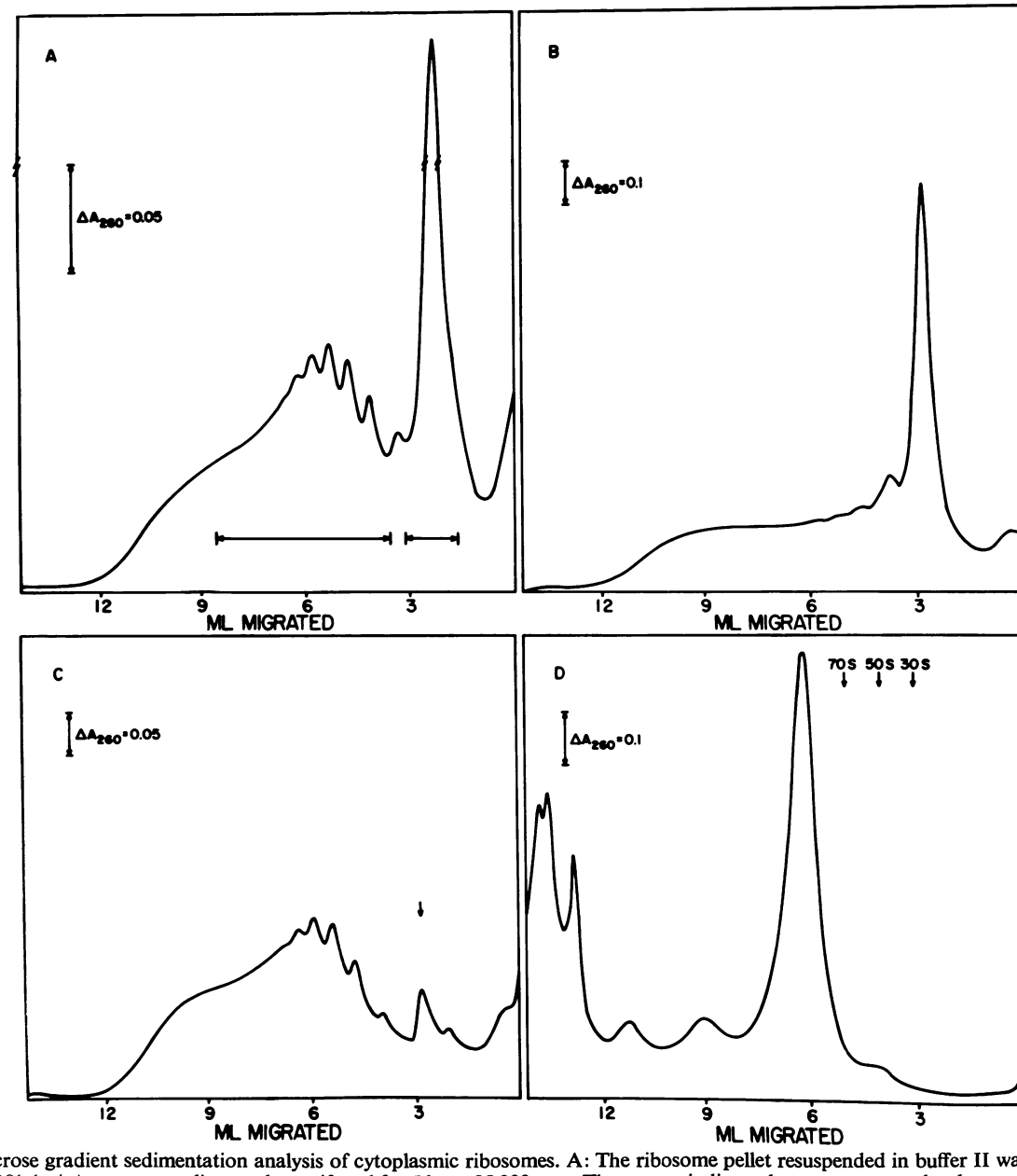

FIG. 1. Sucrose gradient sedimentation analysis of cytoplasmic ribosomes. A: The ribosome pellet resuspended in buffer II was layered over a linear 20 to 50% (w/w) sucrose gradient and centrifuged for 5 hr at 25,000 rpm. The arrows indicate the monosome and polysome fractions which were collected for further purification. B: The monosome fraction (from A) was recovered by overnight centrifugation, resuspended in buffer II and analyzed on a linear 20 to 50% sucrose gradient. C: The polysome fraction (from A) was recovered by overnight centrifugation, resuspended in buffer II, and analyzed on a linear 20 to 50% sucrose gradient. The arrow indicates the position of the monosomes. D: The ribosome pellet resuspended in buffer II was layered over a linear 5 to 20% sucrose gradient and centrifuged for 4 hr at 25,000 rpm. The arrows indicate the positions of the *E. coli* 70S monosome, 50S subunit, and 30S subunit, which were run to calibrate the gradients.

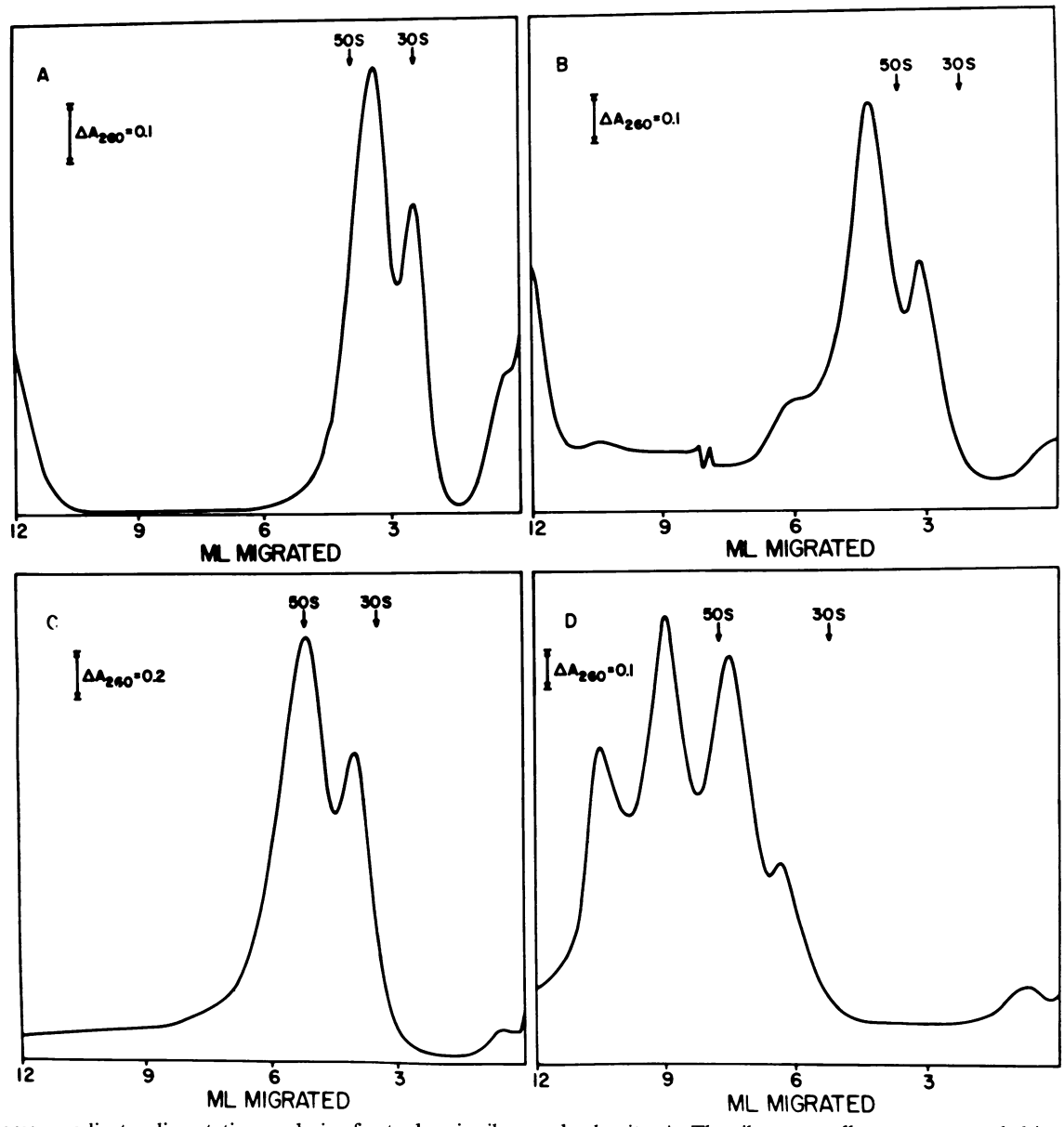

FIG. 2. Sucrose gradient sedimentation analysis of cytoplasmic ribosomal subunits. A: The ribosome pellet was resuspended in tris-HCl, pH 7.6, 10 mM; Na_2EDTA, 1 mM; layered over a linear 5 to 20% sucrose gradient and centrifuged for 4.5 hr at 25,000 rpm. B: The ribosome pellet was resuspended in buffer II (tris-HCl, pH 7.6, 10 mM; KCl, 30 mM; magnesium acetate, 2.0 mM), dialyzed 5 hr against buffer IV (tris-HCl, pH 7.6, 10 mM; magnesium acetate, 0.1 mM; mercaptoethanol 20 mM), layered over a linear 5 to 20% sucrose gradient prepared in buffer V (tris-HCl, pH 7.6, 10 mM; magnesium acetate 0.5 mM) and centrifuged for 4.5 hr at 25,000 rpm. C: The purified ribosome pellet was resuspended in buffer IV, dialyzed for 10 hr against buffer IV, layered over a linear 15 to 35% sucrose gradient prepared in buffer IV, and centrifuged for 14 hr at 20,000 rpm. D: The purified ribosome pellet was resuspended in tris-HCl, pH 7.6, 10 mM; KCl, 30 mM; magnesium acetate, 0.1 mM; dialyzed 8 hr against this buffer, layered over a 10 to 35% linear sucrose gradient prepared in this buffer, and centrifuged 15 hr at 20,000 rpm. Arrows indicate the position of *E. coli* ribosomal subunits (50S and 30S) which were used to calibrate the gradient.

tents of the bag were concentrated by placing the dialysis bag in dry aquacide II at 2 C. The concentrated protein solution was dialyzed overnight against buffer VII at 2 C.

Using the RNAase method, ribosomes were resuspended in buffer VIII or were dialyzed overnight against this buffer at 2 C. Pancreatic ribonuclease (5 μg/ml) and ribonuclease T_1 (500 units/ml) were added, and the mixture was incubated for 1 hr at 37 C. This solution was used directly for electrophoresis or was dialyzed overnight at 2 C against buffer VII.

ELECTROPHORESIS OF RIBOSOMAL PROTEINS

One-Dimensional Urea-Polyacrylamide Gels. The technique is a modification of the method of Fogel and Sypherd (8). Acrylamide, 7.5 g; bis-acrylamide, 0.2 g; TMED,[3] 0.5 ml; ammonium persulfate, 0.160 g; KOH (1.0 N), 6 ml; and glacial acetic acid, 2.16 ml were added to enough 8 M urea solution to make 100 ml. The gels were polymerized in tubes (180 mm × 6 mm) containing 4.7 ml of acrylamide solution pH 4.5. A solution containing 100 to 150 μg of ribosomal proteins and pyronin y was layered on top of the gel. The upper buffer compartment contained β-alanine, 3.12 g and glacial acetic acid, 0.8 ml in 1 liter of water; the lower compartment contained a solution composed of 120 ml of 1.0 N KOH, 43 ml of glacial

[3] Abbreviations: TMED: N,N,N,N'-tetramethylethylenediamine; SDS: sodium dodecyl sulfate.

acetic acid, and 837 ml of water. The 4% acrylamide, 6 M urea, pH 8.6 gel described by Kaltschmidt and Wittmann (12) was used for the analysis of acidic ribosomal proteins with bromphenol blue as marker. In both cases a constant current of 1.7 ma was applied until the dye had reached the bottom of the tube (16–18 hr at 4 C). After electrophoresis the gels were removed from the tubes and fixed for 15 min with 7% acetic acid. They were stained for 3 hr in 0.1% (w/v) amido black in 7% (v/v) acetic acid and destained by washing in a succession of solutions of 7% acetic acid.

Two-Dimensional Urea-Acrylamide Gels. The procedure used was that of Kaltschmidt and Wittmann (12) using samples of 1 to 3 mg/150 μl; electrophoresis took place at room temperature. The first dimension employed an 8% acrylamide gel at pH 8.6 with a running time of 24 hr at 135 v. The second dimension utilized an 18% gel at pH 4.6 with a running time of 26 hr at 105 v.

The gels were stained for 20 min in 0.5% amido black in 5% acetic acid and were destained for 1 hr in running water and then for 4 days in 5% acetic acid circulated through activated charcoal. The protein spots were numbered successively in horizontal lines starting at the upper left of the gel.

SDS-Polyacrylamide Gels. The method of Weber and Osborn (25) was used. Proteins were denatured with 1.0% SDS, 1.0% mercaptoethanol in 10 mM sodium phosphate buffer, pH 7.0, for 45 min at 45 C. The gel solution contained: acrylamide, 10%; bis-acrylamide, 0.27%; SDS, 0.1%; TMED, 0.15%; ammonium persulfate, 0.075%; sodium phosphate buffer (pH 7.0), 10 mM. Ribosomal proteins (50–100 μg) were applied to the top of the gel and electrophoresis took place at room temperature for 12 to 14 hr with a constant current of 8 ma/tube. Bromphenol blue was added as a visible mobility reference.

After removing the gels from the tubes, a small piece of copper wire was inserted to mark the position of the bromphenol blue marker. The gels were then fixed overnight with 50% trichloroacetic acid. The gels were stained with a solution containing: Coomassie brilliant blue, 0.1%; glacial acetic acid, 2.5%; methanol, 40%; and glycerine, 2.5% for 5 hr. Destaining was accomplished with a solution containing: acetic acid, 10%; and methanol, 45%, circulating through activated charcoal.

Scanning and Calibration of Gels. One-dimensional gels (urea and SDS) were scanned on a Joyce-Loebl microdensitometer. To calibrate the SDS gels, five proteins yielding monomers of known mol wt were treated in the same manner as the ribosomal proteins (see above). These standards included: cytochrome *c*, 13,400; myoglobin, 17,800; chymotrypsinogen A, 23,650; ovalbumin, 46,500; and bovine serum albumin, 67,000. The distance of migration plotted against the logarithm of the mol wt yielded a straight line which was used to calculate the mol wt of the ribosomal proteins.

Amino Acid Incorporation into Ribosomal Proteins. The ribosomal proteins of dark-grown resting cells exposed to light to induce chloroplast development were labeled by adding 5 μc of a ^{3}H-amino acid mixture supplied by New England Nuclear Corp. (NET 250) per ml of culture, at appropriate times.

Incorporation into whole cell proteins was determined as follows. The labeled cells were suspended in 5% trichloroacetic acid and were incubated for 20 min at 90 C. The suspension was filtered through a glass fibre filter, and the filter was washed with 3 volumes of 5% trichloroacetic acid, 1 volume of ether-chloroform (3:1), and was then dried and counted in a Beckman LS-150 scintillation counter after addition of 10 ml of a solution containing PPO, 4 g; POPOP, 50 mg; hyamine, 5 g; per liter of toluene, in a standard scintillation vial.

The 15,000*g* ribosomal supernatant prepared from ^{3}H-labeled cells was layered over a 2-ml cushion of 40% (w/w) sucrose prepared in buffer II, and the ribosomes were recovered by centrifugation for 17 hr at 25,000 rpm.

The ribosomal preparation was analyzed on sucrose density gradients, and 0.2-ml fractions were collected. Each fraction received 100 μg of bovine serum albumin and 1 volume of 10% trichloroacetic acid. They were then incubated for 20 min at 90 C, and the precipitate was collected on a glass fiber filter. The filter was washed three times with 5% trichloroacetic acid

Table I. *Effects of Different Treatments on the Sedimentation Rates of Euglena Ribosomal Subunits*

Treatment	Large Subunit		Small Subunit		Reports of Similar Particles
	Intact	Degraded	Intact	Degraded	
					reference
Mg^{2+} 0.1 mM dialyze 5 hr[1]	62		45		1–4, 6
Mg^{2+} 0.1 mM dialyze 10 hr[2]		48		38	9
Mg^{2+} 0.1 mM, KCl 30 mM dialyze 8 hr[3]	62	47	45	40	6
Na_2 EDTA 1 mM[4]		43		29	6

[1] Complete buffer composition and dissociation conditions are given in the legend of Figure 2B.

[2] Complete buffer composition and dissociation conditions are given in the legend of Figure 2C.

[3] Complete buffer composition and dissociation conditions are given in the legend of Figure 2D.

[4] Complete buffer composition and dissociation conditions are given in the legend of Figure 2A.

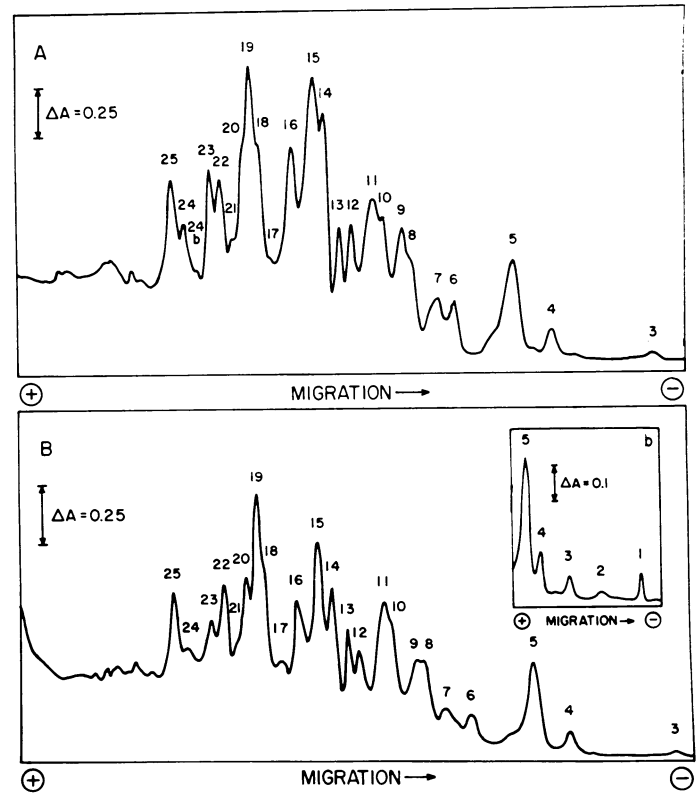

FIG. 3. Densitometer tracings of cytoplasmic ribosomal proteins analyzed on pH 4.5, 8 M urea, 7.5% polyacrylamide gels. A: Cytoplasmic ribosomal proteins from *Euglena gracilis* var. *bacillaris;* B: cytoplasmic ribosomal proteins from the bleached mutant W_3BUL.

and once with 95% ethanol. The filters were then dried and counted as described above. An aliquot of the ribosomal preparation was analyzed in a similar manner in order to determine the recovery from the gradient fractions.

RESULTS AND DISCUSSION

Extraction of Cytoplasmic Ribosome Components. Cells grown at pH 3.5 were broken in a buffer containing 100 mM tris-HCl in order to provide enough buffer capacity to keep the pH of the homogenate above 7.5, since a *Euglena* ribonuclease is active in the range of pH 6 to 7 (11). For the analysis of ribosomal proteins, the ribosomes are usually washed with a buffer high in salt in order to remove adhering nonribosomal proteins. However, this treatment seems to remove essential ribosomal proteins from *Euglena* ribosomes, since smaller subunits are found after cytoplasmic subunits are treated with 500 mM KCl (6). In the present work, various washing buffers were tried: 100 mM KCl was found to dissociate 20% of the monosomes while NH_4Cl used at the same concentration did not appear to induce ribosome dissociation; above 200 mM NH_4Cl ribosome dissociation is observed.

Ribosomes purified as described under "Materials and Methods" and analyzed on a linear 20 to 50% (w/w) sucrose gradient appear as a monosome peak followed by 6 to 7 peaks which represent the polysomes (1, 5, 11) (Fig. 1A). The polysome and monosome fractions can be recovered for further analysis by overnight centrifugation. Upon reanalysis on

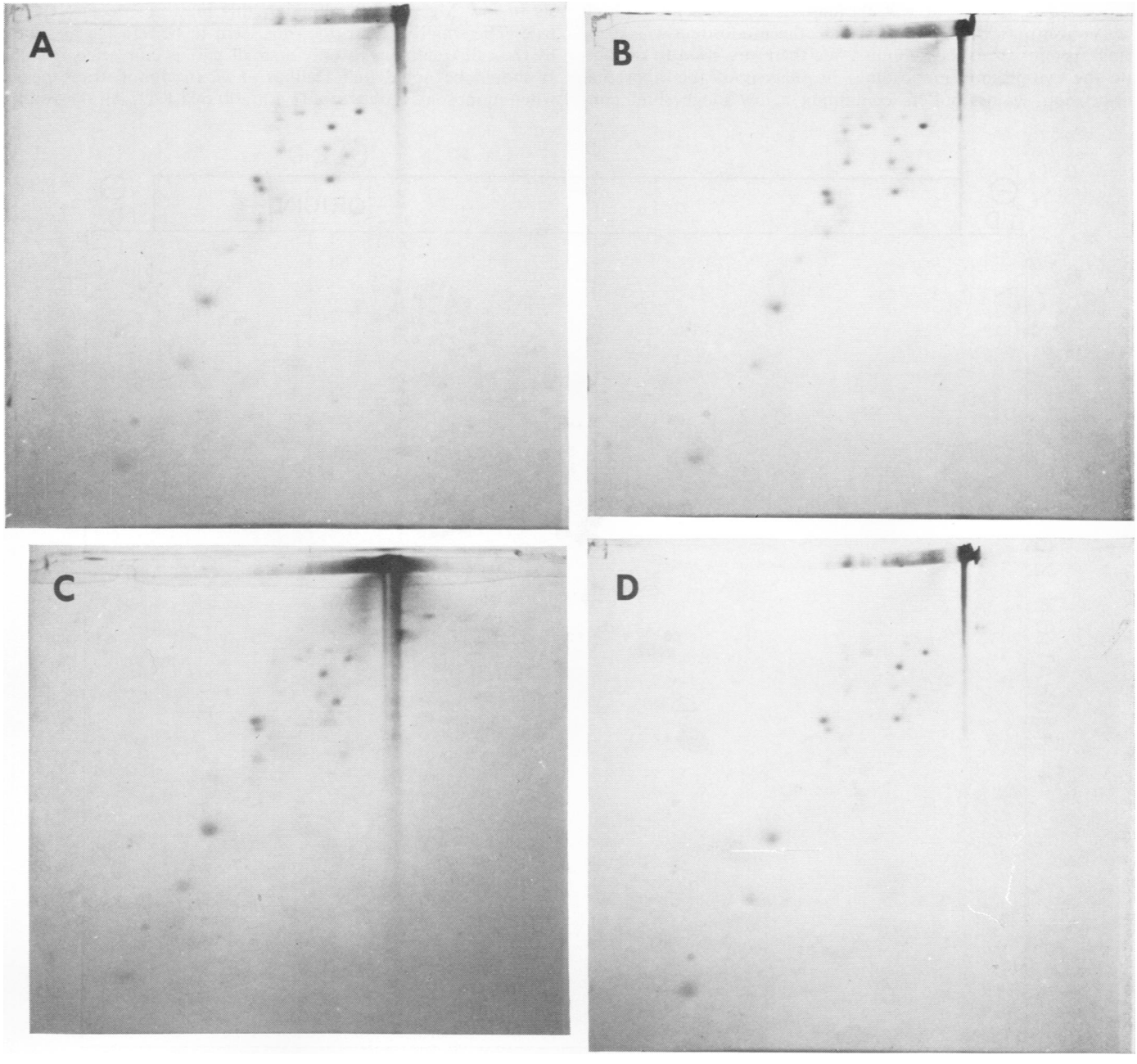

FIG. 4. Two-dimensional analysis of the cytoplasmic ribosomal proteins of *Euglena gracilis*. The anode is at the right for the first dimension and at the top in the second dimension. A: 2 mg of ribosomal proteins extracted from light-grown cells of *Euglena gracilis* var. *bacillaris;* B: 2 mg of ribosomal proteins extracted from dark-grown cells of *Euglena gracilis* var. *bacillaris;* C: 1.5 mg of ribosomal proteins extracted from light-grown cells of the bleached mutant W_3BUL; D: 1.5 mg of ribosomal proteins extracted from dark-grown cells of the bleached mutant W_3BUL.

sucrose gradients, the polysomes are well preserved, and the monosomes have not dissociated (Fig. 1, B and C). If the same preparation is analyzed on a linear 5 to 20% (w/w) sucrose gradient, only the monosome peak is visible; the majority of the polysomes are not detected, since they enter the 40% sucrose cushion (Fig. 1D). All of the strains studied, wild type *bacillaris* and Z, W_3BUL and Sm_1^rBNgL contain monosomes showing an apparent S value of 89S compared with 70S monosomes and 50 and 30S subunits from *E. coli* (Fig. 1D).

When the ribosomal pellet is resuspended in a buffer containing EDTA and analyzed on a sucrose gradient, two small subunits (43S and 29S) (Fig. 2A) were obtained. Delihas *et al.* (6) also described small subunits (35S–29S) after EDTA treatment. Since these values are smaller than expected for the cytoplasmic ribosomal subunits (62–67S and 41–46S, ref. 1 and papers cited therein), it was thought that the EDTA or high salt concentration used to dissociate the monosome was stripping proteins from the subunits. We therefore tried to dissociate the cytoplasmic monosomes by dialysis of the ribosomal suspension against buffers containing a low magnesium concentration. Dialysis of the 89S ribosomes for 5 hr against a low magnesium buffer, in the absence of added KCl yielded two particles, 62S and 45S (Fig. 2B) which represent the intact subunits (1–4, 6). If the dialysis is continued for more than 5 hr, the values of the two particles obtained are 48S and 38S (Fig. 2C), values already reported by Gnanam and Kahn (9) for cytoplasmic ribosomal subunits. If KCl is present in the dialysis buffer, three particles are found in addition to the monosomes (Fig. 2D), 62S, 47S and 40S. Extended dialysis in this buffer causes the amount of the 62S particle to decrease as the 45S particle peak increases, indicating that the large subunit (62S) is progressively converted to a 47S particle due to either a conformational change or to a change in composition. This 47S particle is probably similar to the 43S particle found after EDTA treatment and the 48S particle found after a long dialysis in low Mg^{2+} buffer, although this may not necessarily be true. The small subunit does not seem to be affected as much by the salt treatment as only a small part is converted to 40S. It should be noted that Delihas *et al.* (6) found three peaks when monosomes are treated with 300 mM KCl. All the results

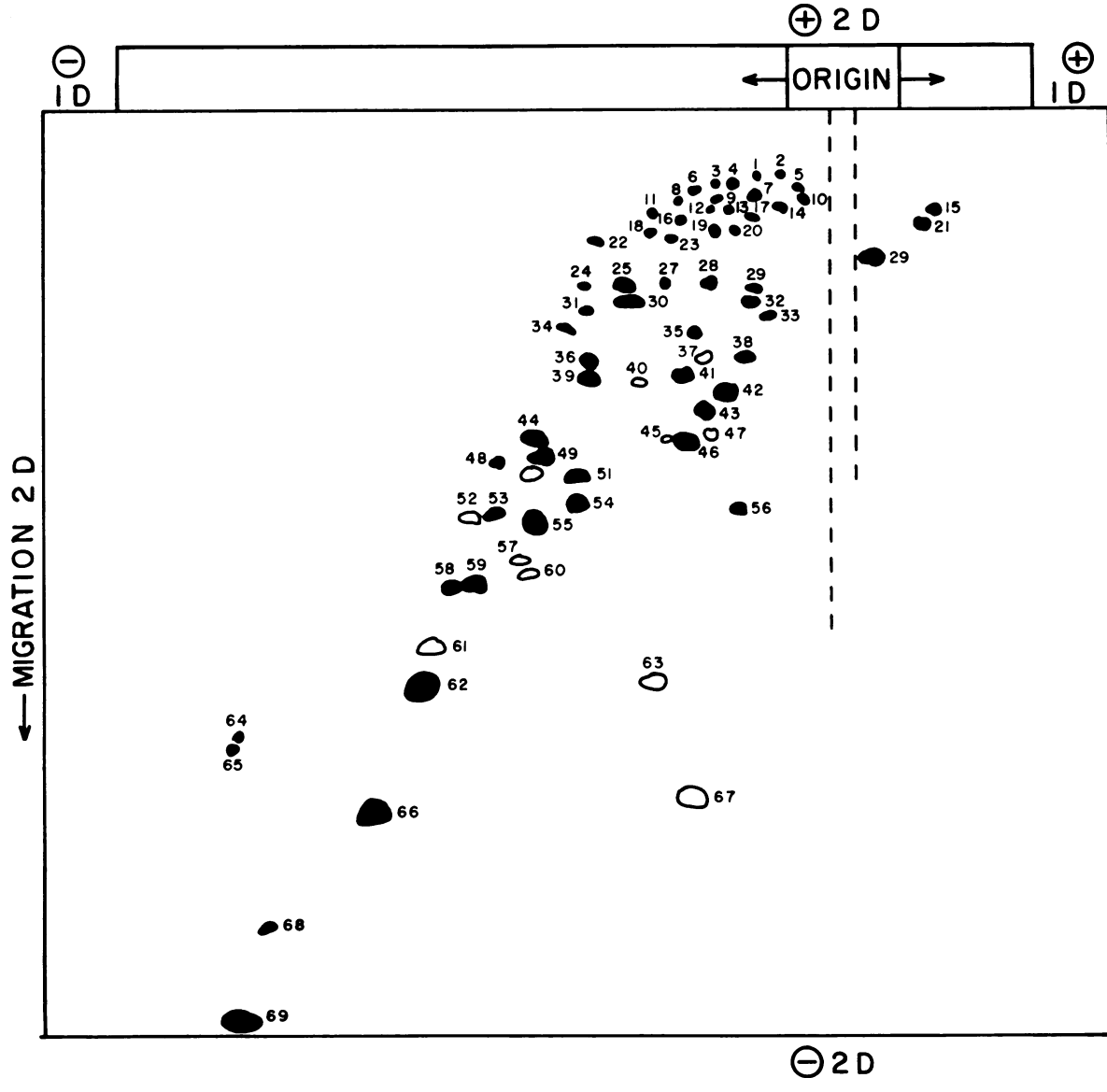

FIG. 5. Diagrammatic representation of the two-dimensional electrophoretogram of the cytoplasmic ribosomes of *Euglena*. The open spots are those which are difficult to see in the photographs of the gels (Fig. 4).

are summarized in Table I. The significance and composition of these particles remain to be determined.

Characterization of Chloroplast and Cytoplasmic Ribosomal Proteins. Twenty-five protein components were identified in extracts of cytoplasmic ribosomes from wild type and W_3BUL using one-dimensional urea-polyacrylamide gel electrophoresis (Fig. 3, A and B). Two rapidly migrating components are only seen if the electrophoresis is carried out for less than 16 hr

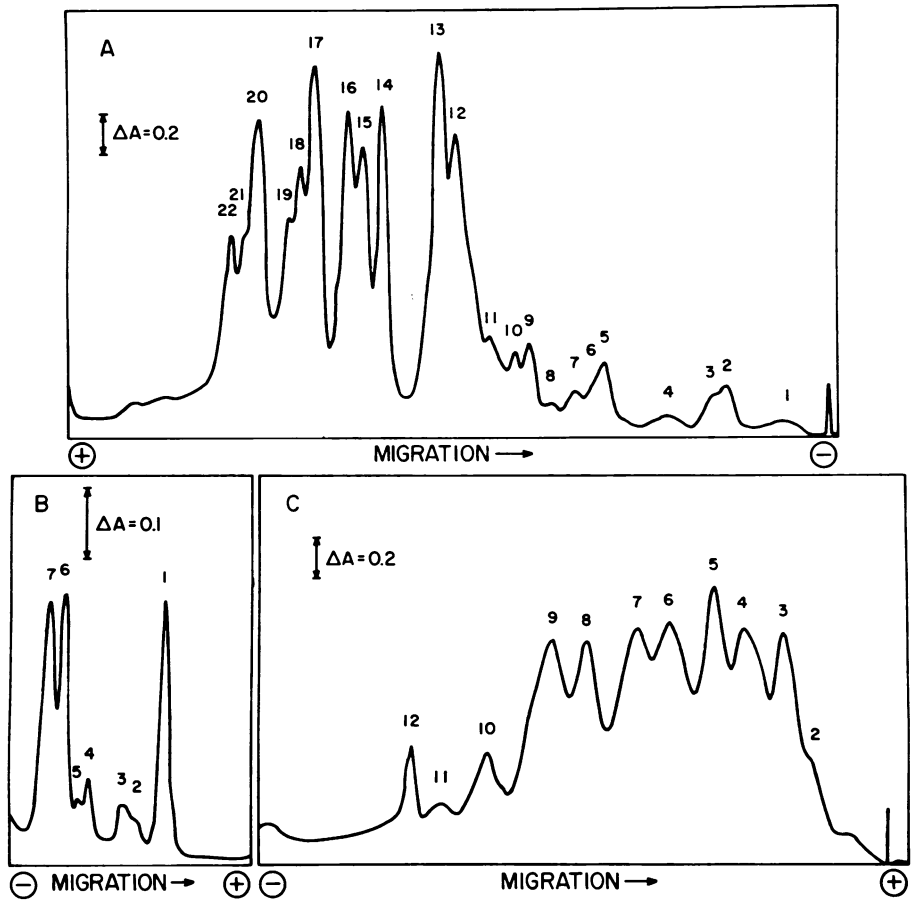

FIG. 6. Analysis of chloroplast ribosomal proteins extracted from light-grown cells of *Euglena gracilis* var. *bacillaris*. A: Densitometer tracing of basic chloroplast ribosomal proteins analyzed on pH 4.5, 8 M urea, 7.5% polyacrylamide gels; B: densitometer tracing of acidic chloroplast ribosomal proteins analyzed on pH 8.6, 6 M urea, 4% polyacrylamide gels; C: densitometer tracings of chloroplast ribosomal proteins analyzed on SDS-polyacrylamide gels.

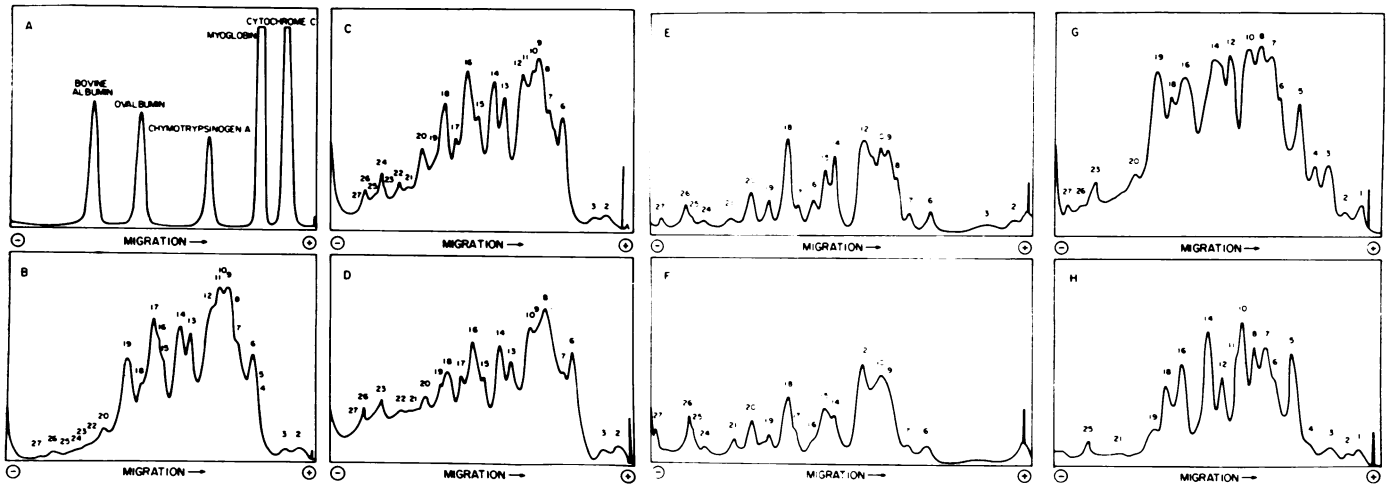

FIG. 7. Densitometer tracings of cytoplasmic ribosomal proteins analyzed on SDS-polyacrylamide gels. A: Proteins of known mol wt which were used to calibrate the gel; B: cytoplasmic ribosomal proteins from *Euglena gracilis* strain Z; C: cytoplasmic ribosomal proteins from light-grown W_3BUL; D: cytoplasmic ribosomal proteins from Sm_1^rBNgL; E: cytoplasmic ribosomal proteins from dark-grown *Euglena gracilis* var. *bacillaris;* F: cytoplasmic ribosomal proteins from light-grown *Euglena gracilis;* G: cytoplasmic ribosomal proteins from the large subunit of light-grown *Euglena gracilis* var. *bacillaris;* H: cytoplasmic ribosomal proteins from the small subunit of light-grown *Euglena gracilis* var. *bacillaris*.

(inset, Fig. 3B). These components are found in both strains. The components which migrate slower than the 25th protein are present in low amounts and may represent contaminants rather than true ribosomal proteins. In electrophoresis of ribosomal proteins derived from subunits or monosomes which have been purified on a sucrose gradient, these components are still present, indicating that if they are contaminants, they are tightly bound to the ribosome. One-dimensional ribosomal protein patterns obtained from monosomes or polysomes were found to be identical.

The cytoplasmic ribosomal proteins extracted from light- and and dark-grown cells of wild type *Euglena gracilis* var. *bacillaris* and W_3BUL (Fig. 4) and those from strain Z and Sm_1^rBNgL (not shown) were analyzed on two-dimensional urea-acrylamide gels and the patterns obtained from all strains were the same. Sixty-nine protein components can be distinguished (Fig. 5) in agreement with the numbers reported for other eucaryotic cytoplasmic ribosomes (24, 26). Three proteins (15, 21, and 29) migrate toward the anode, indicating isoelectric points lower than pH 8.6. These proteins are always present but the extent of their migration is variable. The intensity of staining of these components is never very high and component 29 seems to have an isoelectric point close to 8.6 since it does not move very far from the origin.

When extracts from chloroplast ribosomes are separated on one-dimensional urea-polyacrylamide gels at pH 4.5, 22 basic components are found (Fig. 6). If the electrophoresis is repeated at pH 8.6, 7 acidic components can be distinguished (Fig. 6B). Cytoplasmic ribosomal proteins analyzed under the same conditions at pH 8.6 showed, in most cases, only two lightly staining components. The third component found using the two-dimensional technique may not be resolved under these conditions, as its isoelectric point seems close to 8.6 (Figs. 4 and 5).

With seven acidic components, chloroplast ribosomal proteins behave in a similar manner to ribosomal proteins from *E. coli* (17), while cytoplasmic ribosomal proteins are similar to those from other eucaryotes (17, 19, 20, 24, 26).

Table II. *Molecular Weights of Cytoplasmic Ribosomal Proteins*

Zones	Molecular Weight × 10^{-3}						
	Bacillaris dark grown	*Bacillaris* light grown	*Bacillaris* light grown large subunit	*Bacillaris* light grown small subunit	Sm_1^rBNgL	W_3BUL dark grown	Strain Z light grown
27	98	104	98		97	99	98
26	82	83	88		83	84	86
25	80	81		81		81	81
24	74	75				74	75.5
23			71		71	72	71
22					65	66	65
21	61	61		62	57	60	
20	54	55	57		51	54	57
19	48	49	47	48	47	48	48
18	42.5	43	42	42	43	43.5	43
17	40	41			38.5	39.7	40
16	36	37	38	38	37	37	37
15	33.5	34			35	35	35
14	31.5	32	30	30.6	30.6	32	31
13					28	29	28
12	26	26.4	26.7	28		26.6	26.2
11	24.5	24.3		25		25	25
10	23.3	23.4	23	23.6	24	23.3	23.7
9	22.2	22.7			22.5	22	22
8	21.2		20.8	21.4	20.7	20.4	20.7
7	19.4	19.5	19.1	19.6	17.5	18.2	19.4
6	17	17.3	17.6	18.3	16.7	16.7	17
5			15.2	16			15.7
4			13.3	14		14.7	14.3
3	12.2		11.9	11.8	13	13	12.7
2	10.2		10.2	10.2	11.4	11.5	11.2
1			8.9	9.3			
W_{TN}[1]	29,000	30,400			27,500	29,600 (±1,880)[2]	29,400
W_{TW}[3]	36,500	36,100			34,500	34,800 (±1,820)	35,400
N_S[4]	97.6	92.6			102.5	96.7 (±5.9)	95.7
N_T[5]	77.3	78			81.6	80.9 (±4.2)	79.5

[1] W_{TN}, number average molecular weight = total daltons of protein divided by the total number of proteins.

[2] Numbers in brackets indicate SD.

[3] W_{TW}, weight-average molecular weight = mean molecular weight obtained from the "center of gravity" of the densitometer tracing of Coomassie stained SDS-polyacrylamide gels containing separated proteins.

[4] N_S, the calculation of the number of proteins is based on staining of proteins by Coomassie blue following the technique described by King *et al.* (14).

[5] N_T, the number obtained by dividing the total daltons of proteins by the weight-average molecular weight.

In order to determine the mol wt of the cytoplasmic ribosomal proteins from several strains as well as from their subunits, ribosome extracts were analyzed on SDS-polyacrylamide gels (Fig. 7). The results obtained are summarized in Table II. The mol wt of the cytoplasmic ribosomal proteins range from about 10,200 to about 104,000. The proteins in the large cytoplasmic ribosomal subunit range from 8,900 to 97,900 and those in the smaller cytoplasmic subunit show a range of 9,300 to 81,000. Certain components are not found in all strains or under all conditions (Table II). In all cases these represent bands which are close to the limit of detection and it cannot be concluded at present that they are truly missing.

Analysis of the *Euglena* chloroplast ribosomal proteins on SDS-polyacrylamide gels (Fig. 6C) yielded the mol wt distribution shown in Table III. The mol wt range from 9,700 to 57,900.

The total number of proteins in each of the ribosomes was determined using the technique of King *et al.* (14) employing the following formula to calculate the number of proteins contained in each zone of the gel: (axM)/(Axm) where a and A are the areas subtended by a particular peak and the total area respectively, m is the mol wt of the protein in the zone and M is the total daltons of protein in the ribosome. The sum of the number of proteins in each peak is equal to the total number of proteins in the ribosome. Using the results of Brown and Hazelkorn (3) and Rawson and Stutz (21), the protein content of the cytoplasmic ribosome was calculated to be 2.816×10^6 daltons, while the protein content of the chloroplast ribosome was calculated to be 0.824×10^6 daltons. The results in Tables II and III are based on at least two preparations. In the case of W_3BUL cytoplasmic ribosomes, the standard deviation for three preparations has been calculated (Table II) to indicate the reproducibility of the technique. The cytoplasmic ribosomes contain 90 to 100 proteins whose number average mol wt is 27,500 to 30,400 and whose weight average mol wt is 34,500 to 36,500 (Table II). These values agree with the values reported for other eucaryotic cytoplasmic ribosomes (2, 14). The chloroplast ribosome contains about 40 proteins whose number average mol wt is 19,900 and whose weight average mol wt is 23,400 (Table III). These values agree with those found for *E. coli* ribosomes (2), although the number of proteins found in the *Euglena* chloroplast ribosome, 40, is somewhat lower than the number of proteins, 45 to 56, reported for the *E. coli* ribosome (2). The lower sedimentation coefficient, 68S, of the *Euglena* chloroplast ribosome is probably a reflection of its lower protein content.

Table III. *Molecular Weights of Chloroplast Ribosomal Proteins Extracted from Euglena gracilis var. Bacillaris*

The abbreviations used are defined in Table II.

Zones	Molecular Weight $\times 10^{-3}$
12	58
11	53
10	42
9	32
8	28
7	23
6	20.4
5	16.8
4	14.7
3	12.7
2	11.4
1	9.7
W_{TN}	19,900
W_{TW}	23,400
N_S	41.5
N_T	35.2

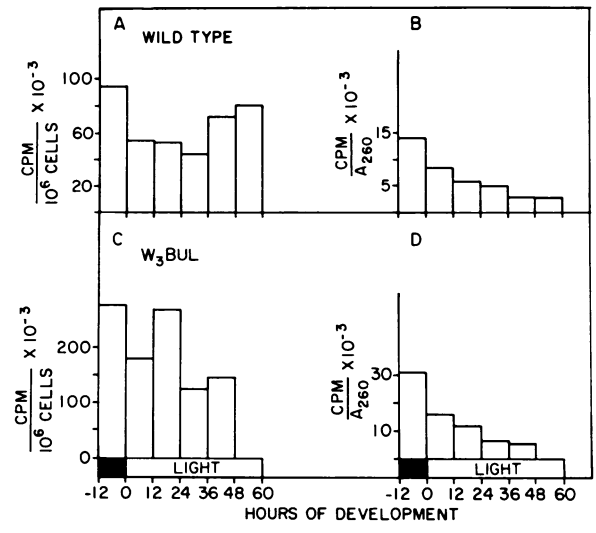

FIG. 8. Incorporation of radioactive amino acids into whole cells and the cytoplasmic ribosomal pellet during light induced chloroplast development. A: Whole cell incorporation in *Euglena gracilis* var. *bacillaris;* B: incorporation into the cytoplasmic ribosomal pellet of *Euglena gracilis* var. *bacillaris;* C: whole cell incorporation in W_3BUL; D: incorporation into the cytoplasmic ribosomal pellet of W_3BUL.

In conclusion, the *Euglena* cytoplasmic ribosomes contain more proteins of higher mol wt than the *Euglena* chloroplast ribosome. This agrees with the finding of Bickle and Traut (2) that procaryotic ribosomes are smaller than eucaryotic cytoplasmic ribosomes because they contain fewer ribosomal proteins and these proteins are smaller in size.

Synthesis of Cytoplasmic Ribosomal Proteins during Light-induced Chloroplast Development. Figure 8 shows the incorporation of ^{3}H-amino acids into cellular proteins during a 12-hr labeling period at various times during development. Consistent with previous work using ^{3}H-leucine (Schwartzbach and Schiff, unpublished data), the rate of incorporation into total cellular protein of wild type is high in the dark. During the first 12 hr of illumination, the rate decreases and then increases after this time (Fig. 8A). Incorporation into W_3BUL shows the initial light-induced decrease in rate, but the subsequent increase in rate observed with wild type cells is not seen (Fig. 8C). Concomitant measurements of incorporation of the amino acids into the ribosomal pellets show a light-induced decrease in the rate of incorporation in both wild type and W_3 (Fig. 8, B and D). It should be noted that the time of onset and the magnitude of the increased rates of incorporation noted after the initial light inhibition in wild type were variable from one experiment to another.

The cytoplasmic polysome content of dark-grown resting cells of wild type and W_3BUL was low (Fig. 9, A and C), as has been previously observed in wild type Z resting cells by Heizmann *et al.* (11). The addition of the labeled amino acid mixture had no effect on the polysome content. On transfer of these cultures to light, a net formation of cytoplasmic polysomes were observed (Fig. 9, B and D). The incorporation of radioactive amino acids into polysomes and monosomes was found to be higher in the dark than in the light (Fig. 9), in agreement with the results obtained with the ribosomal pellets (Fig. 8, B and D). This indicates that most of the protein in

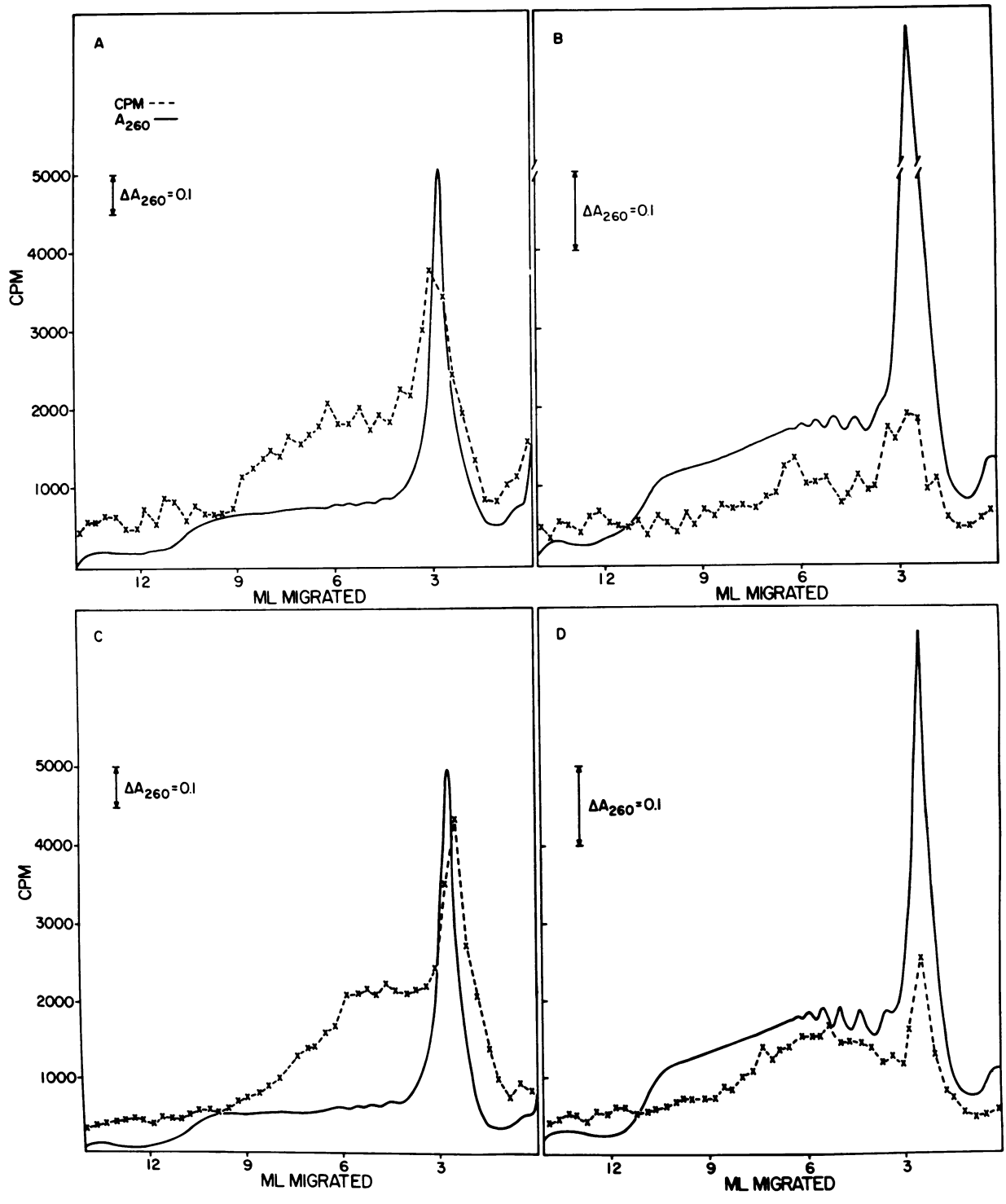

FIG. 9. Sucrose gradient sedimentation analysis of ^{3}H-amino acid labeled cytoplasmic polysomes. pH 4.5 dark-grown resting cells were labeled for 12 hr intervals. Cytoplasmic ribosomes were isolated and analyzed by centrifugation for 5 hr at 25,000 rpm on linear 20 to 50% sucrose gradients. A: Polysomes extracted from *Euglena gracilis* var. *bacillaris* after a 12-hr incubation with ^{3}H-amino acids in the dark; B: polysomes extracted from *Euglena gracilis* var. *bacillaris* after incubation with ^{3}H-amino acids during the first 12 hr of light-induced chloroplast development; C: polysomes extracted from W_3BUL after a 12-hr incubation with ^{3}H-amino acids in the dark; D: polysomes extracted from W_3BUL after incubation with ^{3}H-amino acids during the first 12 hr of light induction.

the ribosomal pellets cosediments with monosomes or polysomes. Since an appreciable amount of the radioactivity of the ribosomal pellet cosediments with the ribosomal subunits, on dissociation (Fig. 10), the major incorporation of label is into ribosomal proteins rather than nascent polypeptides. The amount of contaminating proteins in the ribosomal pellet is low, since only a small amount of radioactivity is found on the 50% sucrose cushion or at the top of the gradient.

The light-induced decrease in the rate of amino acid incorporation into ribosomal proteins and total cellular proteins

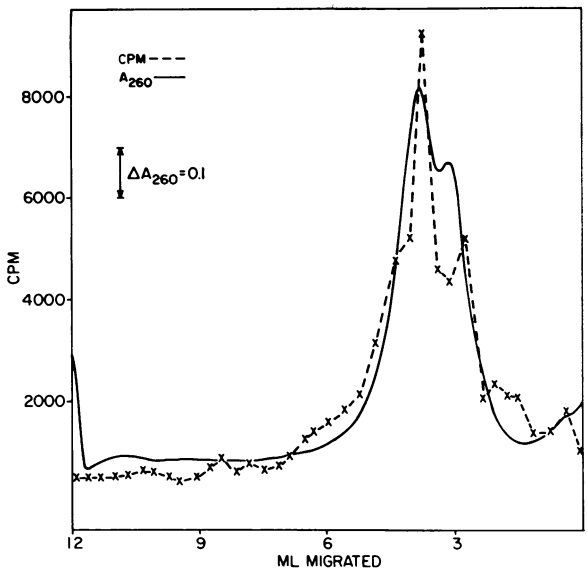

FIG. 10. Sucrose gradient sedimentation analysis of ^{3}H-amino acid labeled cytoplasmic ribosomal subunits. pH 4.5 dark-grown resting cells were labeled for 12 hr as described under "Materials and Methods" and cytoplasmic ribosomes were extracted and converted to subunits as described in Figure 2. The subunits were analyzed by centrifugation for 4.5 hr at 25,000 rpm on linear 5 to 20% sucrose gradients.

is probably not a consequence of a change in the effective specific radioactivity of the precursor related to a change in cellular permeability or amino acid pool size. Kirk and Tilney-Bassett (15) found that there was no marked change in the size of the total amino acid pool during the first 12 hr of light-induced chloroplast development. The rate of amino acid incorporation into the bulk protein of *Euglena* was also decreased to the same extent by light when the amino acids (Schwartzbach and Schiff, unpublished data), leucine, phenylalanine, or a mixture of 15 labeled amino acids (Fig. 8) were used to determine the rate of protein synthesis. This suggests that the pool sizes of the individual amino acids do not expand on light induction to yield a lower specific radioactivity of the precursor pool of amino acids and hence an apparent lowering of the rate of incorporation into protein on light exposure. Another possibility not ruled out is that more than one pool of each amino acid exists in the cell and that some of these represent metabolically active pools which behave differently from the bulk amino acids.

The rate of uptake of the labeled amino acid does not seem to limit the rate of protein synthesis, since the amount of amino acid taken up by the cell is always greater than the amount incorporated into protein. Furthermore, the amount incorporated into protein is decreased to a greater extent by light than is the amount taken up by the cell, indicating that a light-induced permeability change is probably not the cause of the change in protein labeling that is observed (Schwartzbach and Schiff, unpublished data).

Previous work (4, 5, 27) has shown that light induction of chloroplast development in *Euglena* results in an increased rate of labeling of cytoplasmic ribosomal RNA, although the total amount of cytoplasmic ribosomal RNA remains constant. This indicated that the ribosomal RNA is turning over. If we assume that all of the labeled ribosomal RNA is in ribosomes, then light induces an increased rate of cytoplasmic ribosome formation. Since light decreases the rate of incorporation of amino acids into ribosomal proteins, ribosomal proteins would have to be reutilized during turnover in order to provide the proteins for the synthesis of ribosomes containing the newly transcribed ribosomal RNA. During magnesium starvation of *E. coli*, ribosomes cannot be detected. The addition of magnesium results in the synthesis of ribosomes without the synthesis of new ribosomal proteins (16). This indicates that the ribosomal proteins can be reutilized for the synthesis of ribosomes containing newly transcribed RNA.

CONCLUSIONS

The ribosomal proteins of the cytoplasm of *Euglena* resemble those of other eucaryotic cytoplasmic ribosomal proteins in their size and number. The ribosomal proteins of *Euglena* chloroplast ribosomes, on the other hand, are very similar to procaryotic ribosomes such as those of *E. coli*. Thus ribosomal proteins join a long list of properties which suggest that the plastid is procaryotic in nature and which may point in the direction of an endosymbiotic invasion by a procaryote as the origin of the plastid (22).

Although ribosomal RNA synthesis and presumably ribosome synthesis are stimulated by light (4, 5), the rate of incorporation of amino acids into cytoplasmic ribosomal proteins is decreased, indicating that the ribosomal proteins were reutilized. This reutilization allows the cell to devote its protein synthetic machinery to the synthesis of the proteins required for chloroplast development.

Acknowledgments—We thank Dr. Harlyn Halvorson for the loan of the apparatus for two-dimensional gel electrophoresis and Mr. Steven Schwartzbach for his help in the preparation of chloroplast ribosomes.

LITERATURE CITED

1. AVADHANI, N. G. AND D. E. BUETOW. 1972. Isolation of active polyribosomes from cytoplasm, mitochondria and chloroplasts of *Euglena gracilis*. Biochem. J. 128: 353-365.
2. BICKLE, T. A. AND R. R. TRAUT. 1971. Differences in size and number of 80S and 70S ribosomal proteins by dodecyl sulfate gel electrophoresis. J. Biol. Chem. 246: 6828-6834.
3. BROWN, R. D. AND R. HAZELKORN. 1971. Synthesis and maturation of cytoplasmic ribosomal RNA in *Euglena gracilis*. J. Mol. Biol. 59: 491-503.
4. COHEN, D. 1973. Photoregulation of formation and turnover of chloroplast ribosomal RNA and cytoplasmic ribosomal RNA during chloroplast development in *Euglena gracilis* Klebs var. *bacillaris* Pringsheim. Ph.D. thesis. Brandeis University, Waltham, Mass.
5. COHEN, D. AND J. A. SCHIFF. 1973. Photoregulation of formation and turnover of chloroplast-rRNA (chlrRNA) and cytoplasmic-rRNA (cytrRNA) during chloroplast development in *Euglena gracilis* Klebs var. *Bacillaris* Pringsheim. Biophys. Soc. Abstr. 13: 111a.
6. DELIHAS, N., A. JUPP, AND H. LYMAN. 1972. Properties of *Euglena gracilis* cytoplasmic ribosomes in salt. Biochim. Biophys. Acta 262: 344-351.
7. FAZEKAS DE ST. GROTH, S., R. G. WEBSTER, AND A. DAYTNER. 1963. Two new staining procedures for quantitative estimation of proteins on electrophoretic strips. Biochim. Biophys. Acta 71: 377-391.
8. FOGEL, S. AND P. S. SYPHERD. 1968. Extraction and isolation of individual ribosomal proteins from *Escherichia coli*. J. Bacteriol. 96: 358-364.
9. GNANAM, A. AND J. S. KAHN. 1967. Biochemical studies on the induction of chloroplast development in *Euglena gracilis*. III. Ribosome metabolism associated with chloroplast development. Biochim. Biophys. Acta 142: 493-499.
10. GREENBLATT, G. L. AND J. A. SCHIFF. 1959. A pheophytin-like pigment in dark adapted *Euglena gracilis*. J. Protozool. 6: 23-28.
11. HEIZMANN, P., G. TRABUCHET, G. VERDIER, G. FREYSSINET, AND V. NIGON. 1972. Influence de l'éclairement sur l'évolution des polysomes dan des cultures d'*Euglena gracilis* étiolées. Biochim. Biophys. Acta 277: 149-160.
12. KALTSCHMIDT, E. AND H. G. WITTMANN. 1970. Ribosomal proteins. VII. Two-dimensional polyacrylamide gel electrophoresis for finger-printing of ribosomal proteins. Anal. Biochem. 36: 401-412.
13. KALTSCHMIDT, E. AND H. G. WITTMANN. 1972. Ribosomal proteins. XXXII. Comparison of several extraction methods for proteins from *Escherichia coli* ribosomes. Biochimie 54: 167-175.
14. KING, H. W. S., H. J. GOULD, AND J. J. SHEARMAN. 1971. Molecular weight distribution of proteins in rabbit reticulocyte ribosomal subunits. J. Mol. Biol. 61: 143-156.

15. KIRK, J. T. O. AND R. A. E. TILNEY-BASSETT. 1967. The Plastids. W. H. Freeman and Co., London. pp. 448–451.
16. LEFKOVITS, I. AND M. DIGIRALOMO. 1969. Reutilization of ribosomal proteins *in vivo* for the formation of new ribosomal particles in *Escherichia coli* B. Biochim. Biophys. Acta 174: 566–573.
17. LOW, R. B. AND I. G. WOOL. 1967. Mammalian ribosomal protein: analysis by electrophoresis on polyacrylamide gel. Science 155: 330–332.
18. LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR, AND R. J. RANDALL. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265–275.
19. LYTTLETON, J. W. 1968. Protein constituents of plant ribosomes. Biochim. Biophys. Acta 154: 145–149.
20. MARTINI, O. H. W. AND H. J. GOULD. 1971. Enumeration of rabbit reticulocyte ribosomal proteins. J. Mol. Biol. 62: 403–405.
21. RAWSON, J. R. AND E. STUTZ. 1969. Isolation and characterization of *Euglena gracilis* cytoplasmic and chloroplast ribosomes and their ribosomal components. Biochim. Biophys. Acta 190: 368–380.
22. SCHIFF, J. A. The development, inheritance, and origin of the plastid in *Euglena*. *In:* M. Abercrombie, J. Brachet, and T. J. King eds., Advances in Morphogenesis, Vol. 10. Academic Press, New York. pp. 265–312.
23. SCHWARTZBACH, S. D., G. FREYSSINET, AND J. A. SCHIFF. 1974. The chloroplast and cytoplasmic ribosomes of *Euglena*. I. Stability of chloroplast ribosomes prepared by an improved procedure. Plant Physiol. 53: 533–542.
24. SHERTON, C. C. AND I. G. WOOL. 1972. Determination of the number of proteins in liver ribosomes and ribosomal subunits by the two-dimensional polyacrylamide gel electrophoresis. J. Biol. Chem. 247: 4460–4467.
25. WEBER, K. AND M. OSBORN. 1969. The reliability of molecular weight determination by dodecyl-sulfate-polyacrylamide gel electrophoresis. J. Biol. Chem. 244: 4406–4412.
26. WELFE, H., J. STAHL, AND H. BIELKA. 1971. Studies on proteins of animal ribosomes. VIII. Two-dimensional polyacrylamide gel electrophoresis of ribosomal proteins of rat liver. Biochim. Biophys. Acta 243: 416–419.
27. ZELDIN, M. H. AND J. A. SCHIFF. 1967. RNA metabolism during light-induced chloroplast development in *Euglena*. Plant Physiol. 42: 922–932.