MITOCHONDRIAL AND CYTOPLASMIC RIBOSOMES

FROM *TETRAHYMENA PYRIFORMIS*

Correlative Analysis by Gel Electrophoresis

and Electron Microscopy

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ABSTRACT

Mitochondrial and cytoplasmic ribosomes from *Tetrahymena pyriformis* have been isolated and studied by the techniques of polyacrylamide gel electrophoresis and electron microscopy used in conjunction. Although the two ribosome types show the same coefficient of sedimentation (80S) in sucrose gradients, they can be distinguished by gel electrophoresis: mitoribosomes migrate in a single band, considerably slower than the cytoribosome band. Electron microscope observations of negatively stained cytoribosomes show typical rounded or triangular profiles, about 275 × 230 Å; mitoribosome profiles are much larger and clearly elongate, about 370 × 240 Å. An electron-opaque spot delimits two nearly equal size subunits. In mixtures of mito- and cytoribosomes, each type can be recognized by its characteristic electrophoretic mobility and by its distinctive fine structure. Cytoribosomal 60S and 40S subunits each produce a distinct electrophoretic band. On the contrary, neither electrophoretic analysis, using a variety of conditions, nor electron microscopy is able to discern two different subunit types in the single 55S mitoribosomal subunit peak. Electrophoretic analysis of RNA shows that both ribosomal RNA species are present in the mitoribosomal subunit fraction. These results establish that mitoribosomes from *T. pyriformis* dissociate into two subunits endowed with the same sedimentation coefficient, the same electrophoretic mobility, and a similar morphology.

INTRODUCTION

Mitochondrial ribosomes (mitoribosomes) have now been isolated and characterized from a number of organisms (see reviews: Borst and Grivell, 1971; Borst, 1972; Dawid, 1972; Kroon et al., 1972). Their sedimentation coefficients in sucrose gradients vary according to the particular organism, but, in general, they are inferior to that of the homologous cytoplasmic ribosomes (cytoribosomes). As a rule, mitoribosomes may be classed in two groups (Dawid, 1972): animal mitoribosomes which sediment between 55S and 60S and those of several fungi sedimenting between 72S and 75S.

A surprising exception has been reported for the

ciliate *Tetrahymena pyriformis* where both cyto- and mitoribosomes exhibit the same sedimentation coefficient (80S) in sucrose gradients (Chi and Suyama, 1968; 1970; Allen and Suyama, 1972). Mitoribosomes could, however, be distinguished from cytoribosomes by buoyant density determinations in CsCl gradients, and by analysis of their respective ribosomal RNA components. A further unexpected property of these mitoribosomes concerned their dissociation into subunits: while cytoribosomes showed the characteristic dissociation into unequal subunits of 60S and 40S, mitoribosomes dissociated in the presence of ethylenediaminetetraacetate (EDTA)[1] into a single 55S peak. Two different subunits could be demonstrated only in a CsCl density gradient which showed two density species of 1.46 and 1.52 g/cm^3.

The determination of an 80S monomer form of *T. pyriformis* mitoribosomes has not been fully accepted by other workers in this field (Borst and Grivell, 1971; Borst, 1972); the unusual results have been ascribed to possible contaminating membrane fragments and/or dimer formation. From an evolutionary point of view (Schutgens et al., 1973) concerning the relation of *T. pyriformis* mitoribosomes to the two groups of mitoribosomes (Dawid, 1972) as well as from the point of view of ribosome function, the problem merits further study. We have therefore undertaken an examination of *T. pyriformis* ribosomes using two additional analytical techniques: polyacrylamide gel electrophoresis and electron microscopy of negatively stained particles. Both techniques have allowed us to characterize the two ribosomes: mitoribosomes have a unique fine structure and a greater volume than cytoribosomes, mitoribosomes dissociate into particles which behave as a single class in gel electrophoresis and which display similar morphological features. No evidence is found to consider them as the monomer form.

MATERIALS AND METHODS

Cell Culture and Preparation of Mitochondria

Axenic cultures of *T. pyriformis*, ST strain, were grown at 28°C in 1.5% proteose-peptone (Difco

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[1] *Abbreviations used in this paper:* DOC, sodium deoxycholate, EDTA, ethylenediaminetetraacetate, sodium salt; SDS, sodium dodecyl sulfate; TMK, 10 mM Tris-HCl, pH 7.4, 10 mM MgCl$_2$, 100 mM KCl; Tris, tris (hydroxymethyl) aminomethane (Sigma Chemical Co., St. Louis, Mo.).

Laboratories, Detroit, Mich.) and 0.25% yeast extract (Difco) medium until the stationary growth phase was attained, after 3 days growth (approximately 1.2×10^5 cells per ml).

Cells were harvested using a GGT centrifuge at 1,800 rpm for 5 min (model 700, Giovanni Giaccardo-Torino, Italy), and washed in either: (a) TMK-sucrose (10 mM Tris-HCl, pH 7.4, 10 mM MgCl$_2$, 100 mM KCl, and 0.25 M sucrose) if cytoribosomes were to be conserved, or (b) Tris-EDTA-sucrose (10 mM Tris-HCl, pH 7.4, 1 mM Na EDTA, and 0.25 M sucrose) if cytoribosomes were not to be conserved (Chi and Suyama, 1970).

Cells were broken by passage through an emulsion homogenizer (Arthur H. Thomas Co., Philadelphia, Pa.) at 4°C and centrifuged at 500–800 g, 6 min to remove nuclei and cell debris. The supernate (S$_1$) was then centrifuged at 5,000 g, 6 min and the resulting supernate (S$_2$) was retained for cytoribosome purification. The pellet (P$_2$) was resuspended in Tris-EDTA-sucrose and spun at 500–800 g, 6 min to remove cell debris and contaminating membranes (P$_3$). Purified mitochondria (P$_4$) were obtained after three successive centrifugations of the supernate S$_3$ at 5,000 g. If cytoribosomes were not to be conserved, all steps were carried out in Tris-EDTA-sucrose medium. All subsequent operations were performed at 4° C.

Preparation of Ribosomes

CYTORIBOSOMES: To prepare cytoribosomes, the supernate S$_2$ was centrifuged 20–30 min at 56,000 g in the Spinco 40 rotor (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.) to eliminate large contaminants. Triton X-100 (Touzart and Matignon, Paris) was added to the resulting supernate (0.02 ml of 1.4% Triton X-100 per ml of supernate) and 10 ml of the mixture were immediately placed on 2 ml of 1.5 M sucrose (RNase-free, Schwarz/Mann Div., Becton, Dickinson & Co., Orangeburg, N. Y.) in TMK buffer and centrifuged 2.5 h at 140,000 g (Spinco 40 rotor). A transparent pellet of cytoribosomes was obtained.

The ribosomal pellet was resuspended in 1–2 ml of TMK buffer. A quantity corresponding to an absorbancy of 3–4 at 260 nm was layered on 12 ml of a linear sucrose gradient (0.3–1.4 M) made in TMK buffer. Centrifugation was carried out for 3–3.5 h at 35,000 rpm (Spinco SW 36 rotor).

Cytoribosomal subunits were obtained by placing a sample fraction of 0.1–0.5 ml (corresponding to an optical absorbancy of 6–8 at 260 nm) of the ribosomal suspension on 10 ml of a linear (0.3–1.4 M) sucrose gradient made in buffer containing 10 mM Tris-HCl, pH 7.4, 0.5 mM MgCl$_2$, and 50 mM KCl and deposed on a 2-ml sucrose cushion (1.4 M). Centrifu-

gation was carried out for 3–3.5 h at 35,000 rpm (Spinco SW 36 rotor).

MITORIBOSOMES: The mitochondrial pellet P_4 (0.5–1 ml) was resuspended in 2 ml of TMK buffer and lysed with either Triton X-100, sodium deoxycholate (DOC) at final concentrations of 2.5% and 0.4%, respectively, or with a mixture of both, and remained 30 min at 4°C. 1 ml of the lysate was placed directly on 12 ml of a linear sucrose gradient (0.3–1.4 M) in TMK buffer. Centrifugation was carried out for 3–3.5 h at 35,000 rpm (Spinco SW 36 rotor).

Mitoribosomal subunits were obtained by layering the mitochondrial lysate on a 12-ml linear sucrose gradient (0.3–1.4 M) made in buffer containing 10 mM Tris-HCl, pH 7.4, 100 mM KCl, and 1 mM EDTA. Tubes were centrifuged for 3.5 h at 35,000 rpm (Spinco SW 36 rotor).

Fractions of 12 drops were collected by pumping from the bottom of the tubes through capillary tubing. Absorbance at 260 nm was measured with a Gilford spectrophotometer 240 (Gilford Europe S.A., Malakoff, France).

Electron Microscopy

Unfixed ribosomal particles and subunits from the peak fractions of sucrose gradients or from ribosomal pellets were negatively stained with a 1% aqueous solution of uranyl acetate and examined in a Siemens Elmiskop 1A. Copper grids (300 mesh) covered with a thin carbon film only were used throughout. Other details of the technique can be found in Vignais et al. (1972).

Extraction of Ribosomal RNA

Mitochondrial RNA and cytoplasmic RNA were extracted from mitochondria or whole cells, respectively, by a modification of the technique used by Vignais et al. (1972). Pellets of whole cells or mitochondria were resuspended in a buffer containing 10 mM Tris-HCl, pH 7.4, and 0.3 M NaCl. Sodium thioglycollate (B grade, Calbiochem, San Diego, Calif.) was added to a final concentration of 20 mg/ml and the suspension was agitated 10 min at 4°C. Diethylpyrocarbonate (20–30 μl/ml) was added and the suspension was again agitated for 1 or 2 min before deproteinization by sodium dodecyl sulfate (SDS) at a final concentration of 2.5% and by an equal volume of water-saturated, freshly distilled phenol containing 0.1% of 8-hydroxyquinoleine. Tubes were centrifuged 5–10 min at 500 g (Sorvall RC2-B, SS-34 rotor, Ivan Sorvall, Newtown, Conn.). The nucleic acid-containing supernate was deproteinized one or two times and finally treated three times with ethyl ether to remove traces of phenol. After addition of 0.1 vol of 1 M potassium acetate,

pH 5.1, the nucleic acids were precipitated by ethyl alcohol at −20°C.

Polyacrylamide Gel Electrophoresis

Ribosomes and RNA were analyzed by vertical electrophoresis in polyacrylamide gels in quartz tubes according to the technique of Loening (1967). Polyacrylamide gels containing 2.2–3.0% of acrylamide were prepared from a stock solution containing 15% acrylamide (recrystallized from chloroform) and 0.75% bisacrylamide (recrystallized from acetone). The buffer commonly used contained 40 mM Tris, 0.1 mM EDTA, 20 mM sodium acetate, and 2 mM magnesium acetate; the pH was adjusted to 7.8 with acetic acid. The buffer used for the gel was filtered through a 0.45 μm Millipore membrane (Millipore Corp., Bedford, Mass.). Different analyses were made by varying the pH and the concentrations of sodium acetate and magnesium acetate, and by replacing sodium acetate by potassium acetate. All gels were pre-run for at least 1 h before samples were applied.
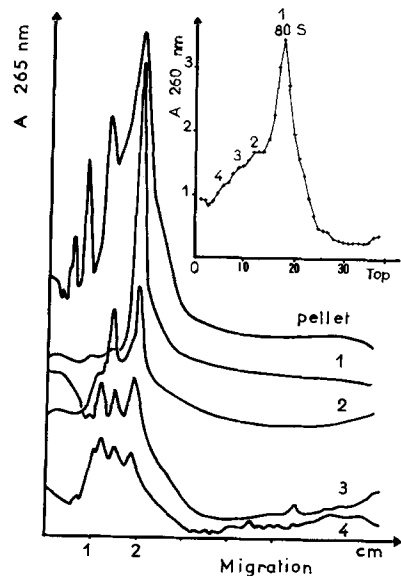

FIGURE 1 Polyacrylamide gel electrophoresis of cytoribosome fractions. Electrophoresis in 2.4% acrylamide gels was carried out for 3 h at 4°C in buffer composed of 40 mM Tris, 0.1 mM EDTA, 20 mM Na acetate, and 2 mM Mg acetate at pH 7.8. Tracings 1–4 correspond to fractions 1–4 from the sucrose gradient (inset); tracing pellet was obtained by electrophoresis of a cytoribosomal pellet (see Materials and Methods). Inset: Sedimentation pattern of cytoribosomes. A ribosomal suspension was layered on a 12-ml linear sucrose gradient made in TMK buffer. Centrifugation was for 3 h at 35,000 rpm and 4°C.

Samples corresponding to an optical absorbancy of 0.2–0.5 at 260 nm were generally used. When comparisons were to be made, equal quantities having a similar absorbancy were applied. Migration times were from 2 to 4 h at 4°C and the direction of migration was toward the anode. The current applied to the tubes had an intensity of 5 mA per tube; the tension varied slightly according to the buffer solution. Gels were scanned directly in the tubes at 265 nm using a Polyfrac UV scanner (Joyce Loebl-

France S.A., Suresnes, France) and were recorded using a Bryans chart recorder (Bryans Ltd., Mitcham, Surrey, England).

RESULTS

Cytoribosomes and mitoribosomes of *T. pyriformis* sediment in linear sucrose gradients in a coincident peak at 80S, as previously reported by Chi and
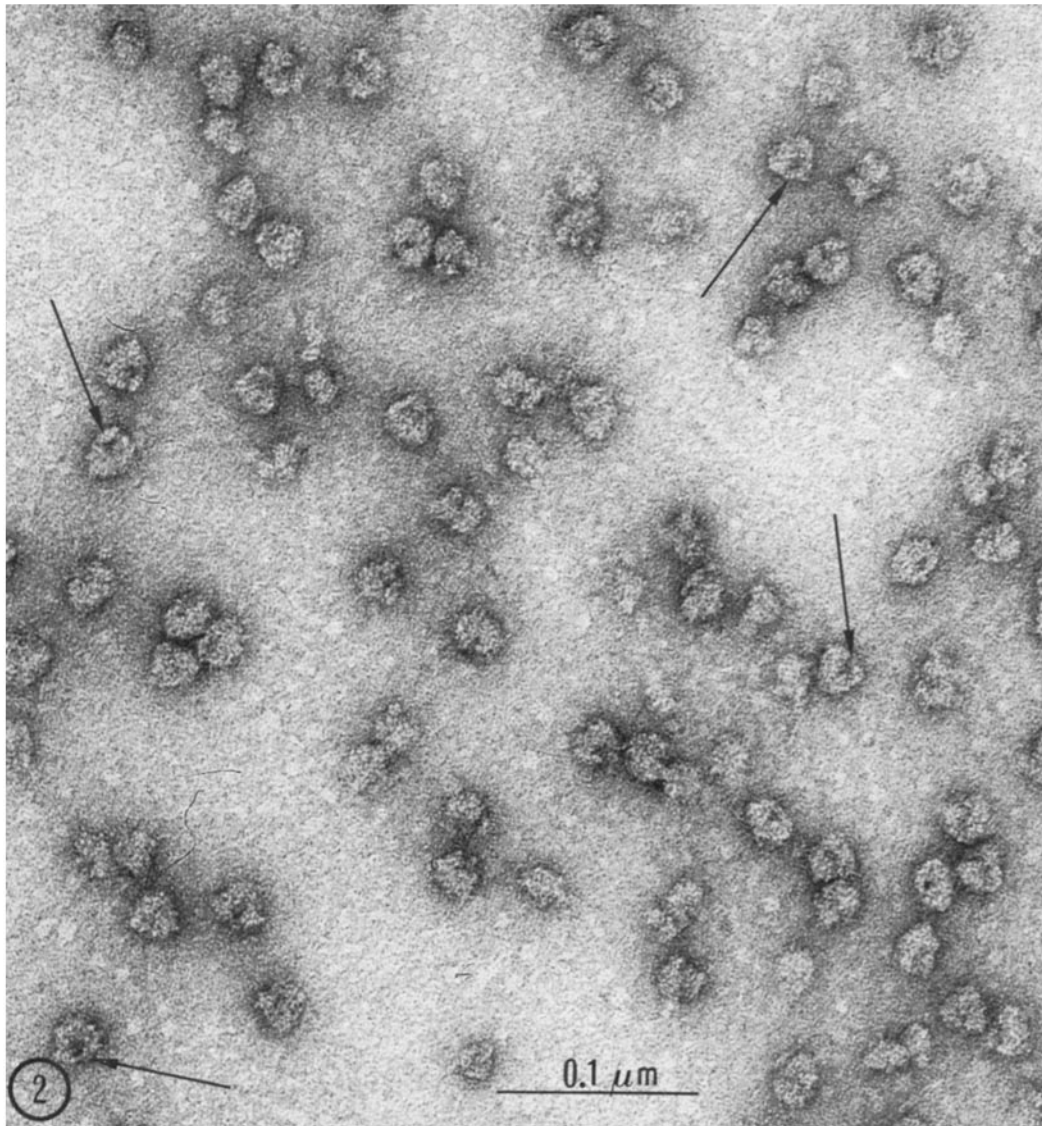

FIGURE 2   General view of a field of negatively stained cytoribosomes. The majority of profiles presents an electron-opaque spot (arrows) which is on the left side when the image is oriented with the small subunit towards the top. Some smaller profiles may represent subunits. × 280,000.

Suyama (1968; 1970), distinctly faster than 70S *Escherichia coli* ribosomes used as reference.

Comparative Analysis of Cytoribosomes and Mitoribosomes by Gel Electrophoresis and Electron Microscopy

CYTORIBOSOMES: Fractions of a cytoribosomal sucrose gradient (Fig. 1 inset) which showed a major component sedimenting at 80S and several shoulders on the heavy side were analyzed by electrophoresis. Fraction 1, the major peak, migrated in a single, narrow band (Fig. 1). Fraction 2 gave two clear bands, the fastest being coincident with the single band from fraction 1. The heavy shoulders 3 and 4 produced the same two bands, of decreasing intensity, a third slower band, and some indication of a still slower band. We interpret these four bands as representing the cytoribosome monomers and three types of polysomes. Evidence supporting this interpretation was obtained by
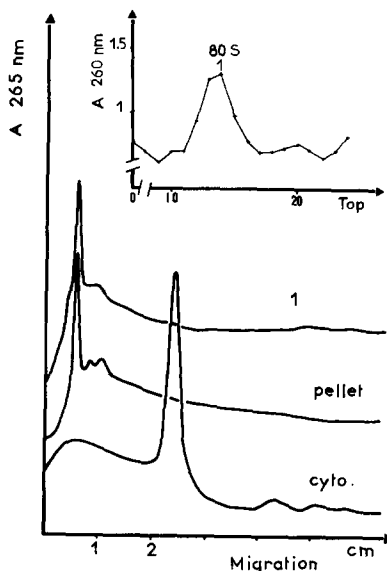

FIGURE 3 Polyacrylamide gel electrophoresis of mitoribosome fractions. Electrophoretic conditions as in Fig. 1; migration was for 4 h. Tracing *1* corresponds to a sample of fraction *1* from the sucrose gradient (*inset*). For the tracing *pellet*, a mitoribosomal pellet, prepared by procedure for cytoribosomes in Materials and Methods was used. The tracing *cyto.* corresponds to cytoribosomes obtained from a sucrose gradient. *Inset*: Sedimentation pattern of mitoribosomes from a mitochondrial lysate. Sedimentation was for 3.5 h at 35,000 rpm and 4°C.

electron microscope observations of these same fractions. While fraction 1 contained monomer ribosomes and some rare dimers, dimer forms were predominant in fraction 2. In fraction 3, trimers were frequent and many tetramer forms appeared in fraction 4. Supplementary bands representing polysomal species have been obtained also by direct electrophoretic analysis of a cytoribosomal pellet (Fig. 1). Five bands of increasing absorbance from the negative toward the positive pole are shown.The superposition of the more rapidly migrating bands with those derived from the 80S peak and heavy shoulders of the sucrose gradient confirms their identification, respectively, as cytoribosomal monomers and polysomes.

Examination in the electron microscope of negatively stained cytoribosomes from fraction 1 in Fig. 1 reveals a homogeneous population of slightly elongated particles measuring about 275 × 230 Å (Fig. 2). The majority of the profiles presents an eccentric electron-opaque spot which is located on the left side when the profile is oriented with the small subunit on top. The small subunit appears wide and flattened; the large subunit assumes a roughly triangular form, with a bluntly pointed end. A series of images selected from larger fields is shown in Fig. 5.

Only rarely do profiles present a different form from this one. Profiles which show the dark spot to the right are infrequent. Occasionally, an opaque cleft appears to separate the two subunits; kidney-shaped profiles having one convex and one concave side are also rare. We interpret the most frequent form as representing a projection of the "dorsal" face of the ribosome; the other, more rare forms would then represent "frontal" and "lateral" projections, respectively, as defined by Vignais et al. (1972). The high frequency of dorsal images suggests a preferential orientation of cytoribosomes as they come to rest on the supporting film. A similar observation was reported by Nonomura et al. (1971) and Lutsch et al. (1972) for rat liver cytoribosomes.

MITORIBOSOMES: When a fraction of the 80S mitoribosomal peak of a sucrose gradient is placed on a polyacrylamide gel, mitoribosomes are observed to migrate in a single, well-defined band, whose electrophoretic mobility is considerably slower than that of cytoribosomes (Fig. 3). Under all the conditions used, the mitoribosomal peak always remains close to the origin. Electrophoresis of a mitoribosomal pellet likewise demonstrates a single, slowly migrating band, coincident
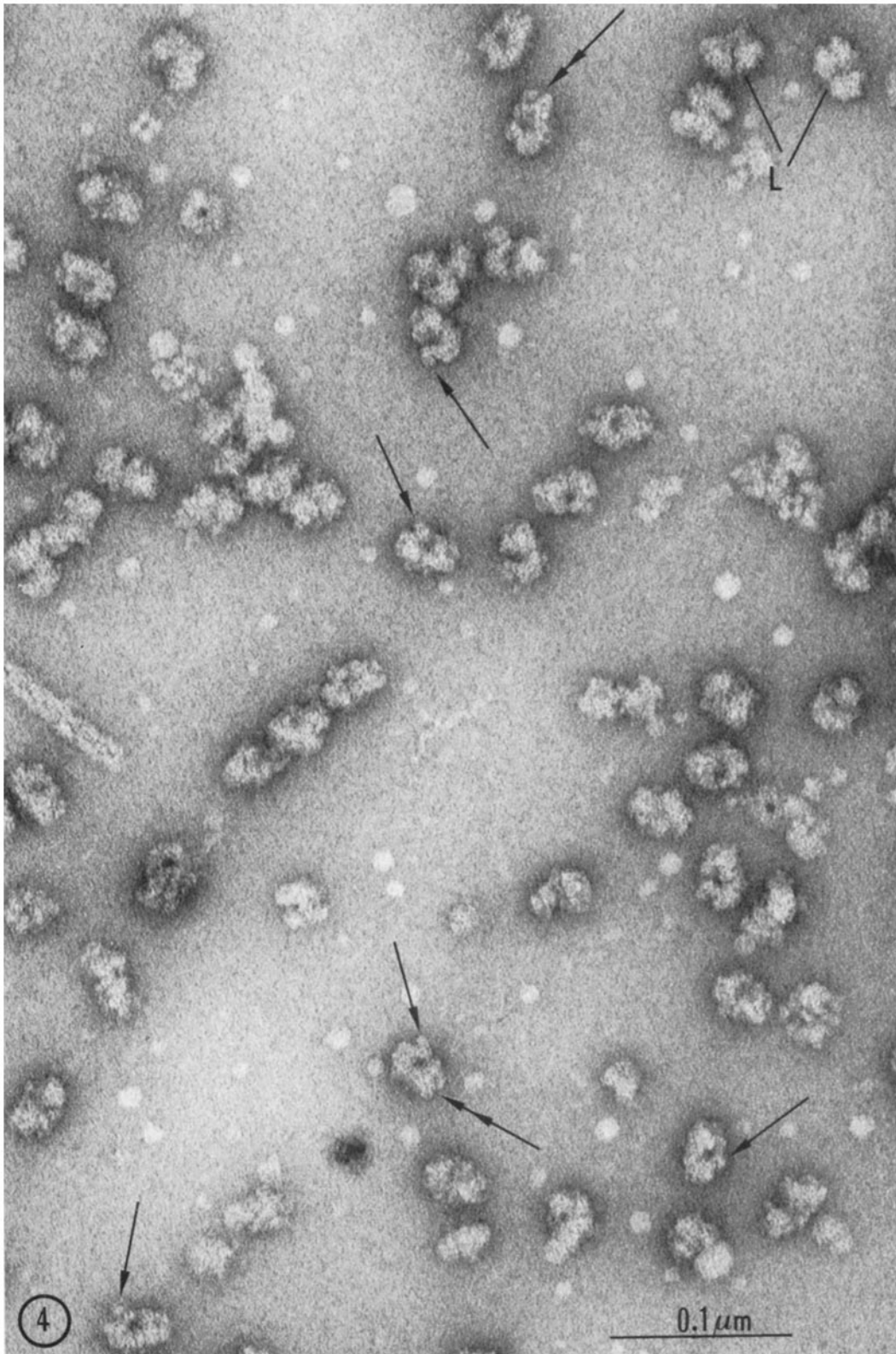
FIGURE 4   General view of a field of negatively stained mitoribosomes. An electron-opaque spot present in most profiles marks a division into roughly equal size subunits. One end shows a definite lobe on the left or right side (arrows); the other end is narrower and shows a notch (double arrows). A dense line appears to divide some profiles into equal size subunits (L). × 280,000.
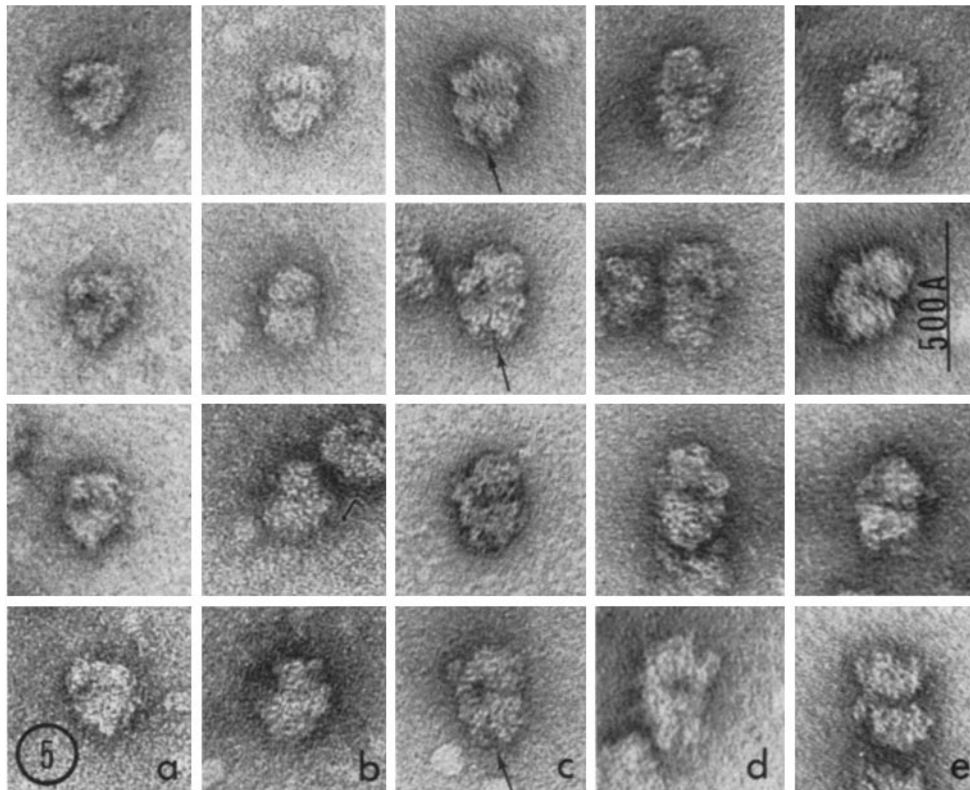
FIGURE 5 Selected images of mito-and cytoribosomes. Vertical columns from left: *a*, principal view (dorsal) of cytoribosomes showing the dense spot on the left and small subunit at top; *b*, rare frontal views (upper two) and lateral views (lower two); *c* and *d*, principal view of mitoribosomes with lobe at right (*c*) and at left (*d*), arrows mark notch in opposite extremity; *e*, mitoribosome images with dense cleft separating subunits of about equal size. × 400,000.

with that achieved from the 80S peak of a sucrose gradient (Fig. 3). It is likely that mitochondrial polysomes either are not conserved during extraction or are unable to penetrate into the gel.

Profiles of negatively stained mitoribosomes are definitely larger (about 370 × 240 Å) and more elongate than those of cytoribosomes (Fig. 4; selected images in Fig. 5). The most frequent image is characterized by an electron-opaque spot at about equal distance from the two ends and closer to one edge. This spot appears to mark the limit between two, nearly equal size subunits. In this view, the two subunits can be distinguished by several differences in form and dimension. One of the subunits appears wider, about 260 Å as measured perpendicularly to the long axis. Its contour is irregularly rounded and a lobe protrudes on one side. When the profile is viewed with the wider subunit on top, the lobe can be

seen, with about equal frequency, on either side. The opaque spot is always located on the side closer to the lobe. The other subunit is narrower, about 210 Å; its rounded end exhibits a notch (Figs. 4 and 5).

Mitoribosomal profiles which do not show the opaque spot sometimes show an electron-opaque cleft separating the profile in two approximately equal size subunits. In this view, the two subunits seem identical (Fig. 5).

Mixtures of Mito- and Cytoribosomes

The efficiency of the electrophoretic separation of mitoribosomes and cytoribosomes was tested on natural and artificial mixtures of the two ribosome species. A lysate of the pellet P₃ was fractionated on a sucrose gradient (Fig. 6, inset) and a portion of the 80S peak was analyzed by gel electrophoresis. The electrophoretic patterns derived from this 80S
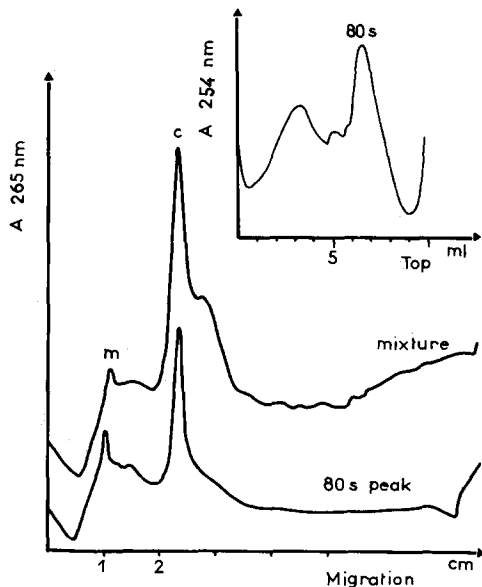
FIGURE 6 Electrophoresis of natural and artificial mixtures of mito- and cytoribosomes. Electrophoretic conditions were as in Fig. 1. The *80S peak* tracing corresponds to the 80S fraction of the sucrose gradient (*inset*); the *mixture* tracing corresponds to a mixture of mito- and cytoribosomes, sedimented previously on separate gradients and intentionally combined just before deposition on the gel. *Inset*: Sedimentation pattern of ribosomes from pellet P_3 prepared according to procedure for cytoribosomes. The ribosomal suspension was layered on a 10-ml linear sucrose gradient (0.3–1.4 M) made in TMK buffer, placed over a 2-ml cushion of 1.4 M sucrose. Centrifugation was carried out for 2 h at 35,000 rpm and 4°C. Profile was obtained by continuous monitoring in an LKB Uvicord II (Laboratorie och Kemikaliska Produkter, Stockholm) at 254 nm. The heavy peak represents membrane fragments (unpublished data).

peak and from an intentional mixture of mito- and cytoribosomes previously sedimented on sucrose gradients are similar and show two well-separated peaks, corresponding to the different mobilities previously determined for the two ribosome species (Fig. 6). Electrophoresis, therefore, is capable of separating mixtures of mito- and cytoribosomes which could not be separated by sucrose gradient centrifugation.

The presence of both types of ribosomes in the 80S fraction of Fig. 6 is confirmed by observation with the electron microscope (Fig. 7). The majority of profiles are typical of cytoribosomes, but mitoribosomal profiles can also be recognized.

Comparative Analysis of Cyto- and Mitoribosomal Subunits

CYTORIBOSOMES: When fractions of the 60S subunit peak from a sucrose gradient with 0.5 mM Mg^{2+} are analyzed by gel electrophoresis, a single band is obtained (Fig. 8). The 40S subunit fraction produces a major band migrating rapidly in the gel, as well as a smaller band in the same position as the large subunit band.

Electron microscope examination of the subunit fractions clearly distinguishes two types of particles (Figs. 9 and 10). The large subunits present rounded profiles, about 245 Å in diameter. Small subunits are slightly elongated and significantly smaller, about 240 × 175 Å. Some small subunits appear bipartite (Fig. 9, arrows).

MITORIBOSOMES: Mitoribosomal subunits from various regions of the 55S peak (Fig. 12 inset, arrows), in sucrose gradients containing 1 mM EDTA, have been analyzed by gel electrophoresis. Under standard conditions (2.6% gel, pH 7.8, 0.1 mM EDTA, 2 h) all fractions demonstrate a unique band whose mobility coincides with that of the 60S cytoribosomal subunit (Fig. 12). Further, when mitoribosome monomers are placed on a gel lacking Mg^{2+} ions, they are shown to dissociate during migration and to produce a coincident band (Fig. 12).

Attempts to demonstrate two types of mito-ribosomal subunits by varying a number of parameters of the electrophoretic conditions have been unsuccessful. Migration of various portions of the 55S peak from a sucrose gradient produced only a single absorption peak when the following variations in the conditions were tried: (*a*) concentration of acrylamide (2.2, 2.4, 2.6, and 3.0%), (*b*) duration of migration (2, 3, and 4 h), (*c*) pH of the buffer (6.8, 7.8, 8.4, and 9.3), (*d*) concentration in Na^+ ions (0, 20, 40, and 80 mM), and (*e*) replacement of Na^+ by K^+ ions, at 20 and 40 mM. In all cases, the large cytoribosomal and the mitoribosomal subunits migrate to the same position. The only indication of some heterogeneity in the mitoribosomal subunit band is given by high concentrations of Na^+ ions. At 40 mM sodium acetate, a shoulder appears on the slow side of the major absorption peak; at 80 mM, some spreading of the peak is observed. A similar spreading of the subunit band also occurred with 40 mM K^+. Under the same conditions, large cytoribosomal subunits always produce a sharp, well-defined peak. The response to pH changes
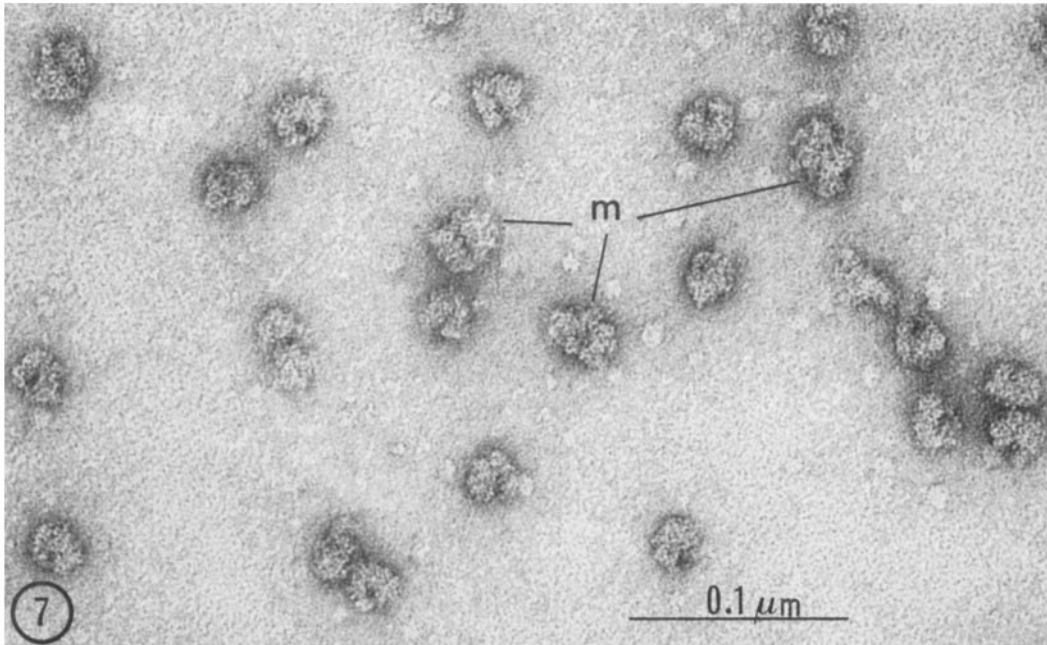
FIGURE 7 Negatively stained preparation of the 80S peak from a natural mixture of ribosomes in pellet 3 (Fig. 6, *inset*). Cytoribosomal profiles are predominant; some mitoribosomal profiles are also present (*m*). × 280,000.

should be noted. At high pH (9.3) the absorption peak is extremely narrow and high, while at lower pH values, the peak is more spread.

Morphological examination of fractions from the 55S peak reveals rounded but irregular profiles with an average maximum diameter of 255 Å (Fig. 11). The contours as well as the overall texture of the profiles are uneven and no recurrent characteristic forms can be noted. The profiles show none of the standard morphological features of monomeric ribosomes, i.e., a dense spot or cleft separating two subunits, a distinction between two possible subunits, or repeatable forms representing projections of the different faces of the particle. A number of profiles do contain a region of high opacity on one edge, recalling the notch in large subunits from various cytoribosomes reported by several authors (Bruskov and Kiselev, 1968; Nonomura et al., 1971; Lutsch et al., 1972). We have not been able to detect two classes of profiles according to size, form, or other criteria.

Electrophoretic Analysis of Ribosomal RNA

CYTORIBOSOMAL RNA: Electrophoretic analysis of phenol-extracted cytoplasmic RNA in gels containing SDS (Loening, 1967), shows two

absorption peaks at 265 nm corresponding to heavy and light ribosomal RNA (Fig. 13). When a portion of the cytoribosome fraction from a sucrose gradient is mixed with SDS at a final concentration of 2.5% and placed immediately on a SDS polyacrylamide gel, the resulting bands (Fig. 13) coincide closely with the bands of phenol-extracted RNA, confirming the validity of the procedure.

MITORIBOSOMAL RNA: Nucleic acids were extracted from whole mitochondria using phenol and were analyzed by electrophoresis in SDS-containing gels (Fig. 14, *1*). The three peaks obtained correspond to DNA, and the two mitoribosomal RNA species. When compared to phenol-extracted cytoplasmic RNA (dashed curve), the two heavy ribosomal RNA species can be clearly distinguished, but under the present conditions, the mobilities of the two light ribosomal RNA species are nearly coincident.

SDS extraction of RNA from purified mitoribosomes produces two main bands corresponding exactly to those produced by phenol extraction (Fig. 14, *4*). Similarly, when RNA is extracted from a fraction of mitoribosomal subunits using SDS, the peaks of absorption again correspond
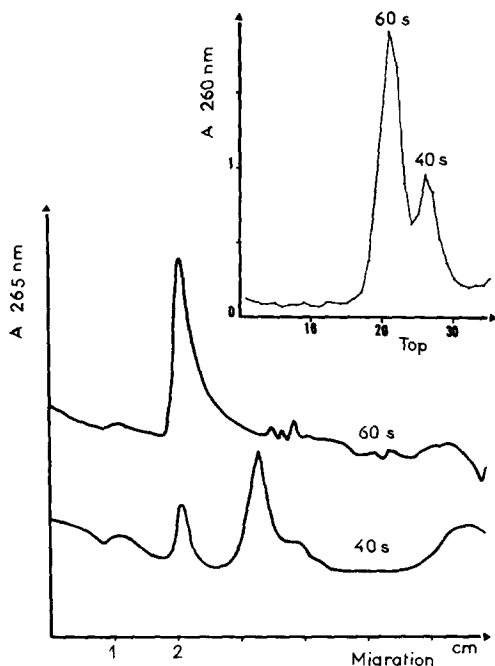
FIGURE 8   Electrophoresis of cytoribosomal subunit fractions from a sucrose gradient containing 0.5 mM Mg^{2+}. The 2.6% acrylamide gel contained 40 mM Tris, pH 7.8, 0.1 mM EDTA, 20 mM Na acetate, and lacked Mg acetate. Migration was for 2 h at 4°C. Tracings *60S* and *40S* correspond to the appropriate fractions from the sucrose gradient (*inset*). Inset: Sedimentation profile of cytoribosomal subunits. Dissociating conditions according to Materials and Methods. Sedimentation was in a linear sucrose gradient (10 ml, 0.3–1.4 M) placed over a 2-ml cushion of 1.4 M sucrose. Centrifugation was carried out for 3 h at 35,000 rpm and 4°C.

(Fig. 14, *2*). Finally, the same pattern is produced when the mitoribosomal fraction of a sucrose gradient is treated with 2 mM EDTA and mixed with SDS before gel electrophoresis (Fig. 14, *3*). Thus we have obtained qualitatively identical mitoribosomal RNA species from all extraction procedures, and we can be assured that our single subunit fraction contains both mitoribosomal RNA species.

DISCUSSION

Electrophoretic Analysis of Ribosomes

A number of previous studies, beginning with that of Hjertén et al. (1965), have explored the property of ribosomes to become negatively charged in appropriate pH medium and to migrate

in polyacrylamide gels. It has been shown that *E. coli* ribosomes and subunits could be distinguished without ambiguity and that electrophoresis is important as a technique complementary to that of sucrose gradient analysis. To date, however, analysis of ribosomes by electrophoresis has been applied only to *E. coli* (Hjertén et al., 1965; Dahlberg et al., 1969; Mikhailova and Bogdanov, 1970; Talens et al., 1970), the blue-green alga, *Anabaena flos-aquae* (Carlton and Herson, 1972), and recently to pea seeds (Thomas, 1973).

Several results in the present work illustrate the precision of the technique. Electrophoretic analysis of the heavy shoulders in a cytoribosomal gradient gave several clear and separate bands corresponding to monomers and polysomes; similar electrophoretic bands were produced from a cytoribosomal pellet (Fig. 1). Electrophoretic analysis of the 40S cytoribosomal subunit peak from a sucrose gradient indicates a mixture of both large and small subunits in this fraction (Fig. 8). Finally, separation of cyto- from mitoribosomes in mixtures sedimenting in a single peak has been achieved by electrophoresis.

Characterization of *T. pyriformis* Mitoribosomes

MONOMERS: Although both mito- and cytoribosomes of *T. pyriformis* sediment at 80S, mitoribosomes migrate only a short distance in polyacrylamide gels whereas the band of cytoribosomes migrates definitely further. This slower mobility contrasts with the identical behavior of the two ribosomes in sucrose gradients. From analysis of RNA present in our mitoribosome fractions and from electron microscope observations of the same fractions, we can safely assume that the electrophoretic band obtained actually represents mitoribosome monomers. According to studies by Tiollais et al. (1972) which indicate that size and molecular configuration are the preponderant factors responsible for the migration of particles in polyacrylamide gels, we could predict that mitoribosome particles are more voluminous than cytoribosomes. Indeed, our electron microscope images demonstrate an important difference in dimensions between mito- and cytoribosomes: the long axis of the mitoribosome is approximately 1.4 times greater and the axial ratio is 1.5 for mito- and 1.2 for cytoribosomes.

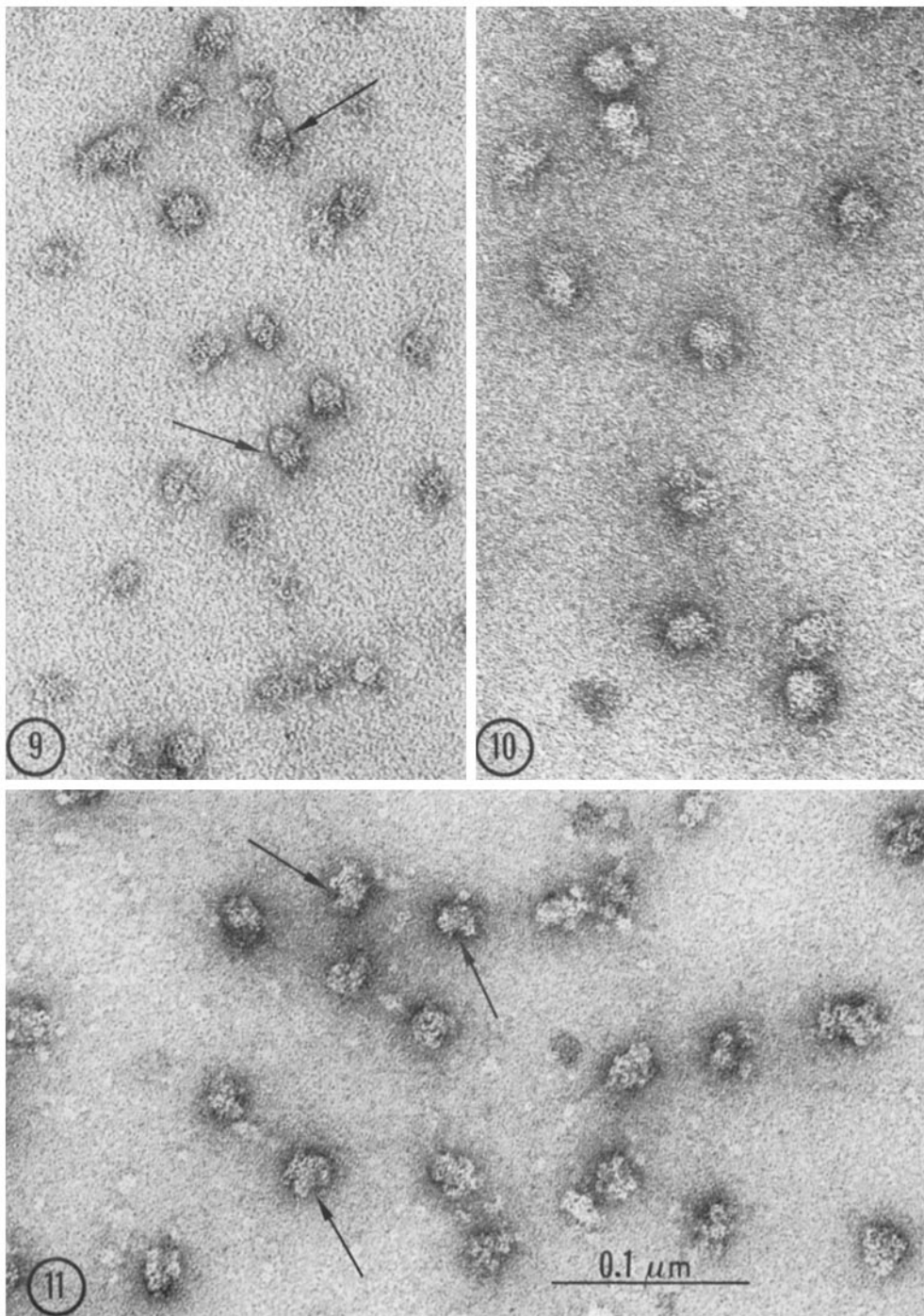Whether the greater volume of mitoribosomes exists in vivo or results from the extraction pro-

FIGURE 9   Small (40S) cytoribosomal subunits. Profiles are small and slightly elongate. Arrows indicate particles appearing bipartite. × 280,000.

FIGURE 10   Large (60S) cytoribosomal subunits. Rounded profiles are predominant. × 280,000.

FIGURE 11   Representative field of negatively stained mitoribosomal subunits from the 55S fraction in Fig. 12, *inset*. The profiles have irregular contours and cannot be classified into two types. No characteristic views of monomeric ribosomes can be found. A dense region, resembling the notch observed in the flattened side of some large ribosomal subunits, is indicated (arrows). × 280,000.
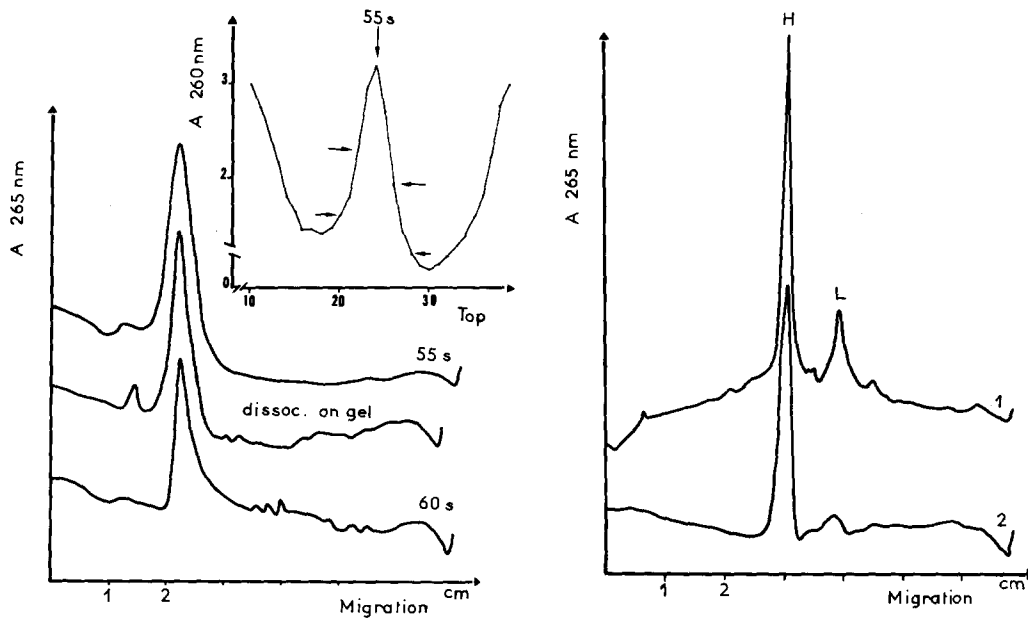
FIGURE 12 Polyacrylamide gel electrophoresis of dissociated mitoribosomes. Standard electrophoretic conditions were as in Fig. 8. Fractions from various regions (arrows) of the 55S peak in the sucrose gradient (*inset*) all gave profiles equivalent to the *55S* tracing. The tracing *dissoc. on gel* is derived from mitoribosomal monomers treated with 2 mM EDTA and then placed on the gel. *60S* corresponds to large cytoribosomal subunits. *Inset*: Sedimentation pattern of mitoribosomes in a sucrose gradient lacking Mg^{2+} but containing 1 mM EDTA (see Materials and Methods). Conditions of centrifugation as in Materials and Methods. (From Stevens et al. *In* The Biogenesis of Mitochondria. A. M. Kroon and C. Saccone, editors. Academic Press, Inc., New York. In press.)

cedures cannot be answered with certainty. Electron microscope observations of thin sections of *Tetrahymena* have indicated that mitoribosomes *in situ* are smaller than cytoribosomes (Swift, 1965); other studies, however, show a similarity in their dimensions (Charret and Charlier, 1973). The more irregular profile and larger dimensions of nega-

FIGURES 13 and 14 Polyacrylamide gel electrophoresis of ribosomal RNA. The 2.4% acrylamide gels contained 40 mM Tris, pH 7.8, 0.1 mM EDTA, 20 mM Na acetate, 2 mM Mg acetate, and 1 g/liter SDS. Migration was for 3 h at room temperature. Letters refer to ribosomal RNA: *H*, heavy; *L*, light; *c*, cytoplasmic; and *m*, mitochondrial.

FIGURE 13 Cytoribosomal RNA. Tracing *1*, total phenol-extracted RNA. See Materials and Methods. Tracing *2*, RNA extracted by 2.5% SDS treatment before deposition on gel.
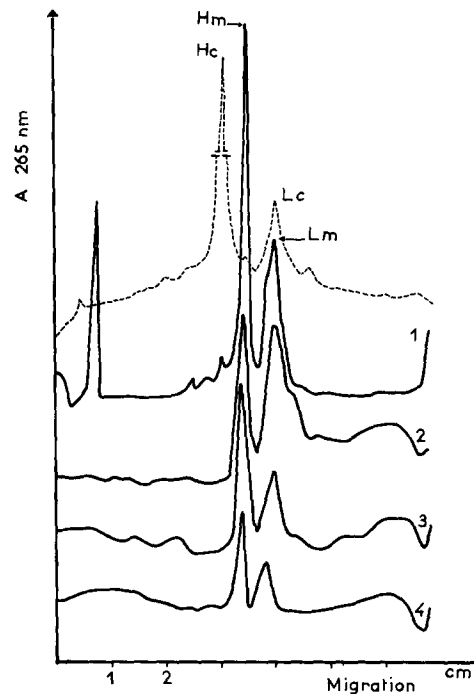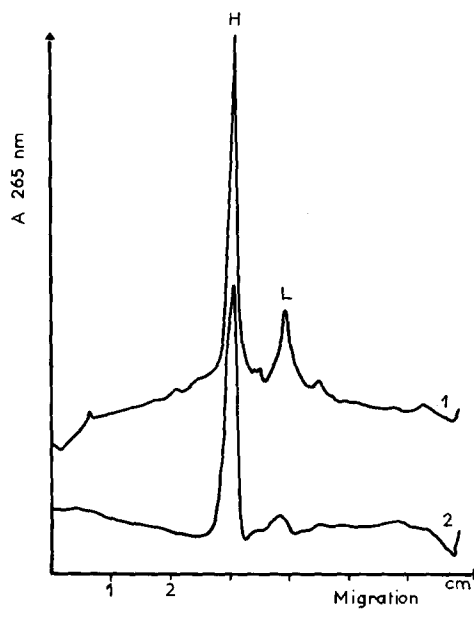
FIGURE 14 Mitoribosomal RNA. Tracing *1*, phenol-extracted RNA from whole mitochondria; *2*, RNA extracted by 2.5% SDS treatment of the 55S fraction of a sucrose gradient as in Fig. 12, inset; *3*, RNA extracted by SDS treatment of mitoribosomes from a sucrose gradient as in Fig. 3, dissociated in the presence of 2 mM EDTA; *4*, RNA extracted by SDS treatment of mitoribosome monomers from the previous gradient; dashed line, cytoribosomal RNA profile *1* from Fig. 13.

tively stained mitoribosomes suggest that the isolated particle has undergone a loosening of its nucleoprotein framework. On the assumption that the configurational changes occurring during the drying of negatively stained preparations are similar for both ribosome types, we conclude that the disparities in dimensions and volume reflect true differences in the molecular structure of the ribosomes.

Negatively stained *T. pyriformis* mitoribosomes demonstrate some unique structural features so far not observed in other ribosomes. The dark spot common to all ribosomes when observed from a given orientation does not appear to delimit a large and a small subunit. Instead, the spot is approximately at the center, between two equally sized subunits. Several characteristics of the profiles are distinct; the only resemblance with other ribosomes is the notch at one end which recalls the partition in eukaryotic small subunits (Nonomura et al., 1971; Lutsch et al., 1972). The lobe at the other end has no counterpart in other ribosomes. Finally, the clearly elongated profile of this mitoribosome is unlike the more spherical or triangular profiles usually observed.

SUBUNITS: It is shown that neither sucrose gradients (Chi and Suyama, 1970) nor gel electrophoresis is able to separate two distinct mitoribosomal subunits. Changes in such variables of the gel electrophoresis technique as pH, acrylamide concentration, and ionic composition have not been successful in separating the 55S peak into two classes of particles. Nor have two morphologically distinct classes of particles been observed in subunit fractions with the electron microscope. The only indication of heterogeneity that we could obtain was a broadened electrophoretic band with high Na^+ or K^+ concentration. The size and charge of the RNA components apparently do not greatly influence the behavior of RNA- and protein-containing particles in gel electrophoresis. The only clear separation has been obtained using CsCl density gradients (Chi and Suyama, 1970). As a whole, it appears well documented that the 55S peak represents both subunits and not a "miniribosome."

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