1967

A Comparison of Ribosomal Ribonucleic Acid using Disc Electrophoresis

Carolyn Gene Mohler
College of William & Mary - Arts & Sciences

A COMPARISON OF RIBOSOMAL RIBONUCLEIC ACID USING DISC ELECTROPHORESIS

A Thesis

Presented to

The Faculty of the Department of Biology

The College of William and Mary in Virginia

In Partial Fulfillment

Of the Requirements for the Degree of

Master of Arts

By

Carolyn Gene Kohler

1968

APPROVAL SHEET

This thesis is submitted in partial fulfillment of

the requirements for the degree of

Master of Arts

Carolyn Gene Mohler
Author

Approved, May 1968

Carl W. Vermeulen, Ph. D.

Robert E. Black, Ph. D.

Martin C. Mathes, Ph. D.

ACKNOWLEDGMENTS

The author wishes to express her sincere appreciation to Dr. Carl W. Vermeulen for his advice, guidance, and criticism throughout this investigation; to Drs. Robert E. Black and Martin C. Mathes for their advice and criticism of the manuscript; to Mr. Robert Gatten for his preparation of the photographs; and to Mr. Arthur F. Conway for his valuable assistance and preparation of the diagrams.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

The electrophoretic patterns of rRNA isolated from a number of different organisms (*E. coli*, *Anacystis nidulans*, *Nostoc muscorum*, bean seedlings, rat liver, and mouse liver) were investigated. Disc electrophoresis in 10% polyacrylamide gels using Tris-boric acid buffer (pH 8.3), followed by visualization of the bands with methylene blue stain, demonstrated that the rRNA of all the organisms investigated was a heterogeneous mixture of components. The heterogeneity appeared in all rRNA preparations, regardless of the purification procedure, i. e., gel filtration or sedimentation analysis of total RNA or isolation of RNA directly from the ribosomes followed by precipitation of the rRNA with 1.5 M NaCl. Control experiments for the presence of endogenous RNase in the RNA preparations indicated that the heterogeneous components were not the result of RNase degradation of RNA. Preincubation of rRNA with DNase, pronase, and alpha amylase for 30 minutes at 37°C produced no significant changes in the electrophoretic banding pattern whereas preincubation of rRNA with RNase under identical conditions abolished all bands. The results of this investigation tend to suggest that the multiple genes for rRNA, as calculated from DNA-rRNA hybridization studies, are not the same.

A COMPARISON OF THE RIBOSOMAL RIBONUCLEIC ACID OF PROCARYOTIC AND EUCARYOTIC ORGANISMS USING DISC ELECTROPHORESIS

INTRODUCTION

A number of studies are in progress to determine the roles of ribosomes in protein synthesis (Eisenstadt and Brawerman, 1967; Ghosh and Khorana, 1967; Igarashi and Kaji, 1967). Recent evidence suggests that ribosomes initiate protein synthesis by attaching to mRNA (Eisenstadt and Brawerman, 1967; Ghosh and Khorana, 1967) and act as binding sites for the transfer RNA-amino acid complexes (Ghosh and Khorana, 1967; Igarashi and Kaji, 1967). There is very little evidence, however, for the roles, if any, of the protein and ribonucleic acid (RNA) components of ribosomes in protein synthesis. More must be known about their structure before the function of ribosomes is determined.

In electronmicrographs ribosomes appear as dense granules 100-200 Å in diameter (cf., Petermann, 1964). Ribosomes have been characterised by their sedimentation rates in the ultracentrifuge. Procaryotic organisms, such as blue-green algae and bacteria, are characterised by 70S* ribosomes (Schachman, Paradee, and Stanier, 1952; Petermann, 1964; Taylor and Storck, 1964) whereas 80S ribosomes are characteristic of the eucaryotic plant and animal cells (Petermann, 1964; Taylor and Storck, 1964). Ribosomes found in chloroplasts and mitochondria of eucaryotic cells appear to be similar to those found in procaryotic organisms (Lyttleton, 1962; Pollard, Stemler, and Klaydes, 1966; Sager

*S(Svedberg unit) represents the sedimentation constant of a molecule, i.e., a measure of the rate of sedimentation in the ultracentrifuge, and is generally assumed to be proportional to the square root of molecular weight.

and Hamilton, 1967; Svetailo, Philippovich, and Sissakian, 1967). Electrophoresis of purified ribosomes shows a single band (Petermann and Pavlovec, 1963). The ribosomes are composed of proteins (40-60%), ribonucleic acid (60-40%), magnesium ions, polyamines, and possibly a small amount of lipids (McQuillen, 1962; Petermann, 1964).

Ribosomal proteins are primarily structural, but there are a few enzymic proteins usually associated with the ribosomes (ribonuclease, latent deoxyribonuclease, non-latent peptidases, and beta-galactosidase) (McQuillen, 1962; Elson, 1964). The structural proteins of ribosomes isolated from a single organism appear to be heterogeneous with respect to chemistry, structure, and function (Waller and Harris, 1961; Traub, Nomura, and Tu, 1966; Traub, Hosokawa, Craven, and Nomura, 1967; Fogel and Sypherd, 1968). Electrophoretic analyses of proteins obtained from the 80S ribosomes of mice have shown 24 protein components (Kedes, Koegel, and Kuff, 1966). Twenty-four to thirty protein components have been identified in the 70S ribosomes of Escherichia coli (Waller and Harris, 1961; Leboy, Cox, and Flax, 1964; Waller, 1964; Traub et al., 1967; Solls and Davis, 1968); 11 to 20 of the different bands are found in the 50S ribosomal subunit (Otaka, Itoh, and Osawa, 1968) and 15 in the 30S ribosomal subunit (Leboy et al., 1964; Fogel and Sypherd, 1968; Otaka et al., 1968). The ribosomal proteins of E. coli (Leboy et al., 1964; Traub et al., 1967; Fogel and Sypherd, 1968) and chicken liver (Lindsay, 1966) have also been separated into the acidic and basic components.

Ribosomal RNA (rRNA) has been shown, by sedimentation and chromatographic studies, to consist of two size classes with molecular weights of 0.5 and 1.0 X 10^6 daltons (cf., Petermann, 1964). The rRNA from procaryotic organisms (Schachman et al., 1952; Mura, 1962; Clark, Matthews, and Ralph, 1964; Click and Tint, 1967), chloroplasts (Boardman, Frenkl, and Wildman, 1966; Loening and Ingle, 1967; Rogers, Preston, Titchener, and Linnane, 1967; Sager and Hamilton, 1967; Stutz and Noll, 1967), and mitochondria (Duro, Epler, and Barnett, 1967), which is designated 16S and 23S by sedimentation studies, has been found to be smaller than the 18S and 28S rRNA of eucaryotic plant (Click and Tint, 1967; Sager and Hamilton, 1967; Stutz and Noll, 1967) and animal (Click and Tint, 1967) cells. Base composition studies (Click and Hackett, 1966; Pollard et al., 1966), competitive deoxyribonucleic acid (DNA)-rRNA hybridization studies (Spiegelman and Yankofsky, 1965; Gibson, 1967), fingerprinting patterns (Aronson and Holowczyk, 1965), and 5' terminal nucleotide analysis (Sugiura and Takanami, 1967) of rRNA from different cellular organelles and organisms have shown that the base composition and base sequences are species-specific.

The technique of DNA-rRNA hybridization has been utilized to determine the DNA sequence homologous to rRNA in the genome of both procaryotic and eucaryotic organisms. Such studies on E. coli (Yankofsky and Spiegelman, 1962a, 1962b; Attardi, Huang, and Kabat, 1965), Bacillus subtilis (Yankofsky and Spiegelman, 1963; Oishi and Sueoka, 1965), Pisum sativum (Chipchase and Birnstiel, 1963) HeLa cells (McConkey and Hopkins, 1964), Drosophila melanogaster (Ritossa and Spiegelman,

1965; Vermeulen and Atwood, 1965), and Xenopus laevis (Wallace and Birnstiel, 1966) have demonstrated that approximately 0.3% (range 0.1% to 0.5%) of the cell's DNA is complementary to the 16S and 23S or 18S and 28S rRNA components. One cistron for 23S rRNA in the E. coli genome would represent 0.02% of the total DNA (Spiegelman and Yankofsky, 1965); therefore the observed percentage (0.3%) of DNA complementary to rRNA ("r-DNA") implies a multiplicity of cistrons for the rRNA of an organism. The DNA-rRNA hybridization studies suggest that there may be 10 complementary cistrons for 23S rRNA in E. coli (Yankofsky and Spiegelman, 1962a, 1962b) and 45 and 35 cistrons for B. subtilis 16S and 23S rRNA respectively (Yankofsky and Spiegelman, 1963). Recent data shows that there are two distinct regions in the E. coli genome which contain cistrons for 16S and 23S rRNA (Vermeulen, 1966; Cutler and Evans, 1967). In B. subtilis nine to ten repetitions have been located in tandem fashion in the DNA sequence for 16S and 23S rRNA (Smith, Dubnau, Morell, and Marmur, 1968). Because of the larger amount of DNA per nucleus in eucaryotic organisms than in procaryotic organisms the 0.3% hybridization implies several hundred cistrons of "r-DNA" (Chipchase and Birnstiel, 1963; McConkey and Hopkins, 1964; Ritossa and Spiegelman, 1965; Vermeulen and Atwood, 1965). Clusters of rRNA genes have been found on at least five of the 22 different HeLa cell chromosomes (Huberman and Attardi, 1967). This raises the question of whether the base sequence of all these cistrons is the same.

Electrophoretic studies have provided inconclusive evidence for the multiplicity of rRNA types suggested by the previous hybridization studies. Microsomal RNA from rabbit liver and lymph nodes (Bachvaroff

and McMaster, 1964) has been resolved into five components and total cytoplasmic RNA from rat liver (Tsanev, 1965) has been resolved into nine components using agar gel electrophoresis. Recently rat liver total RNA and E. coli total RNA have been separated into 15 to 20 components using electrophoresis in polyacrylamide or agarose gels (McIndoe and Munro, 1967; Peacock and Dingman, 1967, 1968; Dingman and Peacock, 1968). Seven bands have been demonstrated in chloroplast RNA from French bean seedlings (Loening and Ingle, 1967). Bromegrass mosaic virus RNA has been resolved into three components (Bishop, Claybrook, and Spiegelman, 1967). Starch gel electrophoresis of total RNA extracted from pigeon liver and pancreas (Beney and Székeley, 1966) and polyacrylamide gel electrophoresis of total RNA from pea root tips, rabbit reticulocytes (Loening, 1967), E. coli (Bishop et al., 1967; Loening, 1967), rat liver (King and Fitschen, 1968), Oscillatoria (Loening and Ingle, 1967), and Drosophila sp. (Grossbach and Weinstein, 1968), however, failed to demonstrate heterogeneity. Only two rRNA components were resolved.

It is difficult to compare and evaluate the results of the above investigations for a number of reasons. A number of different soluble RNAs (sRNA) have been separated by electrophoresis (Richards and Gratzer, 1964; Richards and Coll, 1965); therefore it is probable that a large number of the components detected after electrophoresis of total RNA represent sRNAs rather than rRNAs. Many of the preparation procedures have not included RNase inhibitors. In these cases the action of RNase on the RNA preparations may have contributed to the observed heterogeneity. The absence of a number of bands in some reported investigations may be the result of the use of small amounts of RNA for electrophoretic

analysis (10-100 µg RNA per sample). Investigations of the heterogeneity of rRNA should: 1. include rigorous controls for RNase; 2. select primarily rRNA for the electrophoretic analysis; 3. employ samples large enough to detect minor components if present; and 4. employ standardized conditions for RNA extraction and electrophoresis. Attempts were made to meet these conditions in this investigation of the electrophoretic heterogeneity of rRNA isolated from a number of different organisms (*E. coli*, *Anacystis nidulans*, *Nostoc muscorum*, bean seedlings, rat and mouse liver).

MATERIALS AND METHODS

Escherichia coli A19 (Hfr H, Met$^-$, RNase$^-$)* was cultured in nutrient broth medium (nutrient broth, 10 gm/L; dextrose, 7 gm/L; NaCl, 5 gm/L) at 37°C. The blue-green algae, Anacystis nidulans** and Nostoc muscorum** were cultured in Bristol's medium (Deason and Bold, 1960) under forced aeration. The general conditions for algae culture followed those given by Starr (1960). Bacteria and blue-green algae in exponential growth were harvested by centrifugation and stored in the freezer prior to use. Bean seeds (Burpee's Stringless Greenpod Bush Beans, Southern States Cooperative) were germinated on moist filter paper in three inch petri dishes at room temperature in the dark. Following emergence of the radicle the seedlings were removed to large finger bowls and allowed to grow for approximately one week. The seedlings received 18 hours of light per day and daily waterings with Vigoro plant food. The seedlings were stored in the freezer prior to use. Liver tissue, dissected from adult rats or mice which were killed by over anaesthetizing, was immediately placed in an ice bath and stored at -18°C prior to use. Cell-free preparations were obtained from each organism or tissue and the RNA was extracted either from the cell-free preparations or from ribosomes isolated from these preparations.

*Isolated by Vargo and Watson
**From Indiana University Algae Culture Collection

To prepare homogenates of blue-green algae the cells were suspended in 0.5 M mannitol-0.03 M potassium phosphate, pH 6.8, at approximately four ml per gram of algae; a small amount of bentonite was also added to the suspension. Lysozyme (Sigma Chemical Company) was added to give a final concentration of 0.05% (w/v) and the suspension was incubated at 36 to 38°C for 10 to 12 hours (Biggins, 1967a, 1967b). At the end of this period the suspension was centrifuged in an International Portable Refrigerated Centrifuge, model PR2, at 4°C at 1256 xg for 10 minutes to remove debris and whole cells; the supernatant fluid was collected. The cells were resuspended in twice the original volume of buffer II (see Appendix I) to lyse any remaining protoplasts and recentrifuged as above. [The use of this procedure to lyse blue-green algae has met with limited success depending on the species of algae used.] The supernatant fluids from both centrifugations were pooled and made 0.5% with respect to sodium deoxycholate (DOC) by adding a 20% solution. The mixture was centrifuged in the Beckman Model L preparative ultracentrifuge in the type 30 rotor at 18,000 rpm (28,000 xg average) for 30 minutes to remove insoluble materials. The supernatant fluid was layered over two ml of 1 M sucrose in buffer II and centrifuged in the type 65 rotor at 40,000 rpm (105,000 xg average) for three hours to sediment ribosomes. The supernatant fluid was carefully decanted and the gelatinous ribosome pellets and sides of the tubes carefully rinsed with cold distilled water. The ribosomes were resuspended in buffer III (see Appendix I) by gentle stirring with a wooden applicator stick. The ribosome preparations were either used immediately for RNA extraction or stored overnight at -18°C (Stutz and Noll, 1967).

The bean seedlings were suspended in three ml of buffer I for each gram of material and homogenized in a Waring blendor for five minutes. The homogenate was filtered through a 0.105 mm standard wire sieve and fractionated according to the procedure of Stutz and Noll (1967) as outlined in Appendix I. Both chloroplast ribosomes and cytoplasmic ribosomes were isolated. Rat or mouse liver, suspended in three ml buffer I for each gram of tissue, was homogenized in a Sorval Omni-Mixer homogenizer for three minutes. The cytoplasmic ribosomes were isolated by the Stutz and Noll (1967) procedure. The ribosome preparations were either used immediately for RNA extraction or stored in buffer III overnight.

RNA was isolated either from whole bacterial or algal cells, cell homogenates of bean seedlings or liver tissue, or ribosomes isolated from these organisms according to a modification of the procedure of Peacock and Dingman (1967). All steps were carried out at 4°C. The suspension of cells in 0.1 M sodium acetate-0.1 M NaCl-0.01 M EDTA, pH 5.0 (acetate buffer) and cell homogenates in buffer I were made up to a final concentration of 5% with respect to sodium dodecyl sulfate (SDS) whereas the ribosome suspensions in buffer III were made up to 0.5% with respect to SDS. An equal volume of 0.2 M sodium acetate-0.2 M NaCl-0.02 M EDTA, pH 5.0, was also added to the cell homogenate or isolated ribosome preparations. Bentonite powder (approximately 1 mg/10 ml of solution) was added to adsorb RNase. The mixture was stirred for five minutes, after which one-half volume of acetate buffered phenol was added. The mixture was stirred for 30 minutes followed by centrifugation at 1256 xg for 15 minutes to break the emulsion. The aqueous layer was

removed, mixed with one-half volume of acetate buffered phenol, stirred for 15 minutes, and centrifuged as above. The aqueous layer was placed in a beaker and two to two and one-half volumes of ice cold 95% ethanol were added to precipitate the RNA. The RNA was precipitated overnight at -18°C, collected by centrifugation, and washed twice with 95% ethanol.

For chromatography RNA was dissolved in a small volume (1 - 4% of column volume) of 0.3 M NaCl-0.03 M Tris(hydroxymethyl)amino methane buffer, pH 7.3, and applied to a column (40 X 1.5 cm) of Biogel P-100, exclusion limit 100,000 daltons (Calbiochem). RNA was eluted from the column with the same buffer and five ml fractions were collected using a Gilson Medical Electronics linear fraction collector. The A_{260} of each fraction was measured with a Beckman DB spectrophotometer. The two size classes of rRNA are excluded from the gel and are found in the first peak. Throughout this investigation the concentration of RNA in µg/ml was determined by multiplying the A_{260} by 20. Fractions under the first peak, primarily rRNA, were pooled and reprecipitated with 95% ethanol.

Another method used to select high molecular weight rRNA was that of NaCl precipitation of rRNA. RNA samples were dissolved in 0.1 M NaCl-1 mM EDTA; the solution was then made 1.5 M with respect to NaCl and allowed to stand at 4°C for 12 hours (Dure et al., 1967). The precipitated rRNA was collected by centrifugation at 0°C.

Sedimentation analysis of RNA was performed in a linear gradient of 4 - 20% sucrose in 0.1 M NaCl-1 mM EDTA in the SW25.1 rotor of the Beckman Model L preparative ultracentrifuge (Peacock and Dingman, 1967). The gradient was made by layering six ml each of 4, 8, 12, 16, and 20% sucrose solutions in the 30 ml centrifuge tubes followed by two gentle

stirs with a flat wire coil to eliminate the interfaces. Aliquots of RNA dissolved in 0.1 M NaCl-1 mM EDTA containing two to three mg RNA were applied to the top of the gradient. Sedimentation was performed at 23,000 rpm for 18-20 hours; after which a hole was punched in the bottom of the tube and 32 to 50 drop fractions were collected. Each fraction was made up to three ml with 0.1 M NaCl-1 mM EDTA and the A_{260} was measured. The fractions with sedimentation coefficients of 16-18S and 23-28S were pooled and reprecipitated with 95% ethanol.

Disc electrophoresis of rRNA in polyacrylamide gels followed the methods of Richards and Coll (1965) and Peacock and Dingman (1967) modified for use with the Polyanalyst Disc Electrophoresis apparatus (Buehler Instruments, Inc., Fort Lee, N. J.). Instructions for preparing the polyacrylamide gels and buffer solution used are outlined in Appendix II. Samples of 200 µg of rRNA dissolved in 0.1 ml or less of a solution containing 0.05 M NaCl-1 mM EDTA, pH 6.3, and 30% (w/v) sucrose were layered on top of the spacer gel of each gel column. A small amount of bromophenol blue (BPB) or napthol blue black (NBB) in the same solution was also placed on top of each gel and mixed with the RNA with a stirring rod. Tris-boric acid buffer, pH 8.3, (Tris, 10.8 gm/L; boric acid, 5.5 gm/L; disodium EDTA, 936 mg/L) was placed in the upper and lower buffer chambers of the apparatus. Electrophoresis was conducted at a constant current of six milliamperes (mA) per gel (a total of 36 mA for six gels, 150-200 volts) until the tracking dye reached the end of the gel tubes, approximately 45 minutes with BPB or 55 to 75 minutes with NBB. At the end of the electrophoretic period the gels were removed from the tubes and placed in 1 M acetic acid for 10 to 15

minutes in order to lower the pH of the gels for staining. The gels were stained in either 0.2% (w/v) methylene blue or 0.2% (w/v) acridine orange in 0.2 M sodium acetate-0.2 M acetic acid buffer, pH 4.7, for one hour. Excess stain was removed from the gels by washing in distilled water for several hours (Peacock and Dingman, 1967). Gels stained with acridine orange were examined under ultraviolet light to locate the bands.

In order to determine whether the RNA isolated with phenol was contaminated with other macromolecules which might result in the appearance of extra bands during electrophoresis, several enzymic studies were conducted. Aliquots (not exceeding 0.1 ml volume) containing 200 µg rRNA were incubated for 30 minutes at 37°C with 0.01 ml of each of the following enzymes: bovine pancreatic ribonuclease, A grade (Calbiochem), 100 µg/ml of 0.01 M potassium phosphate, pH 7.0; bovine pancreatic deoxyribonuclease 1 (Sigma Chemical Company), 200 µg/ml of 0.02 M NaCl-0.001 M potassium phosphate-0.001 M $MgCl_2$, pH 7.0; pronase (Calbiochem), self-digested at room temperature for one hour before use, 100 µg/ml of 0.01 M potassium phosphate, pH 7.0; and bacterial alpha-amylase, B grade (Calbiochem), 100 µg/ml of 0.01 M potassium phosphate, pH 7.0. At the end of the incubation period the reaction mixtures were immediately subjected to electrophoresis as described above (Peacock and Dingman, 1967).

RESULTS

Electrophoresis of ribosomal RNA prepared by gel filtration

A typical separation of *E. coli* total RNA by gel filtration on Biogel P-100 is shown in Fig. 1. The larger molecular weight RNAs, composed of mostly rRNA, are eluted from the column in front of the sRNA. The rRNA peak (fractions 8 - 13) was pooled and precipitated with 95% ethanol. The precipitated RNA was redissolved in a solution containing 0.05 M NaCl-1 mM EDTA and 30% sucrose, and aliquots subjected to electrophoresis on 10% polyacrylamide gel at six mA/gel for 45 minutes. The electrophoretic pattern is shown in Fig. 2a; four major bands and four minor bands were resolved. The rRNA from *Anacystis nidulans*, bean chloroplasts, and mouse liver prepared in a similar manner by gel filtration on Biogel P-100 gave electrophoretic patterns shown in Fig. 2b, c, and d. In all cases a number of components are present. Five major bands and three minor bands are shown for *A. nidulans* rRNA; two major and six minor bands for bean chloroplast rRNA; and two major and nine minor bands for mouse liver rRNA. The banding pattern of the various rRNAs appears to be specific for the organism.

Electrophoresis of RNA prepared from isolated ribosomes

Ribosomes were prepared from various organisms according to the procedure of Stutz and Noll (1967). RNA was extracted from these ribosomes, dissolved in a solution containing 0.05 M NaCl-1 mM EDTA and 30% sucrose, and subjected to electrophoretic analysis on 10% polyacrylamide gels.

Fig. 1. Separation of total RNA by gel filtration on Biogel P-100. Approximately six mg of E. coli total RNA were dissolved in one ml of 0.3 M NaCl-0.03 M Tris, pH 7.3, and applied to a column (40 X 1.5 cm) of Biogel P-100. RNA was eluted from the column with the same buffer; five ml fractions were collected. The first and second peaks are ribosomal and soluble RNA respectively.

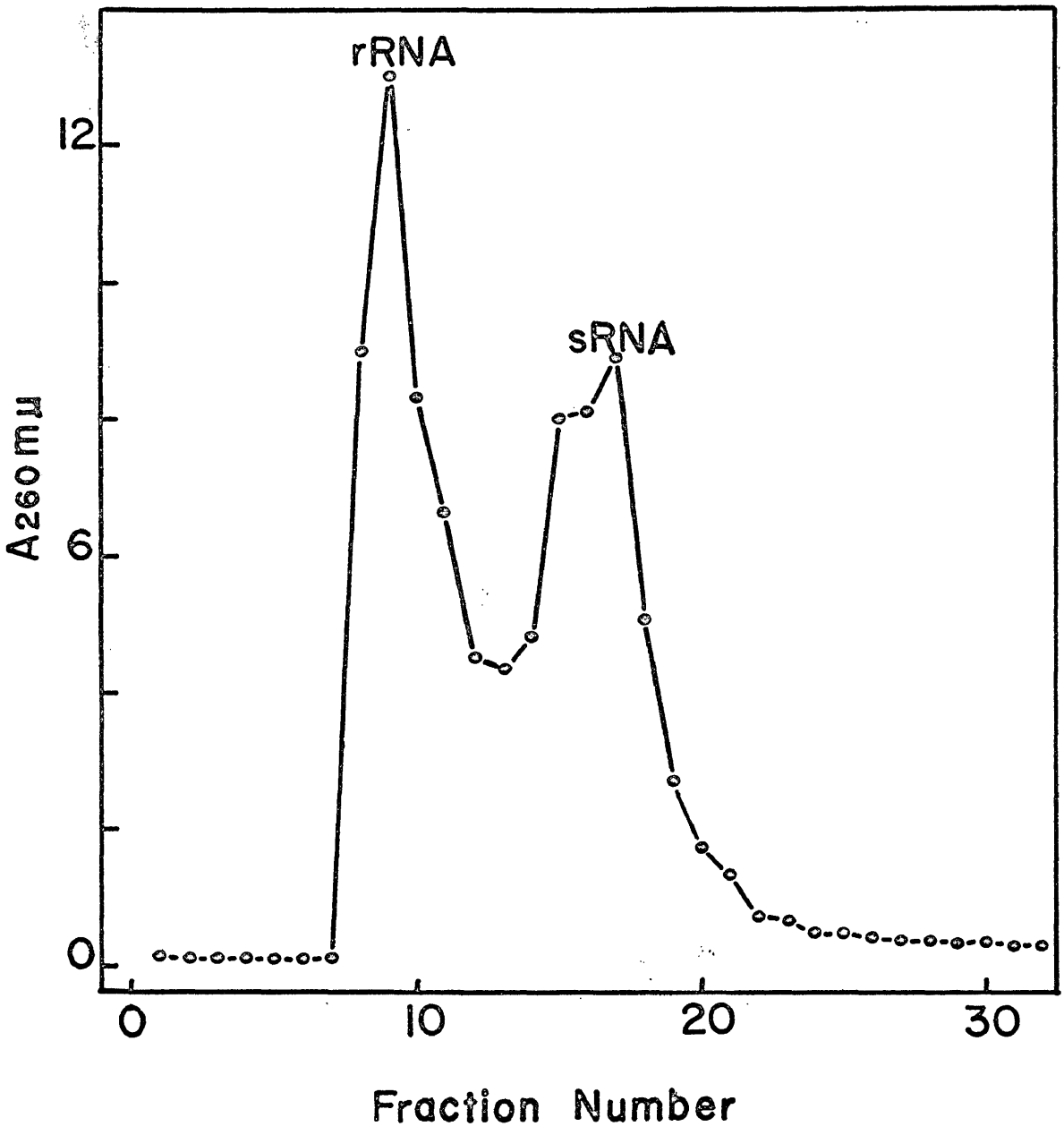
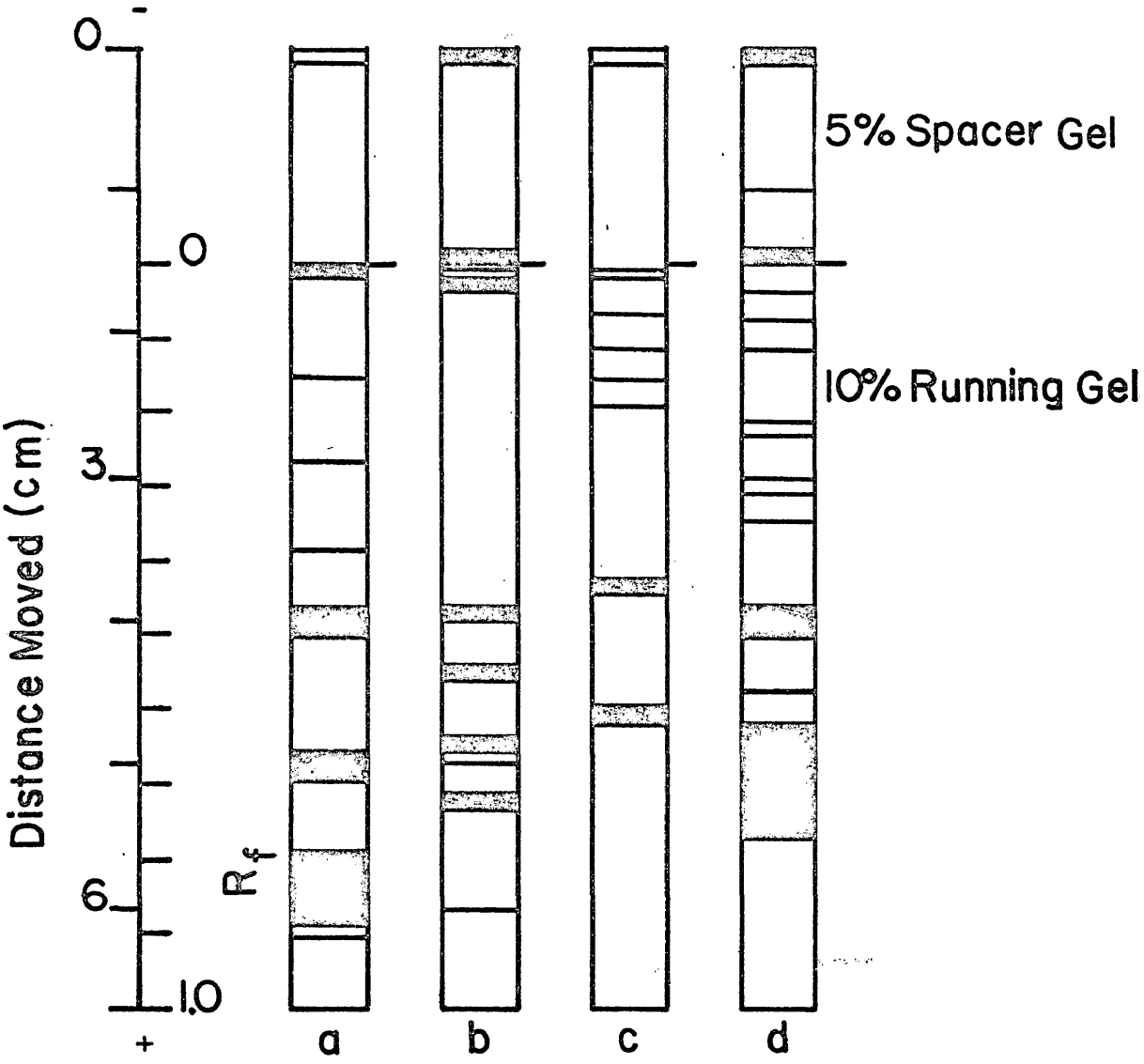

rRNA
sRNA
12
6
0
A260 mμ
0
10
20
30
Fraction Number

Fig. 2. Electrophoretic analyses of rRNAs obtained by gel filtration of total RNA on Biogel P-100. (a) *E. coli*, (b) *Anacystis nidulans*, (c) Bean chloroplasts, (d) Mouse liver. The RNA samples, 200 µg/0.01 ml, were applied at the top (0.0 cm) of each gel column. Electrophoresis was conducted at a constant current of 6 mA/gel for 45 minutes. BPB was used as the tracking dye. Gels were stained for one hour in 0.2% methylene blue or 0.2% acridine orange in 0.2 M sodium acetate-0.2 M acetic acid buffer, pH 4.7.


0
-
0
3
6
1.0
+
Distance Moved (cm)
R_f
a
b
c
d
5% Spacer Gel
10% Running Gel

In all cases studied the rRNAs appear to be heterogeneous mixtures of components. The result of the electrophoretic analysis of RNA obtained from ribosomes isolated from Nostoc muscorum is shown in Fig. 3; 20 major bands and seven minor bands are seen. The 13 components separated by electrophoresis of RNA from mouse liver ribosomes are shown in Fig. 4. RNA from bean seedling cytoplasmic ribosomes was resolved by electrophoresis into 19 major components and two minor components (Fig. 5a) whereas the RNA from bean seedling chloroplast and mitochondrial ribosomes was resolved into 18 components (Fig. 5b).

In evaluating the above results, it was necessary to determine whether the apparently large number of RNA components separated using electrophoresis could represent contamination of the RNA preparations with proteins, DNA, or polysaccharides. The possibility also existed that the numerous components resulted from the degradation of the RNA by endogenous ribonuclease. The purity of the RNA, isolated from the ribosomes with phenol, was determined by incubating aliquots containing 200 µg of Nostoc muscorum rRNA at 37°C for 30 minutes with RNase, DNase, pronase, and alpha amylase prior to electrophoretic analysis. The results shown in Fig. 6 show that the RNA appears to be relatively pure, without extensive contamination by other macromolecules. Incubation of RNA with DNase, pronase, and alpha amylase (Fig. 6c, d, and e) did not produce major changes in the banding pattern. The significance of the loss of a few bands in each case is questionable since the enzymes were not RNase free. In contrast, preincubation with RNase (Fig. 6b) completely abolished all bands because the length of the electrophoretic

Fig. 3. Electrophoretic analysis of RNA obtained from isolated ribosomes of Nostoc muscorum. RNA samples, 200 µg/0.1 ml, were applied at the top (0.0 cm) of the gel column. Electrophoresis was conducted at a constant current of 6 mA/gel for 1½ hours; NBB was used as tracking dye. Gels were stained for one hour in 0.2% methylene blue staining buffer.

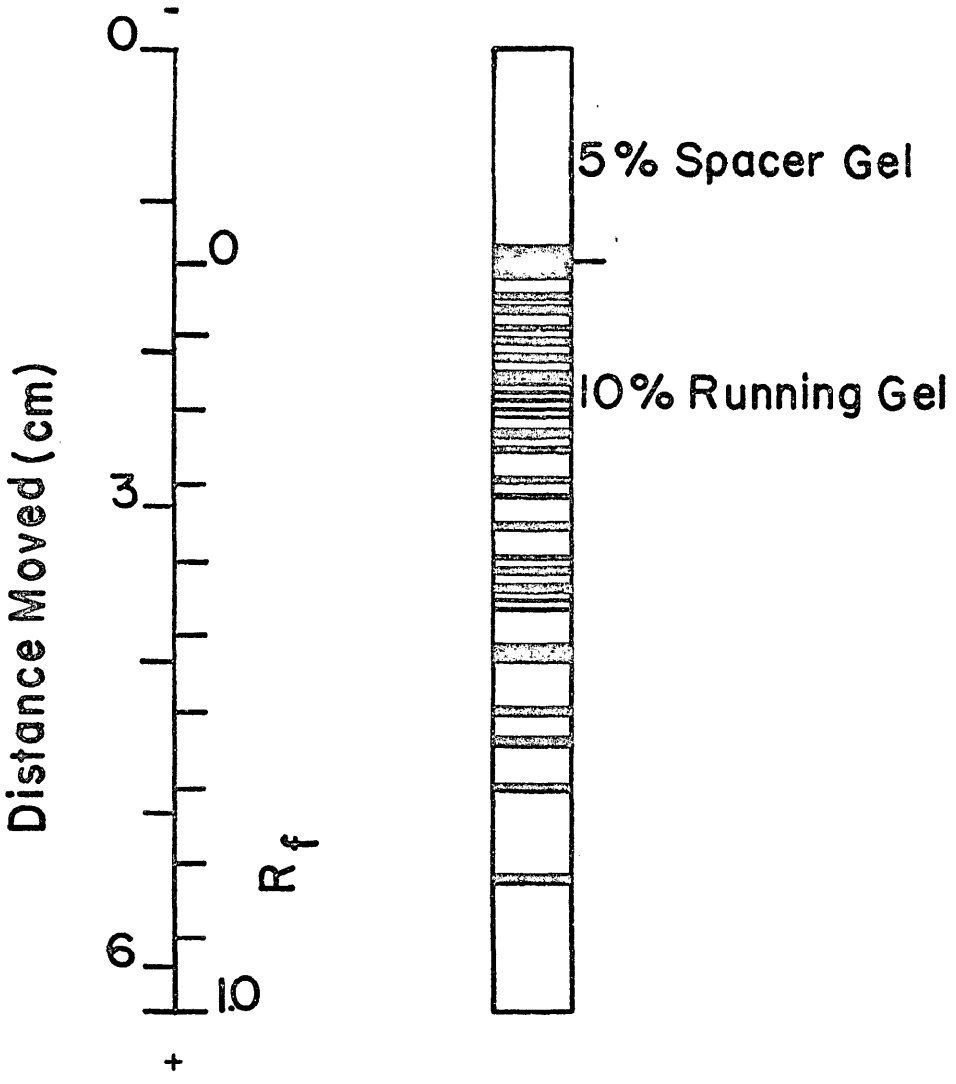

0
0
3
6
10
Distance Moved (cm)
R_f
5% Spacer Gel
10% Running Gel
-
+

Fig. 4. Electrophoretic analysis of RNA obtained from isolated ribosomes of mouse liver. RNA samples, 200 µg/0.1 ml, were applied at the top (0.0 cm) of the gel column. Electrophoresis was conducted at a constant current of 6 mA/gel for 1½ hours; NEB was used as tracking dye. Gels were stained for one hour in 0.2% methylene blue staining buffer.

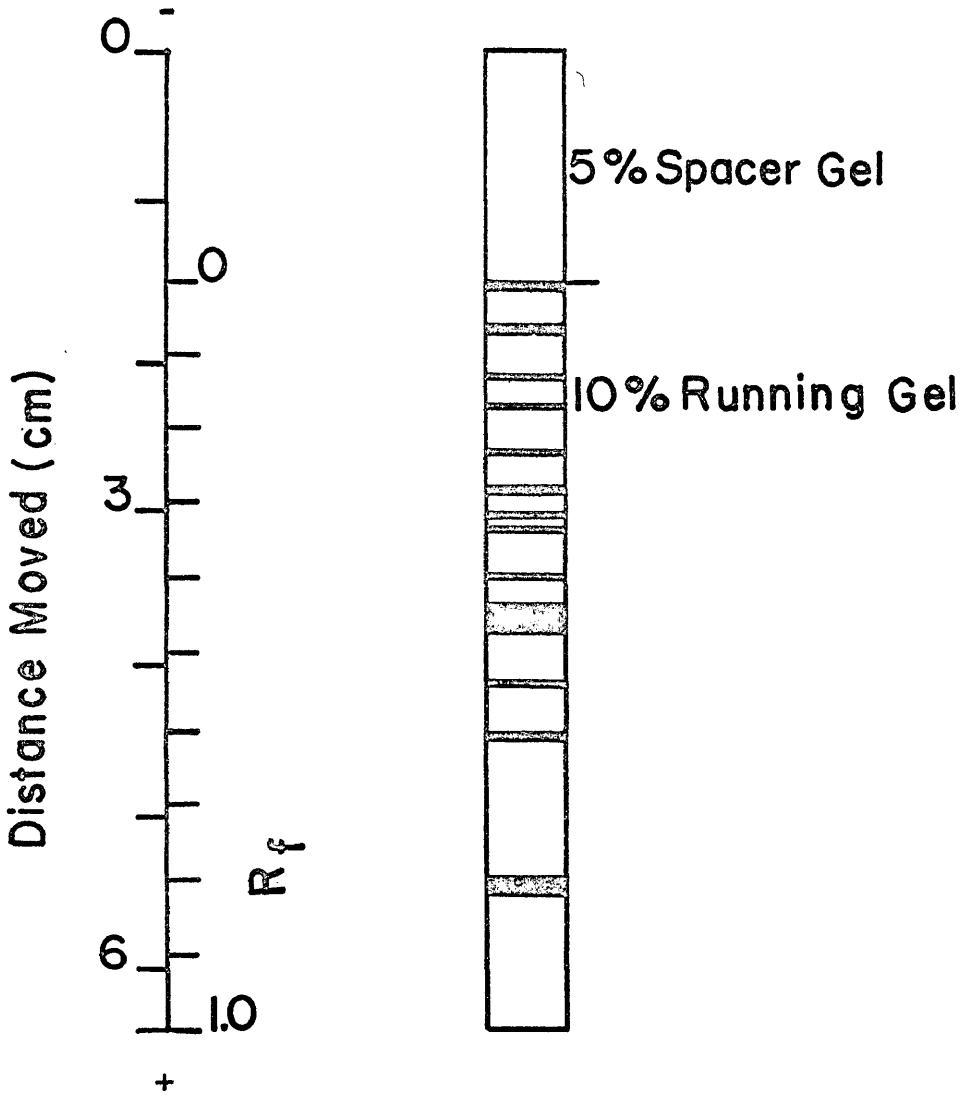

0
-
0
3
6
1.0
+
Distance Moved (cm)
R_f
5% Spacer Gel
10% Running Gel

Fig. 5. Electrophoretic analyses of bean seedling RNA obtained from isolated cytoplasmic, chloroplast and mitochondrial ribosomes. Prior to electrophoresis the rRNA was dissolved in 0.1 M NaCl-1 mM EDTA; the solution was then made 1.5 M with respect to NaCl and allowed to stand at 4°C for 12 hours. The precipitated rRNA was collected by centrifugation and dissolved in 0.05 M NaCl-1 mM EDTA-30% sucrose. (a) Cytoplasmic rRNA, 200 µg/0.1 ml; and (b) Chloroplast-mitochondrial rRNA, 100 µg/0.1 ml, were applied at top (0.0 cm) of the gel column. Electrophoresis was conducted at a constant current of 6 mA/gel for 1½ hours; BBB was used as the tracking dye. Gels were stained in 0.2% methylene blue staining buffer.

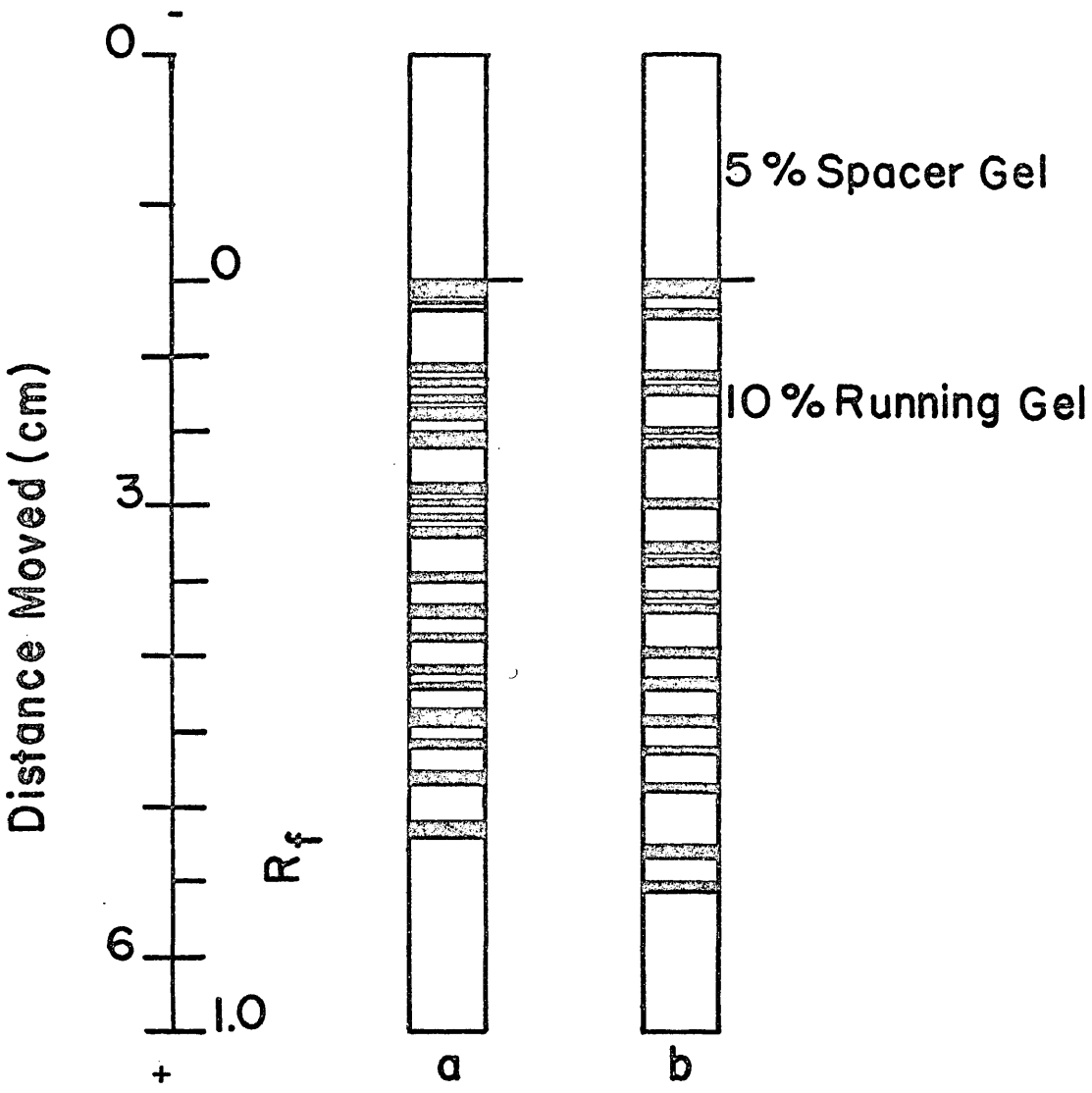
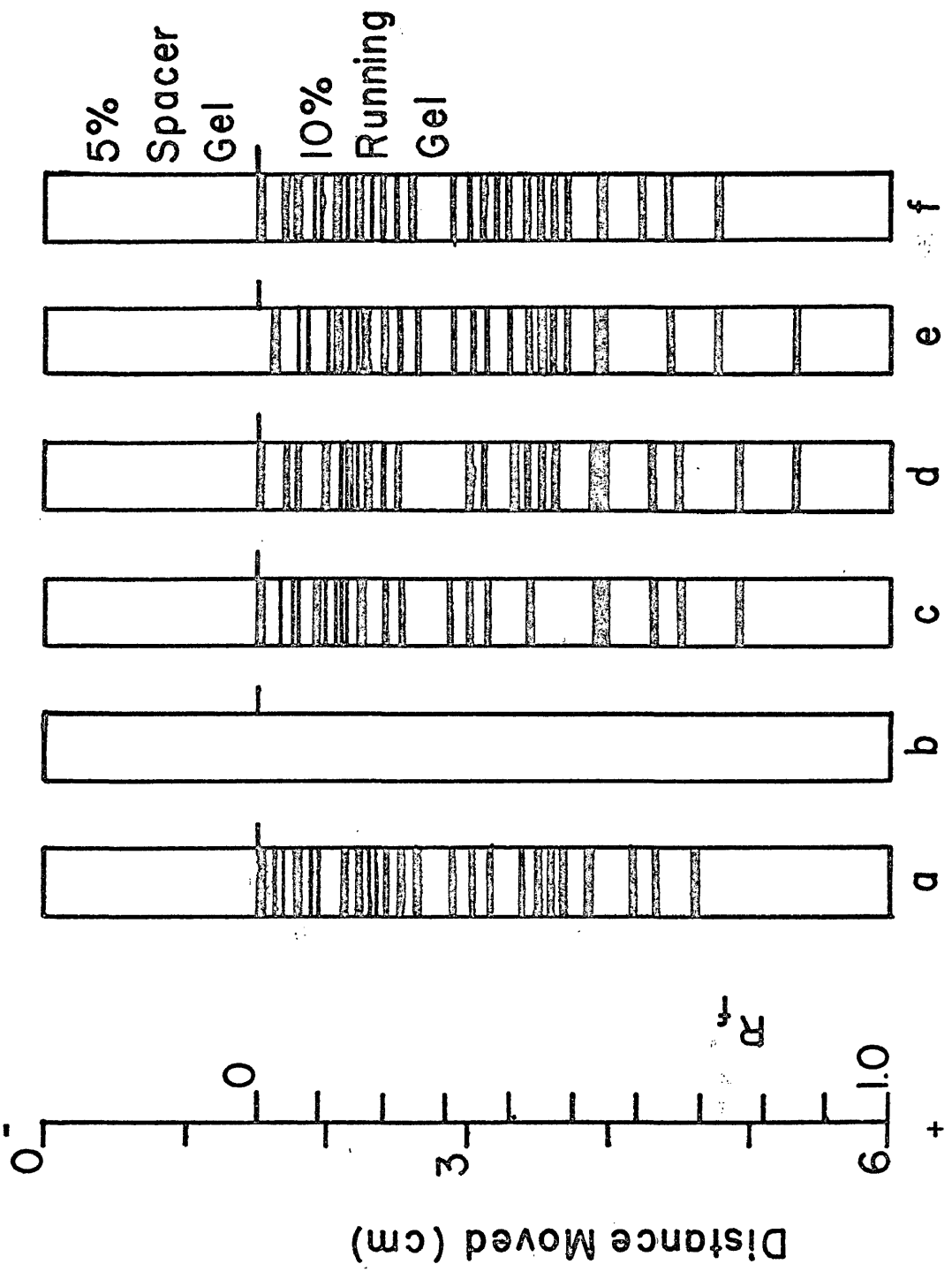

0
-
0
3
6
1.0
+
Distance Moved (cm)
R_f
a
b
5 % Spacer Gel
10 % Running Gel

Fig. 6. Electrophoretic analyses of Nostoc muscorum RNA obtained from isolated ribosomes and treated with enzymes for 30 minutes at 37°C: (a) Incubated control, no enzymes; (b) RNase; (c) DNase; (d) pronase; (e) alpha amylase; and (f) unincubated control, no enzymes. Aliquots of RNA, 200 µg/0.1 ml, were subjected to enzymic digestion prior to applying at the top (0.0 cm) of the gel columns. Electrophoresis was conducted at a constant current of 6 mA/gel for 1½ hours; MBB was used as the tracking dye. Gels were stained for one hour in 0.2% methylene blue staining buffer.


5% Spacer Gel
10% Running Gel
a
b
c
d
e
f
0
3
6
9
10
R_f
0
+
Distance Moved (cm)

period (1½ hours) was sufficient to allow all the oligonucleotides to run off the end of the gels. The RNA preparations did not appear to have endogenous RNase activity since the banding pattern of RNA incubated at 37°C for 30 minutes (Fig. 6a) before electrophoresis did not differ from that of unincubated RNA (Fig. 6f).

Electrophoresis of rRNA prepared by sedimentation

Total RNA isolated from rat liver was subjected to sedimentation in a 4 - 20% (w/v) sucrose gradient. Four components (Fig. 7) with sedimentation coefficients of 28S, 18S, 5S, and 4S were partially separated. The 28S (fractions 1-5) and 18S (fractions 18-22) peaks were collected separately and precipitated with 95% ethanol. The precipitated RNA was redissolved in a solution containing 0.05 M NaCl-1 mM EDTA and 30% sucrose and aliquots containing 200 µg RNA were analyzed electrophoretically on 10% polyacrylamide gels. The results are shown in Fig. 8a and b; 28S rRNA was resolved into 16 bands (Fig. 8a) while 18S rRNA was resolved into six components (Fig. 8b). The rRNAs from bean seedling cytoplasmic ribosomes and E. coli A19 purified by sedimentation in a 4 - 20% sucrose gradient in a similar manner gave the electrophoretic patterns shown in Figs. 9 and 10. Bean seedling cytoplasmic 27S and 18S rRNAs were resolved into five (Fig. 9a) and 11 (Fig. 9b) components respectively. E. coli A19 23S rRNA showed five bands (Fig. 10a) while the 16S rRNA showed three bands (Fig. 10b). The overall banding pattern for each size class appears to be unique although the most rapidly moving major bands may be identical.

Fig. 7. Sedimentation profile of rat liver total RNA in 4 - 20% (w/v) sucrose. Approximately two mg of rat liver total RNA dissolved in one ml 0.1 M NaCl-1 mM EDTA, pH 6.3, was applied to the top of a 30 ml gradient. Sedimentation was performed at 23,000 rpm for 20 hours in the SW25.1 rotor of the Beckman Model L ultracentrifuge. Fractions of 50 drops each were collected from the gradient and the A_{260} of each fraction measured. The four components have sedimentation coefficients of 28S, 18S, 5S, and 4S.

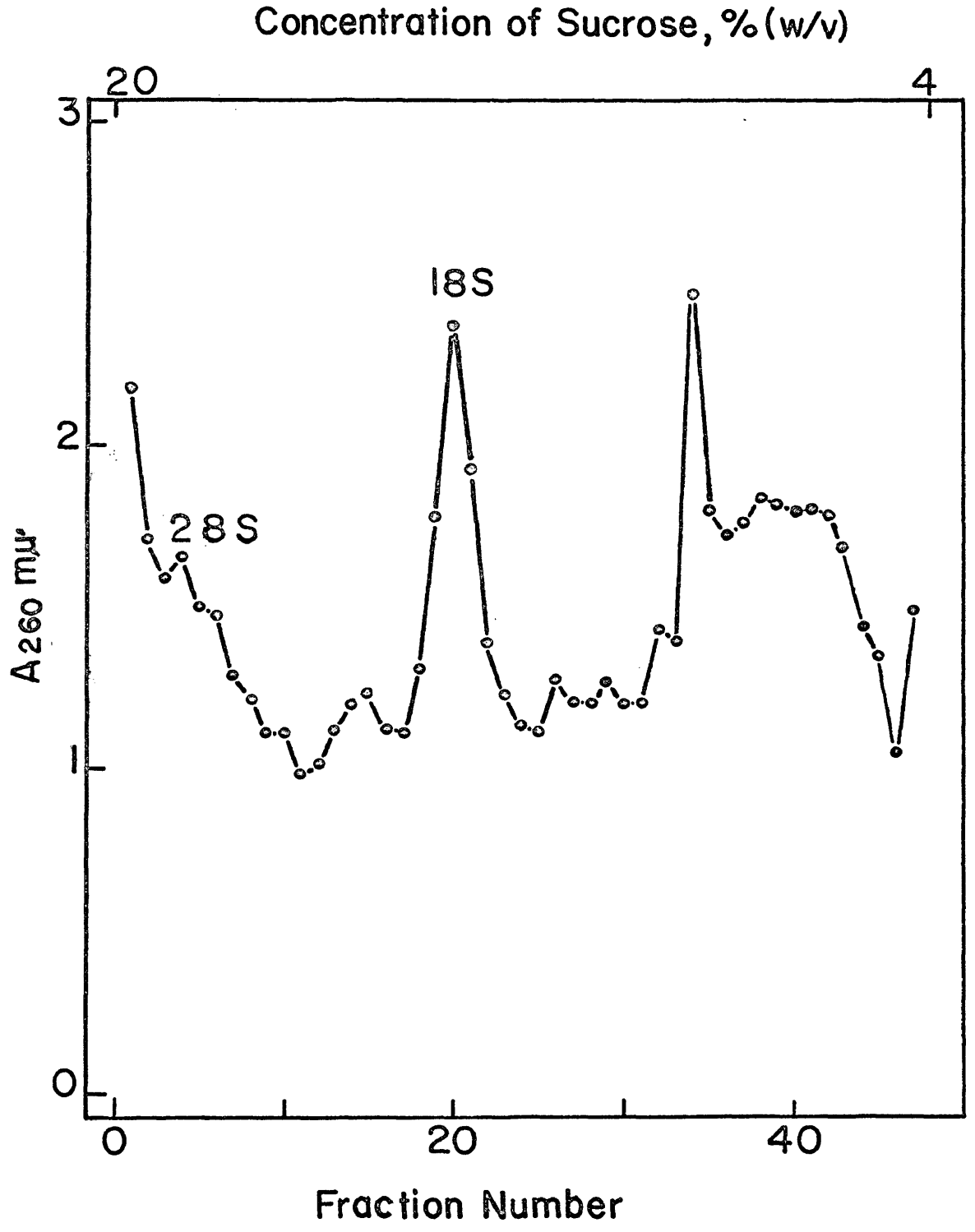

Concentration of Sucrose, % (w/v)
20
4
3
2
1
0
18S
28S
A260 mμ
0
20
40
Fraction Number

Fig. 8. Electrophoretic analysis of rat liver RNA after purification by sedimentation. (a) 28S ribosomal RNA, (b) 18S ribosomal RNA. RNA samples, 200 µg/0.01 ml, were applied at the top (0.0 cm) of each gel column. Electrophoresis was conducted at a constant current of 6 mA/gel for 45 minutes; BPB was used as the tracking dye. Gels were stained for one hour in 0.2% methylene blue staining buffer.

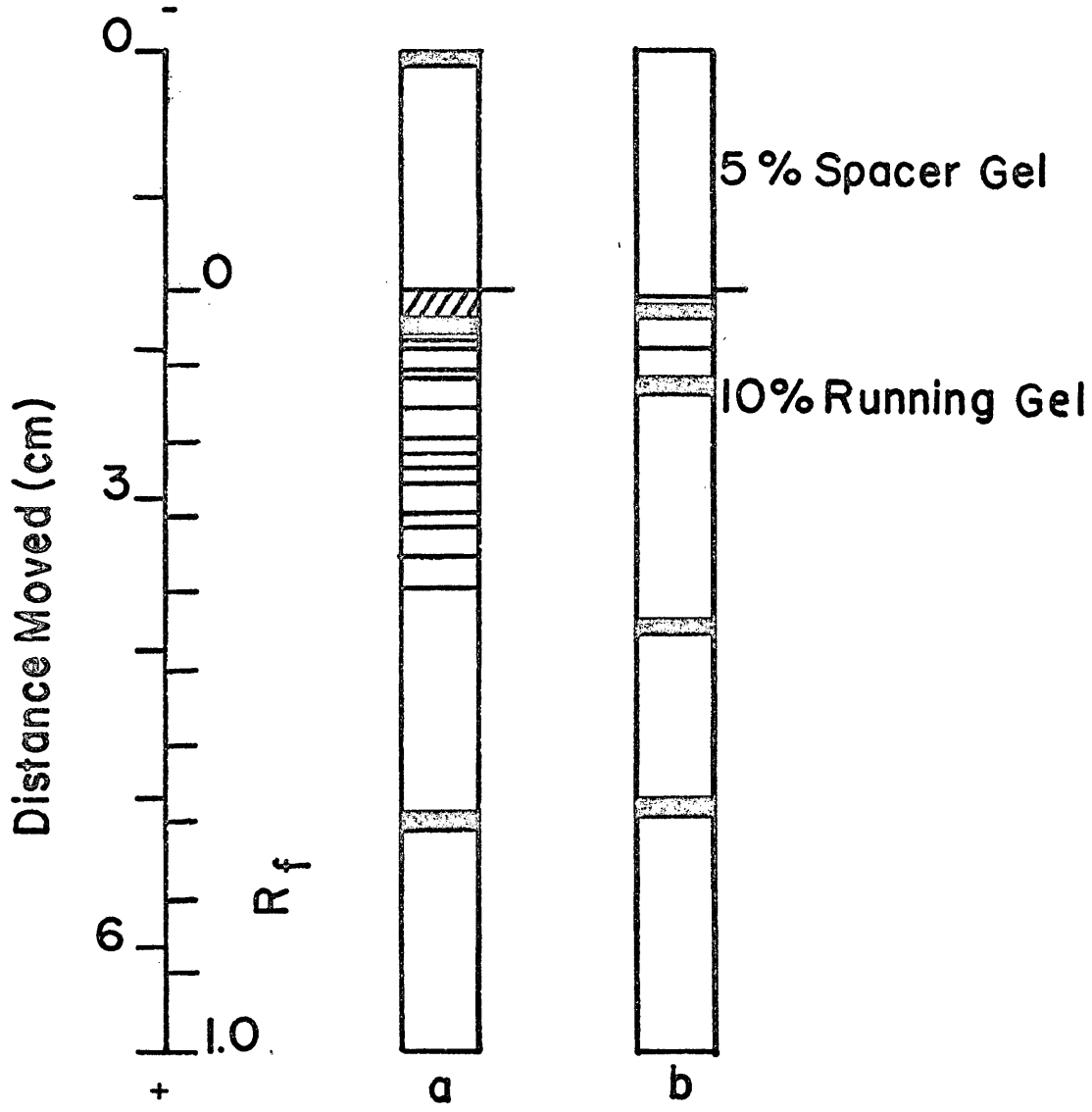

0
-
0
3
6
1.0
+
Distance Moved (cm)
R_f
a
b
5% Spacer Gel
10% Running Gel

Fig. 9. Electrophoretic analysis of bean seedling cytoplasmic rRNA after purification by sedimentation. (a) 27S ribosomal RNA, (b) 18S ribosomal RNA. RNA samples, 200 µg/0.01 ml, were applied at the top (0.0 cm) of each gel column. Electrophoresis was conducted at a constant current of 6 mA/gel for 55 minutes; NBB was used as the tracking dye. Gels were stained for one hour in 0.2% methylene blue staining buffer.

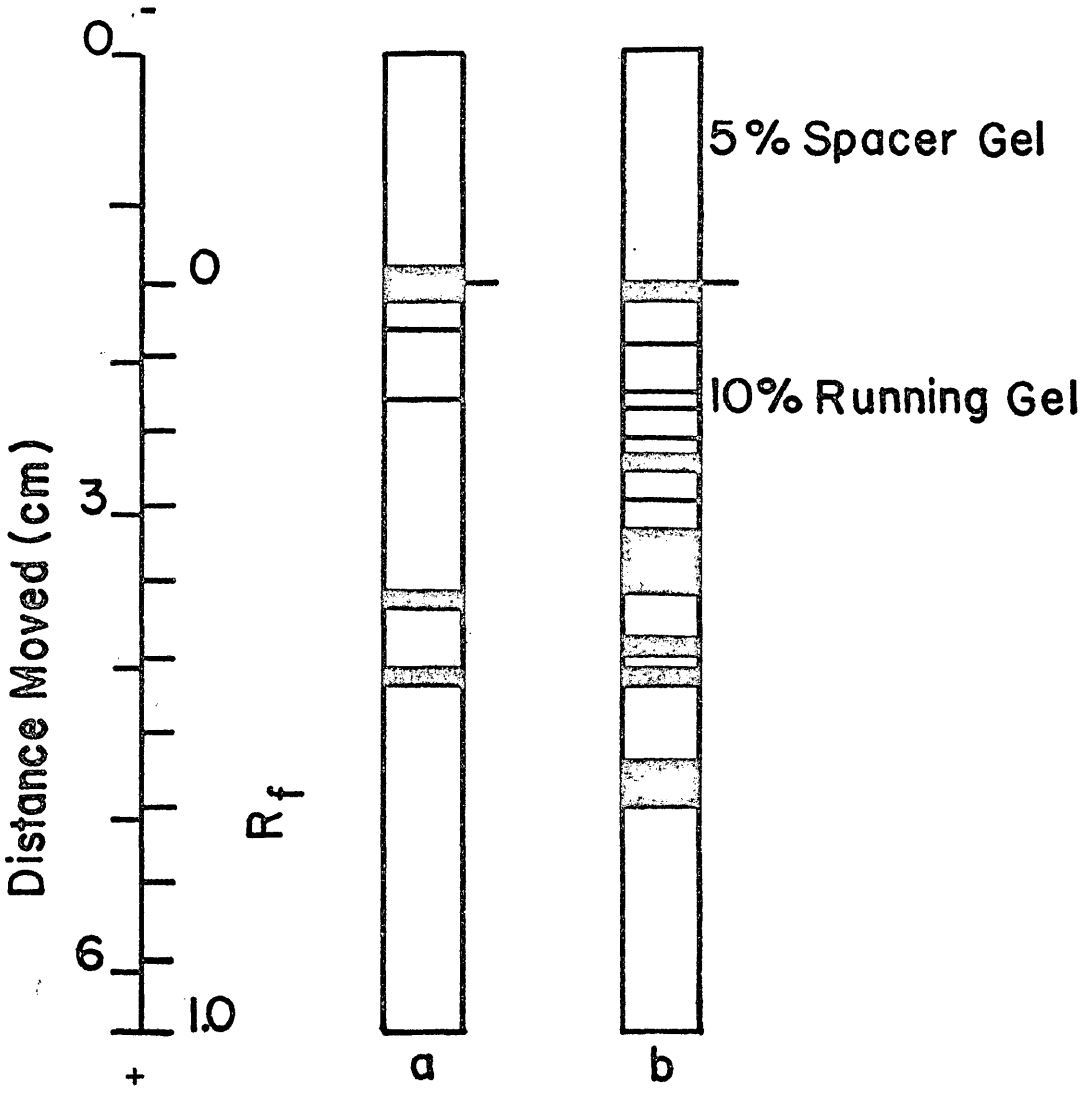
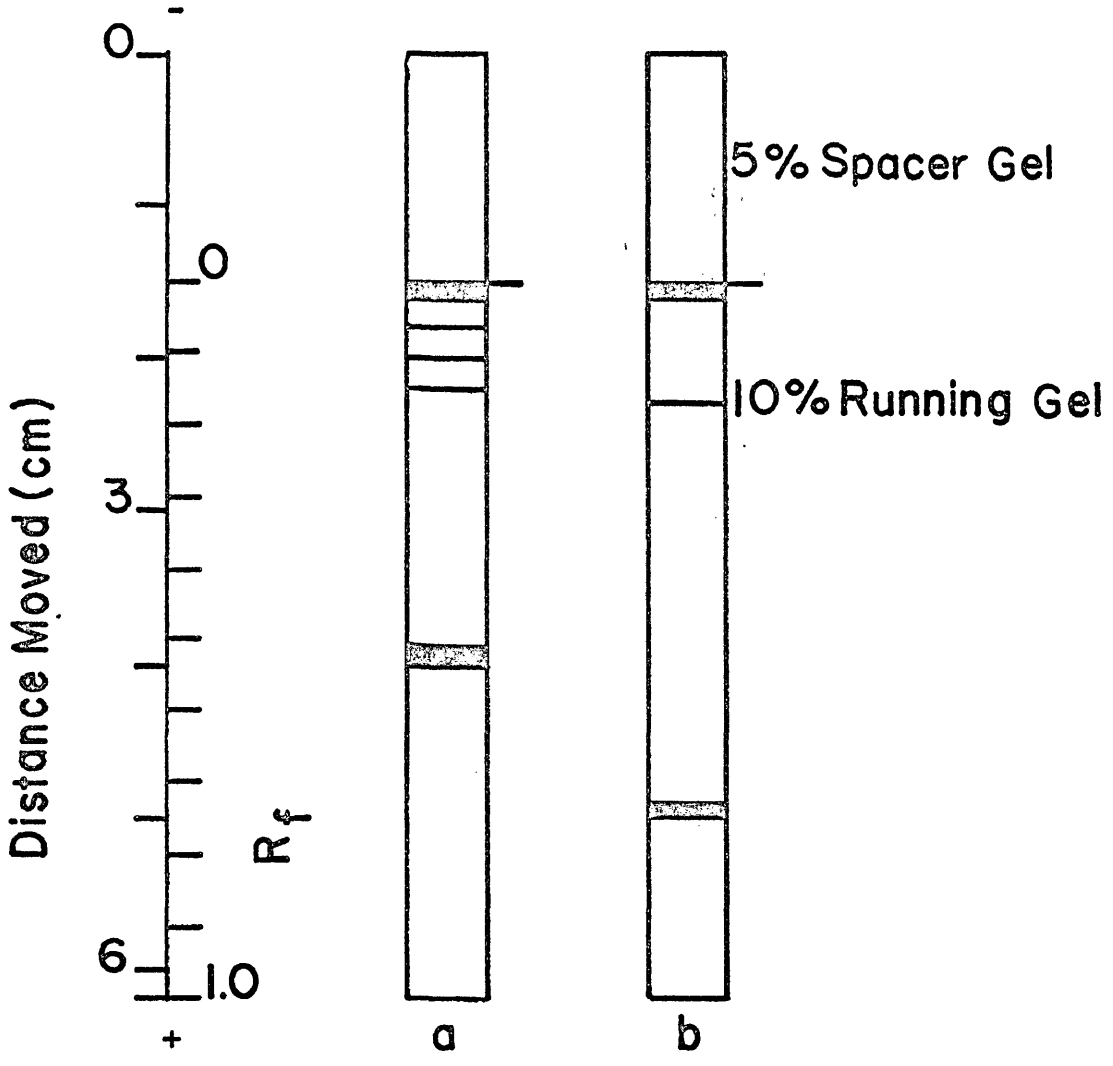

0
-
0
3
6
10
+
Distance Moved (cm)
R_f
a
b
5% Spacer Gel
10% Running Gel

Fig. 10. Electrophoretic analysis of E. coli A19 RNA after purification by sedimentation. (a) 23S ribosomal RNA, (b) 16S ribosomal RNA. RNA samples, 200 µg/0.01 ml, were applied at the top (0.0 cm) of each gel column. Electrophoresis was conducted at a constant current of 6 mA/gel for 55 minutes; NBB was used as the tracking dye. Gels were stained for one hour in 0.2% methylene blue staining buffer.


-
0
0
3
6
1.0
+
Distance Moved (cm)
R_f
a
b
5% Spacer Gel
10% Running Gel

Summary of the results

This investigation has shown that the larger molecular weight RNA which is found in ribosomes is a heterogeneous mixture of components. The results of a number of experiments are shown in Fig. 11. A comparison the the banding patterns of the different species indicates organism specificity of rRNA. Table I includes a summary of the electrophoretic results indicating the organisms investigated, type of purification of rRNA, and the number of bands resolved. The high resolution technique of disc electrophoresis in polyacrylamide gels made it possible to detect a heterogeneity which appeared in all rRNA preparations, regardless of the different purification procedures.

Fig. 11. Comparison of electrophoretic patterns of rRNA from a number of organisms purified by different methods:

(a) Anacystis nidulans - gel filtration
(b) Nostoc muscorum - ribosome isolation
(c) E. coli A19 - gel filtration
(d) E. coli A19 23S - sedimentation
(e) E. coli A19 16S - sedimentation
(f) Bean seedling chloroplasts - gel filtration
(g) Bean seedling chloroplasts and mitochondria - ribosome isolation
(h) Bean seedling cytoplasmic ribosomes - ribosome isolation
(i) Bean seedling 27S - sedimentation
(j) Bean seedling 18S - sedimentation
(k) Mouse liver - gel filtration
(l) Mouse liver - ribosome isolation
(m) Rat liver 28S - sedimentation
(n) Rat liver 18S - sedimentation

5% Spacer Gel
10% Running Gel
a
b
c
d
e
f
g
h
i
j
k
l
m
n
Distance Moved (cm)
0
3
6
10
+
-
R_f

TABLE I

SUMMARY OF ELECTROPHORETIC ANALYSES OF RIBOSOMAL RIBONUCLEIC ACID

Organism	Type of RNA Preparation	Total No. of Bands	R_f Value 0.0-0.3	0.3-0.7	0.7-1.0
Anacystis nidulans	Gel filtration of total cellular RNA	8	2	4	2
Nostoc muscorum	RNA isolated from ribosomes	27	15	11	1
E. coli A19					
a. Total RNA	Gel filtration of total cellular RNA	8	3	3	2
b. 23S rRNA	Sedimentation of total cellular RNA	5	4	1	0
c. 16S rRNA	Sedimentation of total cellular RNA	3	2	0	1
Bean seedlings					
a. Chloroplast fraction	Gel filtration of total RNA	8	6	2	0
b. Combined chloroplast and mitochondrial fractions	RNA isolated from ribosomes	18	7	9	2
c. Cytoplasmic ribosomes	RNA isolated from ribosomes	21	10	10	1
d. Cytoplasmic 27S rRNA	Sedimentation of RNA from ribosomes	5	3	2	0
e. Cytoplasmic 18S rRNA	Sedimentation of RNA from ribosomes	11	7	4	0
Mouse liver					
a. Total RNA	Gel filtration of total cellular RNA	11	5	5	1
b. Cytoplasmic ribosomes	RNA isolated from ribosomes	13	6	6	1
Rat liver					
a. 28S rRNA	Sedimentation of total cellular RNA	16	12	4	1
b. 18S rRNA	Sedimentation of total cellular RNA	6	4	2	0

DISCUSSION

An interpretation of the preceding results relies on the assumption that the integrity of RNA is preserved during the course of the experiments. This investigation has demonstrated the heterogeneity of rRNA prepared using a number of different purification procedures. These results are in agreement with those of Loening and Ingle (1967), McIndoe and Munro (1967), and Peacock and Dingman (1967). It is interesting to note here that the 16S rRNA of Bacillus megaterium (Yankofsky and Spiegelman, 1963) and the 18S rRNA of Neurospora crassa (Michelson and Suyama, 1968) have been separated into two components by chromatography on methylated-albumen-kieselguhr columns. There are some differences in the number and R_f values of the rRNA bands prepared by the different methods. This probably is a reflection of the quality of the techniques in producing and maintaining high integrity RNA. There appears to be no difference in the electrophoretic pattern of rRNA incubated at 37°C for 30 minutes and control samples (see Fig. 6a and f); whereas electrophoresis of RNase digests shows a lack of components (see Fig. 6b). Electrophoresis of rRNA from the mutant of E. coli lacking ribonuclease (E. coli A19) showed five components for 23S and three components for 16S rRNAs. It is unlikely that the numerous bands resolved in the rRNA preparations resulted from break down of rRNA by endogenous RNase.

Electrophoresis on polyacrylamide gels has been used to separate the high-molecular weight RNA fragments produced by T_1 ribonuclease digestion of rabbit reticulocyte rRNA (Gould, Bonanae, and Kanagalingham, 1966; Gould, 1967). The conditions used, however, were slightly different from the ones employed here and the study did not include undigested controls. No comparisons can be made between these investigations because it is uncertain whether the nine components (Gould et al., 1966; Gould, 1967) really represent fragments of digested RNA.

There is a possibility of some contamination of the rRNA preparations in this investigation with high-molecular weight messenger RNAs (mRNA) even after purification by sedimentation. Future studies of this nature should include labelling of RNA with C^{14}-uridine followed by a chase with cold uridine, isolation of ribosomes, and the improved-cresol RNA extraction method (Bishop et al., 1967) which would eliminate sRNA and mRNA from the rRNA preparations.

It can be concluded from the results that the numerous bands resolved during the electrophoresis are RNA molecules with similar molecular weights but of different sizes or configurations causing different mobilities. The heterogeneity of rRNA reported here is probably a result of different base sequences within the rRNA. This however must be tested experimentally. This would tend to support the idea that the numerous cistrons (as calculated from DNA-rRNA hybridization studies) for rRNA are not identical.

Experiments with the bacterial species E. coli and Pseudomonas aeruginosa have demonstrated that the physical and chemical properties

of ribosomes vary with the phase of the growth cycle and type of culture medium (Mendelsohn and Tissières, 1959; Santer, Teller, and Skilna, 1961; Aronson and Holowzyk, 1965). Sedimentation studies have shown that resting bacterial cells consist almost entirely of one class of ribosomes (100 S) whereas four classes of smaller ribosomes (85S, 70S, 50S, 30S) are present in exponentially growing cells (McCarthey, 1960; Ley, 1964). In addition the rRNA of early log phase cells, late log phase cells, and stationary phase cells of the same culture exhibit differences in base composition (Santer et al., 1961) and RNA to protein ratio (Mendelsohn and Tissières, 1959) differences. The transfer of E. coli to buffer appears to result in changes in the ribonucleoprotein particles as indicated by changes in the RNA to protein ratio (Mendelsohn and Tissières, 1959).

There appear to be small differences between the nucleotide base composition of rRNA of E. coli grown on nutrient broth-yeast extract media and of E. coli grown on glucose-salts medium. In glucose-$K_2HP^{32}O_4$ medium there is a difference in the uptake of P^{32} into rRNA by E. coli previously grown in nutrient broth-yeast extract medium when compared with bacteria previously grown in glucose (Santer et al., 1961). Aronson and Holowczyk (1965), studying the pattern of rRNA synthesis in E. coli and Ps. aeruginosa under different growth conditions, found differences in the nucleotide sequences of rRNA of steady state cultures and step-up (addition of a better carbon source) cultures. Differences were detected in the P^{32} labelling pattern of the 15 different fragments produced by RNase digestion of the rRNA synthesized under the different culture conditions.

This difference in base composition and sequence associated with the changes in culture media has been correlated with changes in the 3' OH terminal nucleotide sequence of rRNA (Midgley and McIlreavy, 1967). E. coli cells grown on glucose show 71% adenine and 16% uridine at the 3' OH terminal of 16S rRNA and 48% uridine and 26% adenine at the 3' OH terminal of 23S rRNA. Broth or casamino acid grown cultures in contrast show 57% and 76% uridine at the 3' OH termini of 16S and 23S rRNA respectively (McIlreavy and Midgley, 1967; Midgley and McIlreavy, 1967).

Studies on salt-resistant yeast also suggest the occurence of differentiated ribosomes (Yamamato, 1967) synthesized under different culture conditions. Saccharomyces cerevisiae grown under strontium chloride hypertonicity produces ribosomes which are sensitive to strontium concentration. These ribosomes form polysomes only in medium of high strontium concentrations; amino acid incorporation into protein also occurs under these conditions. When these yeast cells which have been acclimatized to high strontium concentration are transferred to medium lacking strontium, no amino acid incorporation into protein occurs until a second set of ribosomes, not dependent on strontium concentration, are synthesized. Yamamato (1967) has proposed that there may be two different types of ribosomal information and that one of them is activated under specific growth conditions.

There are a number of studies which suggest that differences in rRNA are correlated with changes in development and growth. There is a difference in the base composition of rRNA of green and etiolated cotton plants, Gossypium hirsutum (Katterman and Ergle, 1966). During

cytodifferentiation in the cellular slime mold Polyspondelium pallidum a considerable proportion of the RNA of the vegetative amoebae is degraded and replaced by newly synthesized rRNA (Sussman, 1967). Base composition studies show that the ribosomal RNA of the later embryonic stages is different from that of the earlier stages. Lerner, Bell, and Darnell (1963) found a slight difference between the base composition of rRNA in three and seven day chick embryos. Comb and Brown (1964) showed differences in the base composition of rRNA from Lytechinus variegatus in early blastula and late gastrula stages. The rRNA base ratios of unfertilized eggs and stage 45 (swimming stage) embryos of Xenopus laevis are also different (Brown and Gurdon, 1964). During the development of chloroplasts in Euglena gracilis a specific type of ribosomes (70S) with rRNA of a characteristic base composition appear (Brawerman, 1963). Electrophoretic studies have demonstrated the appearance of new ribosomal RNA components on consecutive days of development of the French bean leaf (Loening and Ingle, 1967).

The results all suggest that the ribosomal fraction is a heterogeneous collection of particles containing RNAs with varying base compositions and sequences. Changes in growth conditions, environmental factors, and development appear to produce changes in the types of rRNA synthesized, suggesting that there may be specialized classes of ribosomes. Spiegelman and Yankofsky (1965) have hypothesized that the ribosomes of an organism are of many distinct species and possibly of function. The function of the heterogeneous rRNAs demonstrated in this and other investigations is unknown at present. It is possible that these different RNAs are involved in altering the structure of the ribosomes

prior to the attachment of the ribosomes to the cellular membranes or attachment of the mRNA to the ribosomes. This alteration of ribosomes might be a prerequisite for the synthesis of different types of proteins, e.g., enzymic or structural proteins associated with the nucleus, cytoplasm, or cell organelles. Further electrophoretic investigations of rRNA from cells under some of these different growth and developmental conditions may provide more direct information concerning specialized types and functions of rRNA.

APPENDICES

APPENDIX I

ISOLATION OF RIBOSOMES

Modified from Stutz and Noll, 1967.

Reagents:

Buffer I - 0.7 M sucrose, 0.1 M Tris-HCl, pH 7.5, 0.005 M $MgCl_2$, 0.05 M KCl, 0.005 2-mercaptoethanol

Buffer II - 0.01 M Tris-HCl, pH 7.5, 0.005 M $MgCl_2$, 0.05 M KCl, 0.005 M 2-mercaptoethanol

Buffer III - 0.001 M Tris-HCl, pH 7.5, 0.004 M $MgCl_2$

Procedure:

1. Homogenize tissue in Waring Blendor or Sorvall Omnimixer homogenizer in 3 ml of cold buffer I/g material. This and all subsequent steps are to be carried out at approximately 4°C.

2. Filter homogenate through 0.1 mm filter and centrifuge at 600 xg for 2 minutes to remove nucleic and cell debris.

3. (For eucaryotic plant material only) Centrifuge supernatant from step 2 at 1100 xg for 12 minutes to remove chloroplasts.

4. (For eucaryotic material only) Supernatant from step 2 for animal preparations or step 3 for plant preparations centrifuge in Beckman type 30 rotor at 18,000 rpm (28,000 xg average) for 30 minutes to sediment mitochondria.

5. Supernatant from step 4 made 0.5% with respect to sodium deoxycholate by adding a 20% solution. Centrifuge for 30 minutes at 18,000 rpm in type 30 rotor.

6. Supernatant from step 5 layered over 2 ml of 1 M sucrose in buffer II and centrifuged in Beckman type 65 rotor at 40,000 rpm (105,000 xg average) for 2½ hours. Yellowish ribosome pellets and walls of tubes were repeatedly and carefully rinsed with cold distilled water.

7. Ribosome pellets resuspended by gentle stirring with glass rod in buffer III. Store at -18°C.

8. (To obtain chloroplast ribosomes)
   a. Chloroplast pellet from step 3 resuspended in buffer I and step 3 repeated.
   b. Pellet taken up in buffer II (1/10 of original volume) and a 20% solution of sodium deoxycholate added to a final concentration of 5%.
   c. Dark green lysate centrifuged in Beckman type 30 rotor at 18,000 rpm for 30 minutes to remove starch granules and insoluble green material.
   d. Supernatant treated as in step 6.
   e. Treat ribosomes as in step 7.

APPENDIX II

DISC ELECTROPHORESIS IN POLYACRYLAMIDE GELS

The method of disc electrophoresis in polyacrylamide gel utilizes the sieving properties of the medium and a discontinuous buffer system to sharpen the electrophoretic zones thus increasing the resolving power of the system. The theoretical aspects of this method have been discussed by a number of workers (Davis, 1964; Orstein, 1964; Raymond, 1964; Williams and Reisfeld, 1964).

The method I have adopted for the separation of RNA is a modification of the technique described by Richards and Coll (1965) using the gel and buffer system of Peacock and Dingman (1967). In the large pore spacer gel the components of the sample become stacked in a thin zone according to their various mobilities, whereas they are separated in the running gel due to electrophoretic mobility and the sieving properties of the gel (Orstein, 1964).

Disc electrophoresis was carried out in the Polyanalyst Disc Electrophoresis Apparatus (Buchler Instruments, Inc., Fort Lee, N. J.). Instructions for operating this instrument are given in the instruction manual (Buchler Instruments, Inc., 1966).

Reagents for gel preparation: Store in refrigerator.

A. 20% acrylamide (19g acrylamide, 1 g N, N'-methylene bis acrylamide) in 100 ml distilled water.

B. Dimethylamino proprionitrile (DMAPN), 6.4% in distilled water.

C. Ammonium persulfate, 1.6% in distilled water. (Make fresh each week).

D. Stock buffer, pH 8.3
108 g Tris
9.3 g disodium EDTA
55 g boric acid
Make up to 1 liter with glass distilled water. Use this buffer undiluted to prepare gels. Dilute 100 ml of buffer to 1 liter to fill upper and lower buffer reservoirs of electrophoresis apparatus.

Gel Preparation:

1. a. For 10% running gel mix as follows:
8 ml A
1 ml B
1.6 ml D
4.4 ml distilled water
1 ml C
b. For 5% running gel mix as follows:
4 ml A
1 ml B
1.6 ml D
8.4 ml distilled water
1 ml C

2. Gel tubes should be marked 5 cm from rubber capped end. Fill tubes to mark with running gel. Overlayer with 0.5 cm distilled water, and allow polymerization to proceed for at least 20 minutes. Pour off liquid after polymerization.

3. a. For 5% spacer gel to be used with 10% running gel, mix as follows:
2 ml A
0.5 ml B
0.8 ml D
4.2 ml distilled water
0.5 ml C
b. For 3% spacer gel to be used with 5% running gel, mix as follows:
1.2 ml A
0.5 ml B
0.8 ml D
5 ml distilled water
0.5 ml C

4. Add 0.3 ml of spacer gel mixture to tubes containing the appropriate polymerized running gel. Overlayer with 0.5 cm distilled water and continue as in step 2.

Electrophoresis:

1. Place gel tubes into tube holder assembly. Be sure the top of the gels extend the same distance into the upper chamber.

2. Fill lower buffer chamber with 1:10 dilution of stock buffer so that the tubes will be submerged approximately ¼ inch into the buffer. Place a hanging drop of buffer at the bottom of each tube prior to placing the tube holder assembly in place.

3. Dissolve RNA in a solution of 0.05 M NaCl-1 mM EDTA, pH 6.2 and 30% (w/v) sucrose at a concentration of 200 µg or more per 0.1 ml. An aliquot containing 200 ug RNA is layered on top of each gel. A small amount of bromophenol (BPB) or napthol blue black (NBB) in the same solution is also placed on top of the gel and mixed with the RNA with a wooden applicator stick.

4. Carefully fill the well of each gel tube with buffer using a pasteur pipette. Using a long stemmed funnel add enough buffer to upper chamber so that the upper electrode will be covered when the cover of the apparatus is in place.

5. Place the cover on the apparatus, level, connect the electrodes. Apply a constant current of 6 mA/tube until the tracking dye reaches the end of the tube.

6. Shut off the power supply, disconnect electrodes, decant off buffer, and remove tubes from assembly. Remove gels from tubes; cut off any gel which extends beyond the position of the tracking dye.

Staining:

1. Rinse gels in 1 M acetic acid for 10 to 15 minutes to lower the pH of the gels.

2. Staining buffer, pH 4.7: 0.2 M Na acetate-0.2 M acetic acid made 0.2% (w/v) with respect to methylene blue or acridine orange. This stain may be reused for several weeks.

3. Stain for one hour.

4. Remove excess stain by rinsing the gels for several hours in distilled water.

BIBLIOGRAPHY

BIBLIOGRAPHY

Aronson, A. I., and M. A. Holowczyk. 1965. Composition of bacterial ribosomal RNA. Heterogeneity within a given organism. Biochim. Biophys. Acta 95:217-231.

Attardi, G., P. Huang, and S. Kabat. 1965. Recognization of ribosomal RNA sites in DNA. I. Analysis of the E. coli system. Proc. Natl. Acad. Sci., USA 53:1490-1498.

Bachvaroff, R., and P. R. B. McMaster. 1964. Separation of microsomal RNA into five bands during agar electrophoresis. Science 143:1177-1178.

Benoy, L., and M. Szekely. 1966. Electrophoretic separation of cytoplasmic ribonucleic acid fractions of pancreas and liver. Biochem. J. 100:17c-19c.

Biggins, J. 1967a. Preparation of metabolically active protoplasts from the blue-green alga, Phormidium luridum. Plant Physiol. 42:1442-1446.

Biggins, J. 1967b. Photosynthetic reactions by lysed protoplasts and particle preparations from the blue-green alga, Phormidium luridium. Plant Physiol. 42:1447-1456.

Bishop, D. H. L., J. R. Claybrook, and S. Spiegelman. 1967. Electrophoretic separation of viral nucleic acids on polyacrylamide gels. J. Mol. Biol. 26:373-387.

Boardman, N. K., R. I. B. Franki, and S. G. Wildman. 1966. Protein synthesis by cell-free extracts of tobacco leaves. III. Comparison of the physical properties and protein synthesizing activities of 70S chloroplast and 80S cytoplasmic ribosomes. J. Mol. Biol. 17:470-489.

Brawerman, G. 1963. The isolation of a specific species of ribosomes associated with chloroplast development in Euglena gracilis. Biochim. Biophys. Acta 72:317-331.

Brown, D. D., and J. B. Gurdon. 1964. Absence of ribosomal RNA synthesis in the anucleolate mutant of Xenopus laevis. Proc. Natl. Acad. Sci., USA 51:139-146.

Buchler Instruments, Inc. 1966. Polyanalyst. An analytical temperature-regulated disc electrophoresis apparatus. Fort Lee, N. J. 10 p.

Chipchase, M. I. H., and M. L. Birnstiel. 1963. On the nature of nucleolar RNA. Proc. Natl. Acad. Sci., USA 50:1101-1106.

Clark, M. F., R. E. F. Matthews, and R. K. Ralph. 1964. Ribosomes and polyribosomes in Brassica pekinensis. Biochim. Biophys. Acta 91:289-304.

Click, R. E., and D. P. Hackett. 1966. Evidence that the two ribosomal RNA species of plant tissues are synthesized on different genetic loci. J. Mol. Biol. 17:279-284.

Click, R. E., and B. L. Tint. 1967. Comparative sedimentation rates of plant, bacterial, and animal ribosomal RNA. J. Mol. Biol. 25:111-122.

Comb, D. G., and R. Brown. 1964. Preliminary studies on the degradation and synthesis of RNA components during sea urchin development. Exp. Cell Res. 34:360-370.

Cutler, R. G., and J. E. Evans. 1967. Relative transcription activity of different segments of the genome throughout the cell division cycle of Escherichia coli. The mapping of ribosomal and transfer RNA and the determination of the direction of replication. J. Mol. Biol. 26:91-105.

Davis, B. J. 1964. Disc electrophoresis. II. Method and application to human serum proteins. Ann. N. Y. Acad. Sci. 121:404-427.

Deason, T. R., and H. C. Bold. 1960. Phycological studies. I. Exploratory studies of Texas soil algae. Univ. of Texas Pub. 6022.

Dingman, C. W., and A. C. Peacock. 1968. Analytical studies on nuclear ribonucleic acid using polyacrylamide gel electrophoresis. Biochemistry 7:659-668.

Dure, L. S., J. L. Epler, and W. E. Barnett. 1967. Sedimentation properties of mitochondrial and cytoplasmic ribosomal RNA's from Neurospora. Proc. Natl. Acad. Sci., USA 58:1883-1887.

Eisenstadt, J. M., and G. Brawerman. 1967. The role of the native subribosomal particles of E. coli in polypeptide chain initiation. Proc. Natl. Acad. Sci., USA 58:1560-1565.

Elson, D. 1964. Studies on ribosomes, p. 92-105. In New perspectives in biology. Amer. Elsevier Publ. Co., N. Y. Vol. 4.

Fogel, S., and P. S. Sypherd. 1968. Chemical basis for heterogeneity of ribosomal proteins. Proc. Natl. Acad. Sci., USA 59:1329-1336.

Ghosh, H. P., and H. G. Khorana. 1967. Studies on polynucleotides. LXXXIV. On the role of ribosomal subunits in protein synthesis. Proc. Natl. Acad. Sci., USA 58:2455-2461.

Gibson, I. 1967. RNA homologies between protozoa. J. Protozool. 14:687-690.

Gould, H. J. 1967. The nature of high molecular weight fragments of ribosomal RNA. J. Mol. Biol. 29:307-313.

Gould, H. J., Bonanno, S., and K. Kanagalingham. 1966. Structural characterisation of ribosomal ribonucleic acids from various species by a new 'fingerprinting' technique. J. Mol. Biol. 22:397-399.

Grossbach, U., and I. B. Weinstein. 1968. Separation of ribonucleic acids by polyacrylamide gel electrophoresis. Anal. Biochem. 22:311-320.

Huberman, J. A., and G. Attardi. 1967. Studies of fractionated HeLa metaphase chromosomes. I. The chromosomal distribution of DNA complementary to 28S and 18S ribosomal RNA and to cytoplasmic messenger RNA. J. Mol. Biol. 29:487-505.

Igarashi, K., and A. Kaji. 1967. On the nature of two ribosomal sites for specific sRNA binding. Proc. Natl. Acad. Sci., USA 58:1971-1976.

Katterman, F. R. H., and D. R. Ergle. 1966. RNA composition in cotton. Plant Physiol. 41:553-556.

Kedes, L. H., R. J. Koegel, E. L. Kuff. 1966. Relationship between structural proteins of derived ribosomal subunits and cytoplasmic post ribosomal particles of mammalian cells. J. Mol. Biol. 22:359-363.

King, H. W. S., and W. Fitschen. 1968. Characterisation of RNA from the smooth endoplasmic reticulum of rat liver. Biochim. Biophys. Acta 155:32-37.

Leboy, P. S., E. C. Cox, and G. Flax. 1964. The chromosomal site specifying a ribosomal protein in Escherichia coli. Proc. Natl. Acad. Sci., USA 52:1367-1374.

Ley, J. de. 1964. On the unity of bacterial ribosomes. J. Gen. Microbiol. 34:219-227.

Lerner, A. M., E. Bell, and J. E. Darnell, Jr. 1963. Ribosomal RNA in the developing chick embryo. Science 141:1187-1188.

Lindsay, D. T. 1966. Electrophoretically identical histones from ribosomes and chromosomes of chicken liver. Arch. Biochem. Biophys. 113:687-694.

Loening, U. E. 1967. The fractionation of high-molecular-weight ribonucleic acid by polyacrylamide gel electrophoresis. Biochem. J. 102:251-257.

Loening, U. E., and J. Ingle. 1967. Diversity of RNA components in green plant tissues. Nature 215:363-367.

Lyttleton, J. W. 1962. Isolation of ribosomes from spinach chloroplasts. Exp. Cell Res. 26:312-317.

McCarthey, B. J. 1960. Variation in bacterial ribosomes. Biochim. Biophys. Acta 39:563-564.

McConkey, E. H., and J. W. Hopkins. 1964. The relationship of the nucleolus to the synthesis of ribosomal RNA in HeLa cells. Proc. Natl. Acad. Sci., USA 51:1197-1204.

McIlreavy, D. J., and J. E. M. Midgley. 1967. The chemical structure of bacterial ribosomal RNA. I. Terminal sequences of Escherichia coli ribosomal RNA. Biochim. Biophys. Acta 142:47-64.

McIndoe, W., and H. N. Munro. 1967. Species of RNA from liver cell fractions separated on agarose gels. Biochim. Biophys. Acta 134:458-460.

McQuillen, K. 1962. Ribosomes and the synthesis of proteins. Prog. Biophys. and Biophys. Chem. 12:67-106.

Mendelsohn, J., and A. Tissières. 1959. Variations in the amount of ribonucleoprotein particles in Escherichia coli. Biochim. Biophys. Acta 35:248-250.

Michelson, E. I., and Y. Suyama. 1968. Evidence for two types of 18S ribosomal RNA in Neurospora crassa. Biochim. Biophys. Acta 157:200-203.

Midgley, J. E. M., and D. J. McIlreavy. 1967. The chemical structure of bacterial ribosomal ribonucleic acid. II. Growth conditions and polynucleotide distribution in E. coli ribosomal RNA. Biochim. Biophys. Acta 142:345-354.

Miura, K. I. 1962. The nucleotide composition of ribonucleic acids of soluble and particulate fractions in several species of bacteria. Biochim. Biophys. Acta 55:62-70.

Oishi, M., and N. Sueoka. 1965. Location of genetic loci of ribosomal RNA on Bacillus subtilis chromosome. Proc. Natl. Acad. Sci., USA 54:483-491.

Orstein, L. 1964. Disc electrophoresis. I. Background and theory. Ann. N. Y. Acad. Sci. 121:321-349.

Otaka, E., T. Itoh, and S. Osawa. 1968. Ribosomal proteins of bacterial cells: Strain- and species-specificity. J. Mol. Biol. 33:93-107.

Peacock, A. C., and C. W. Dingman. 1967. Resolution of multiple ribonucleic acid species by polyacrylamide gel electrophoresis. Biochemistry 6:1818-1827.

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.

Petermann, M. L. 1964. The physical and chemical properties of ribosomes. Amer. Elsevier Publ. Co., N. Y. 258 p.

Petermann, M. L., and A. Pavlovec. 1963. Ribonucleoprotein from a rat tumor, the Jensen Sarcoma. III. Ribosomes purified without deoxycholate but with bentonite as ribonuclease inhibitor. J. Biol. Chem. 238:318-323.

Pollard, C. J., A. Stemler, and D. F. Elaydes. 1966. Ribosomal ribonucleic acids of chloroplastic and mitochondrial preparations. Plant Physiol. 41:1323-1329.

Raymond, S. 1964. Acrylamide gel electrophoresis. Ann. N. Y. Acad. Sci. 121:350-364.

Richards, E. G., and J. A. Coll. 1965. Disc electrophoresis of ribonucleic acid in polyacrylamide gels. Anal. Biochem. 12:452-471.

Ritossa, R. M., and S. Spiegelman. 1965. Localization of DNA complementary to ribosomal RNA in nucleolus organizer region of Drosophila melanogaster. Proc. Natl. Acad. Sci., USA 53:737-745.

Rogers, P. J., B. N. Preston, E. B. Titchener, and A. W. Linnane. 1967. Differences between the sedimentation characteristics of the ribonucleic acids prepared from yeast cytoplasmic ribosomes and mitochondria. Biochem. Biophys. Res. Commun. 27:405-411.

Sager, R., and M. G. Hamilton. 1967. Cytoplasmic and chloroplast ribosomes of Chlamydomonas: Ultracentrifugal characterization. Science 157:709-711.

Santer, M., D. C. Teller, and L. Skilna. 1961. Variation in base composition of ribonucleic acid in Escherichia coli. Proc. Natl. Acad. Sci., USA 47:1384-1392.

Schachman, H. K., A. B. Paradee, and R. Y. Stanier. 1952. Studies on the macromolecular organization of microbial cells. Arch Biochem. Biophys. 38:245-260.

Sells, B. H., and F. C. Davis, Jr. 1968. Ribosome biogenesis: Nonrandom addition of structural proteins to 50S subunits. Science 159:1240-1242.

Smith, I., D. D. Dubnau, P. Morell, and J. Marmur. 1968. Chromosomal location of DNA base sequences complementary to transfer RNA and to 5S, 16S, and 23S ribosomal RNA in Bacillus subtilis. J. Mol. Biol. 33:123-140.

Spiegelman, S., and S. A. Yankofsky. 1965. The relation of ribosomal RNA to the genome, p. 537-579. In V. Bryson and J. Vogel [ed.] Evolving genes and proteins. Academic Press, N. Y.

Starr, R. C. 1960. The culture collection of algae at Indiana University. Amer. J. Bot. 47:67-86.

Stutz, E., and H. Noll. 1967. Characterization of cytoplasmic and chloroplast polysomes in plants: Evidence for three classes of ribosomal RNA in nature. Proc. Natl. Acad. Sci., USA 57:774-781.

Sugiura, M., and M. Takanami. 1967. Analysis of the 5'-terminal nucleotide sequences of ribonucleic acids. II. Comparison of the 5'-terminal nucleotide sequences of ribosomal RNA's from different organisms. Proc. Natl. Acad. Sci., USA 58:1595-1600.

Sussman, R. R. 1967. RNA metabolism during cytodifferentiation in the cellular slime mold, Polyspondylium pallidum. Biochim. Biophys. Acta 149:407-421.

Svetailo, E. N., I. I. Philippovich, and N. M. Sissakian. 1967. Differences in sedimentation properties of chloroplast and cytoplasmic ribosomes from pea seedlings. J. Mol. Biol. 24:405-415.

Taylor, M. M., and R. Storck. 1964. Uniqueness of bacterial ribosomes. Proc. Natl. Acad. Sci., USA 52:958-965.

Traub, P., K. Hosokawa, C. R. Craven, and M. Nomura. 1967. Structure and function of E. coli ribosomes. IV. Isolation and characterization of functionally active ribosomal proteins. Proc. Natl. Acad. Sci., USA 58:2430-2436.

Traub, P., M. Nomura, and L. Tu. 1966. Physical and functional heterogeneity of ribosomal proteins. J. Mol. Biol. 19:215-218.

Tsanev, R. 1965. Direct spectrophotometric analysis of ribonucleic acid fractionation by agar-gel electrophoresis. Biochim. Biophys. Acta 103:374-382.

Vermeulen, C. W. 1966. The genetic mapping of the ribosomal RNA loci in E. coli and the direction of chromosome replication in Hfr strains. Doctoral dissertation. Univ. of Illinois.

Vermeulen, C., and K. C. Atwood. 1965. The proportion of DNA complementary to ribosomal RNA in Drosophila melanogaster. Biochem. Biophys. Res. Comm. 19:221-226.

Wallace, H., and M. L. Birnstiel. 1966. Ribosomal cistrons and the nucleolar organizer. Biochim. Biophys. Acta 114:296-310.

Waller, J. P. 1964. Fractionation of the ribosomal protein from Escherichia coli. J. Mol. Biol. 10:319-336.

Waller, J. P., and J. I. Harris. 1961. Studies on the composition of the protein from Escherichia coli ribosomes. Proc. Natl. Acad. Sci., USA 47:18-23.

Williams, D. E., and R. A. Reisfeld. 1964. Disc electrophoresis in polyacrylamide gels: Extension to new conditions of pH and buffer. Ann. N. Y. Acad. Sci. 121:373-381.

Yamamoto, T. 1967. Evidence of heterogeneous ribosomal particles in salt-resistant yeast. Biochem. Biophys. Res. Commun. 29:21-27.

Yankofsky, S. A., and S. Spiegelman. 1962a. The identification of the ribosomal RNA cistron by sequence complementarity. I. Specificity of complex formation. Proc. Natl. Acad. Sci., USA 48:1069-1078.

Yankofsky, S. A., and S. Spiegelman. 1962b. The identification of the ribosomal RNA cistron by sequence complementarity. II. Saturation of and competitive interaction at the RNA cistron. Proc. Natl. Acad. Sci., USA 48:1466-1472.

Yankofsky, S. A., and S. Spiegelman. 1963. Distinct cistrons for the two ribosomal RNA components. Proc. Natl. Acad. Sci., USA 49:538-544.

VITA

Carolyn Gene Mohler

Born in Roanoke, Virginia, March 5, 1944. Graduated from Andrew Lewis High School, Salem, Virginia, June 1962. Received B. S. degree with honor and distinction in biology from Longwood College, Farmville, Virginia, June 1966.

In September 1966, the author entered the College of William and Mary as a graduate teaching assistant in the Department of Biology. She received a NSF traineeship during the summer of 1967 and a graduate fellowship from the Department of Biology for the academic year 1967-68.