Hirshfield, I., and Koritz, S. B. (1964), *Biochemistry 3*, 1994.
Hochberg, R. B., vander Hoeven, T. A., Welch, M., and Lieberman, S. (1974), *Biochemistry 13*, 603.
Jacobsohn, G. M., and Hochberg, R. B. (1968), *J. Biol. Chem. 243*, 2985.
Kelly, W. G., Bandi, L., Shoolery, J. N., and Lieberman, S. (1962), *Biochemistry 1*, 172.
Kobayashi, S., and Ichii, S. (1969), *J. Biochem. (Tokyo) 66*, 51.
Lehninger, A. L. (1964), The Mitochondrion; Molecular Basis of Structure and Function, New York, N. Y., W. A. Benjamin, p 88.
Lowry, D. H., Rosenbrough, N. J., Farr, A. L., and Randall, R. J. (1951), *J. Biol. Chem. 193*, 265.
Mickan, H., Dixon, R., and Hochberg, R. (1969), *Steroids 13*, 477.
Oertel, G. W., and Eik-Nes, K. (1959), *Anal. Chem. 31*, 98.
Okita, G. J., Kabara, J. J., Richardson, F., and Leroy, G. V. (1957), *Nucleonics 15*, 111.
Raggatt, P. R., and Whitehouse, M. W. (1966), *Biochem. J. 101*, 819.
Roberts, K. D., Bandi, L., Calvin, H. I., Drucker, W. D., and Lieberman, S. (1964), *Biochemistry 3*, 1983.
Roberts, K. D., Bandy, L., and Lieberman, S. (1967), *Biochem. Biophys. Res. Commun. 29*, 741.
Roberts, K. D., Bandy, L., and Lieberman, S. (1969), *Biochemistry 8*, 1259.
Roberts, K. D., and Lieberman, S. (1970), *in* Chemical and Biological Aspects of Steroid Conjugation, Bernstein, S., and Solomon, S., Ed., New York, N. Y., Springer Verlag, p 223.
Roy, A. B. (1970), *in* Chemical and Biological Aspects of Steroid Conjugation, Bernstein, S., and Solomon, S., Ed., New York, N. Y., Springer Verlag, p 100.
Sauer, L. A., and Mulrow, P. J. (1969), *Arch. Biochem. Biophys. 134*, 486.
Simpson, E. R., Jefcoate, C. R., Brownie, A. C., and Boyd, G. S. (1972), *Eur. J. Biochem. 28*, 442.
Yago, N., and Ichii, S. J. (1969), *J. Biochem. (Tokyo) 65*, 215.
Young, D. G., and Hall, P. F. (1969), *Biochemistry 8*, 2987.
Young, D. G., Holroyd, J. D., and Hall, P. F. (1970), *Biochem. Biophys. Res. Commun. 381*, 184.

Comparison of Proteins of Ribosomal Subunits and Nucleolar Preribosomal Particles from Novikoff Hepatoma Ascites Cells by Two-Dimensional Polyacrylamide Gel Electrophoresis†

Archie W. Prestayko, G. Robert Klomp, David J. Schmoll, and Harris Busch*

ABSTRACT: Proteins have been isolated from Novikoff hepatoma polyribosomes, ribosomal subunits, and nucleolar preribosomal particles and compared by two-dimensional polyacrylamide gel electrophoresis. The large and small ribosomal subunits contain 35 and 23 proteins, respectively. Nucleolar particles contain approximately 60 proteins, 21 of which are present in the large ribosomal subunit and 10 of which are present in the small ribosomal subunit. Nucleolar preribosomal particles isolated in the presence of poly(vinyl sulfate) contain specific proteins which are removed when these particles are isolated in media containing sodium ethylenediaminetetraacetate. One of these proteins, B 13′, has identical electrophoretic mobilities in two-dimensional gel electrophoresis to a protein removed from ribosomes during dissociation of ribosomes into subunits with sodium ethylenediaminetetraacetate. A processing mechanism is proposed for the removal of nucleolar preribosomal associated proteins not present in cytoplasmic ribosomes by which some of these proteins are conserved in the nucleolus.

It has been well established that the nucleolus is the site of ribosomal precursor RNA synthesis (Perry, 1962; Scherrer and Darnell, 1963; Darnell, 1968; Busch and Smetana, 1970). Several studies (Tamaoki, 1966; Warner and Soeiro, 1967; Liau and Perry, 1969; Mirault and Scherrer, 1971; Auger and Tiollais, 1973) have shown that newly transcribed 45S ribosomal precursor RNA becomes associated with protein and can be isolated as a rapidly sedimenting ribonucleoprotein complex. It has also been shown that the granular component of the nucleolus is largely composed of ribonucleoprotein complexes which largely are precursors to the large ribosomal subunit (Shankarnarayan and Birnstiel, 1969; Busch and Smetana, 1970; Das *et al.*, 1970; Koshiba *et al.*, 1971). There are many common proteins in nucleolar ribonucleoprotein particles and the large ribosomal subunit (Kumar and Warner, 1972; Tsurugi *et al.*, 1973).

The nature of the nucleolar precursor to the small ribosomal subunit is not clear although studies of tryptic digestion products of nucleolar proteins (Shepherd and Maden, 1972) suggest that the nucleolar ribonucleoprotein particles containing 45S RNA also contain proteins of the small ribosomal subunit.

Since it has been shown that an increased resolution of proteins of mammalian ribosomes (Kaltschmidt and Wittman, 1970; Martini and Gould, 1971; Welfle *et al.*, 1971; Delaunay and Schapira, 1972; Delaunay *et al.*, 1972; Hulten and Sjoquist, 1972; Sherton and Wool, 1972; Welfle *et al.*, 1972; Rodgers, 1973), nuclei (Yeoman *et al.*, 1973), and nucleoli (Ballal and Busch, 1973; Orrick *et al.*, 1973) can be obtained by two-dimensional polyacrylamide gel electrophoresis, a

† From the Department of Pharmacology, Baylor College of Medicine, Houston, Texas 77025. *Received November 19, 1973.* This work was supported by a grant from the National Cancer Institute (CA-10893, P.3).

comparison of nucleolar and ribosomal proteins was made by this method.

It was found in this study that 21 proteins of the larger ribosomal subunit and 10 proteins of the small ribosomal subunit can be found in nucleolar ribonucleoprotein particles. These findings provide further evidence that in addition to ribosomal precursor RNA, the nucleolar ribonucleoprotein particles contain proteins of both ribosomal subunits and therefore are designated preribosomal particles. These findings suggest that one of the initial steps in the processing of ribosomal precursor RNA is the formation of a ribonucleoprotein complex with precursor RNA and specific proteins of both ribosomal subunits. It is clear that other proteins are added in the nucleoplasm or cytoplasm.

Materials and Methods

Preparation of Polysomes and Ribosomal Subunits. Novikoff hepatoma ascites cells were obtained by drainage of the ascites fluid through an abdominal incision 6 days after intraperitoneal implantation of the tumors in male Sprague-Dawley rats. The ascites cells (50 g) were filtered through cheesecloth, collected by centrifugation at 10,000*g* for 10 min, washed gently in TKM buffer (0.06 M Tris-acetate (pH 7.4)–0.05 M KCl–0.005 M $MgCl_2$) containing 0.25 M sucrose, and centrifuged for 10 min at 10,000*g* in a Sorvall Model RC-2 centrifuge.

To prepare polysomes, the washed Novikoff hepatoma cells were homogenized (1 g/3 ml) in the TKM buffer containing 0.25 M sucrose in a glass homogenizer with a tight-fitting Teflon pestle. After centrifugation at 16,000*g* for 15 min, the pellet was rehomogenized and centrifuged. The combined supernatant was adjusted to a final concentration of 0.5% sodium deoxycholate and centrifuged at 30,000*g* for 15 min. The supernatant was then underlayered with two sucrose layers (a) 4 ml of 1.0 M sucrose in TKM buffer and (b) 4 ml of 2.0 M sucrose in TKM buffer and centrifuged at 29,000 rpm (75,000*g*, av) in the Spinco No. 30 rotor for 16 hr. Sucrose density gradient centrifugation analyses showed that the polysome preparations were of high purity. Polysomal pellets (50–75 mg/gram of cells) were used immediately or frozen at −80°.

Ribosomal subunits were prepared from the polysome pellets. In some experiments, the ribosomal subunits were prepared with the 0.5 M KCl–puromycin method (Bloebel and Sabatini, 1971) and in others by treatment with EDTA. Inasmuch as no major differences were found in the protein patterns on the two-dimensional gels, the EDTA method was more frequently used for more satisfactory subunit protein comparisons to those of the nucleolar particles. For preparation of ribosomal subunits with EDTA, the polysome pellets were resuspended at a concentration of 25 OD/ml at 260 nm in 0.005 M Tris-acetate (pH 7.4). Insoluble material, if any, was removed by centrifugation at 3000*g* for 5 min. The supernatant was made 0.008 M with respect to EDTA and spun at 35,000 rpm for 16 hr in the Zonal ultracentrifuge (Beckman Model LZU) through a 5–45% sucrose gradient. The absorbance in the gradient was monitored at 254 nm with an ISCO Model D fractionator and uv analyzer. Sucrose fractions corresponding to the small and large ribosomal subunit peaks were collected, precipitated with 2 volumes of 100% ethanol, and stored at −20°. These subunits were shown to contain 18S and 28S rRNA, respectively.

Preparation of Preribosomal Particles from Isolated Nucleoli. Nuclei were prepared from Novikoff hepatoma ascites cells by a modification (Steele and Busch, 1966) of the sucrose method. Nucleoli (25 mg/50 g of cells) were isolated from these nuclei by the sonication method (Busch, 1967). To avoid aggregation of nucleolar particles, the method of isolation of these nucleolar particles used was essentially that of Warner and Soeiro (1967) and Prestayko *et al.* (1973).

Nucleoli were suspended at a concentration of approximately 1 mg/ml wet weight in a buffer containing 0.01 M NaCl–0.005 M $MgCl_2$–0.01 M Tris-HCl (pH 7.4). Deoxyribonuclease I (Worthington, Freehold, N. J.) was added to a concentration of 10 μg/mg of nucleoli and stirred slowly for 15 min at 0°. The mixture was centrifuged at 20,000*g* for 10 min and the pellet was resuspended at 1 mg/ml of original nucleoli in NEB buffer (0.01 M NaCl–0.01 M EDTA–0.01 M Tris-HCl (pH 7.4)). After centrifugation at 20,000*g* for 10 min, the pellet was resuspended at 1 mg/ml of original nucleoli in NEC buffer (NEB buffer containing 0.01 M dithiothreitol). The mixture was stirred slowly at room temperature for 20 min and centrifuged at 20,000*g* for 15 min. The supernatant containing nucleolar particles was underlayered with one-third the volume of 1.0 M sucrose in NEC buffer and centrifuged at 90,000*g* for 16 hr. The pellet contained nucleolar preribosomal particles (5 mg/50 g of cells).

Poly(vinyl sulfate) Procedure. An alternate procedure used in the extraction of preribosomal particles was a modification (Prestayko *et al.*, 1972) of the method of Liau and Perry (1969). Nucleoli were suspended at a concentration of approximately 1 mg/ml in acetate buffer containing 0.25 M sucrose–0.002 M $MgCl_2$–0.05 M KCl–0.01 M sodium acetate (pH 6.0). Deoxyribonuclease I (Worthington, Freehold, N. J.) was added to a concentration of 10 μg/ml of nucleolar suspension and incubated at 0° for 10 min. The mixture was centrifuged at 20,000*g* for 10 min and the pellet was resuspended to the same concentration in the above acetate buffer containing 40 μg/ml of poly(vinyl sulfate). After standing at 0° for 10 min, the mixture was centrifuged at 20,000*g* for 10 min. The pellet was then resuspended in 0.01 M KCl–0.0005 M $MgCl_2$–0.01 M Tris-HCl (pH 7.4)–0.02 M dithiothreitol at a concentration of 3 mg/ml of starting nucleolar preparation. After centrifugation at 20,000*g* for 15 min, the supernatant was underlayered with one-third volume of 1.0 M sucrose containing 0.0005 M $MgCl_2$–0.01 M KCl–0.001 M dithiothreitol–0.01 M triethanolamine-HCl (pH 7.4) and centrifuged for 16 hr at 90,000*g*. The pellet of preribosomal particles was used for protein extraction. No significant differences in proteins were seen between pelleted preribosomal particles and those prepared by sucrose density gradient centrifugation as previously described (Prestayko *et al.*, 1972).

Acetic Acid Extraction of Proteins. Precipitated ribosomal subunits were collected from the ethanol by centrifugation at 27,000*g* for 15 min and the ethanol was evaporated in air. Whole ribosomes, ribosomal subunits, and nucleolar particles were extracted twice in 40 volumes of 66% acetic acid containing 0.1 M magnesium acetate and 0.005 M dithiothreitol; this extraction medium has been shown to extract ribosomal proteins quantitatively (Hardy *et al.*, 1969; Tsurugi *et al.*, 1973). The extraction mixture was incubated each time at 4° with continuous stirring for 8 hr, after which insoluble RNA precipitate was removed by centrifugation at 27,000*g* for 15 min. Supernatants were combined and precipitated overnight with 4 volumes of 100% ethanol, after which the precipitate which contained more than 95% of the protein was collected by centrifugation, washed twice with 100% ethanol, air-dried, and stored as dry powder. An alternate procedure in which the acid extract was dialyzed against 0.9 N acetic acid contain-

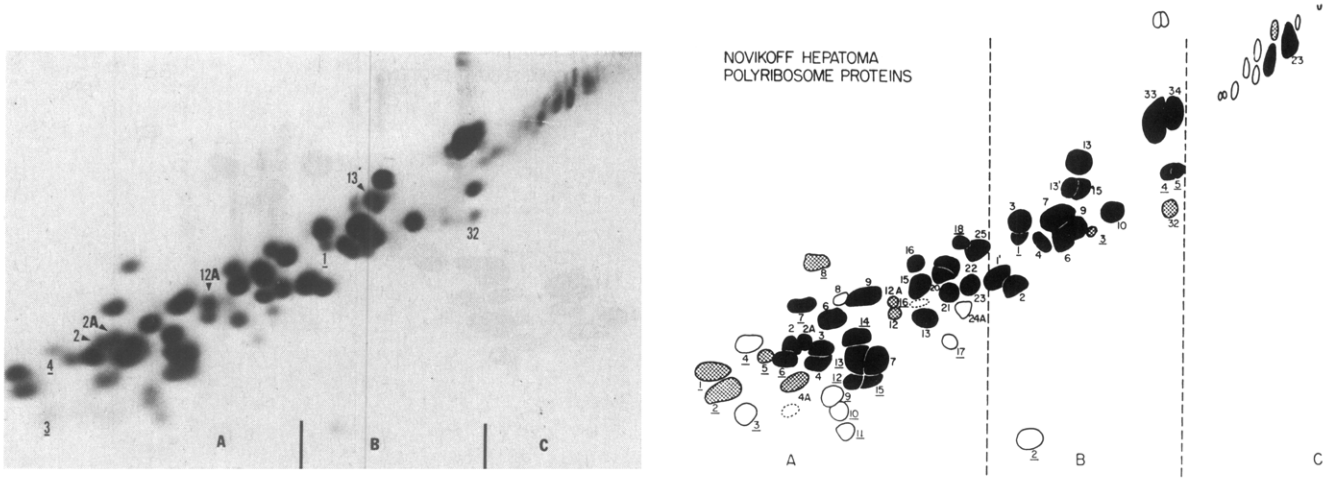

FIGURE 1: (a) Two-dimensional polyacrylamide gel electrophoresis of 250 μg of Novikoff hepatoma polyribosomal protein (left diagram). Samples were run in the first dimension on disc gels of 10% acrylamide, 6 M urea, and 0.9 N acetic acid (pH 2.5) at 120 V for 5.0 hr. For the second dimension, a 12% acrylamide, 0.1% sodium dodecyl sulfate slab gel was run for 14 hr at 50 mA/slab. Gels were stained with Coomassie Brilliant Blue R. (b) Diagrammatic representation of the electrophoretic pattern of Figure 1a (right diagram). The most dense spots are black, the less dense spots are crosshatched, the even less dense spots are open circles, and minor or faint spots are broken circles. The gel was arbitrarily divided into A, B, and C regions (Orrick *et al.*, 1973) on the basis of mobility of spots A 24 and B 34. The ribosomal spots with underlined numbers were not found in nucleoli. The spots with prime numbers were found in nucleolar preribosomal proteins but were not present in sufficient concentration to be detected in whole nucleolar acid extracts.

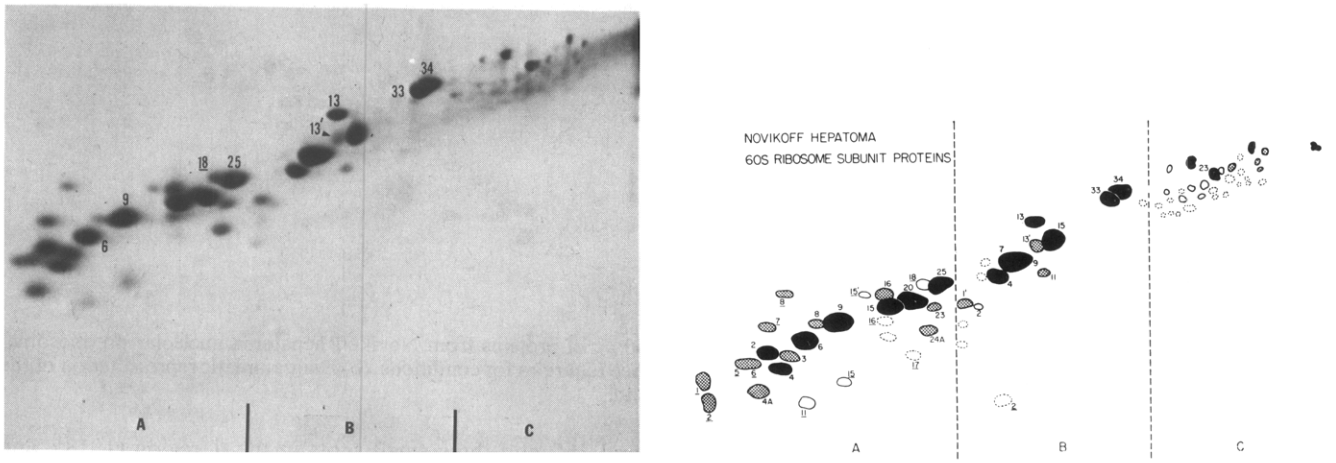

FIGURE 2: (a) Two-dimensional gel electrophoresis of 250 μg of Novikoff hepatoma ribosomal large (60 S) subunit proteins (left diagram). See Figure 1a for conditions. (b) Diagrammatic representation of the electrophoretic pattern of Figure 2a (right diagram). See Figure 1b for legend.

ing 4 M urea and then concentrated by Amicon filtration gave the same results. When comparable extractions were performed with 0.4 N H_2SO_4, the gel patterns obtained were essentially the same.

Two-Dimensional Polyacrylamide Gel Electrophoresis. Protein samples obtained by ethanol precipitation were dissolved 18–24 hr prior to electrophoresis at a concentration of 5 mg/1.0 ml in 0.9 N acetic acid containing 10 M urea. Samples prepared by Amicon filtration were layered directly on the gel. Two-dimensional polyacrylamide gels were prepared and run by the method of Orrick *et al.* (1973); the pH of the first dimension was 2.5 and in the second dimension, in sodium dodecyl sulfate, migration is inversely proportional to size. Although this system has some limitations in resolving power (Busch *et al.*, 1974), it is the only satisfactory system available at present for resolution of the many nucleolar proteins (Orrick *et al.*, 1973).

Results

Ribosomal Proteins. The acetic acid extractable proteins of polyribosomes were separated by two-dimensional gel electrophoresis (Figure 1a). A composite drawing of the stained gel is shown in Figure 1b. Approximately 60 protein spots were found and divided into the A, B, and C regions with greatest, intermediate, and lowest electrophoretic mobilities, respectively, and assigned numbers corresponding to those described by Orrick *et al.* (1973).

To determine which proteins are localized to the individual subunits, the subunits were isolated and the migration of their individual proteins was determined. The two-dimensional electrophoresis patterns of proteins of the large (60 S) ribosomal subunit are shown in Figure 2a[1] and b and those of the small (40 S) ribosomal subunit are shown in Figure 3a and b. The large and small ribosomal subunits contain 35 and 23 proteins, respectively, when prepared by the EDTA procedure. Most of the polyribosomal proteins were found in

[1] Rapidly migrating proteins 1 and 2 were run off the gel in an attempt to resolve better the proteins of the B and C regions. The nomenclature of 60 S and 40 S for ribosomal subunits is used as representative values of mammalian large and small ribosomal subunits, respectively. It is realized that the sedimentation values for these subunits prepared with EDTA is considerably less, *i.e.*, 50 S and 30 S as reported by Hamilton *et al.* (1971).

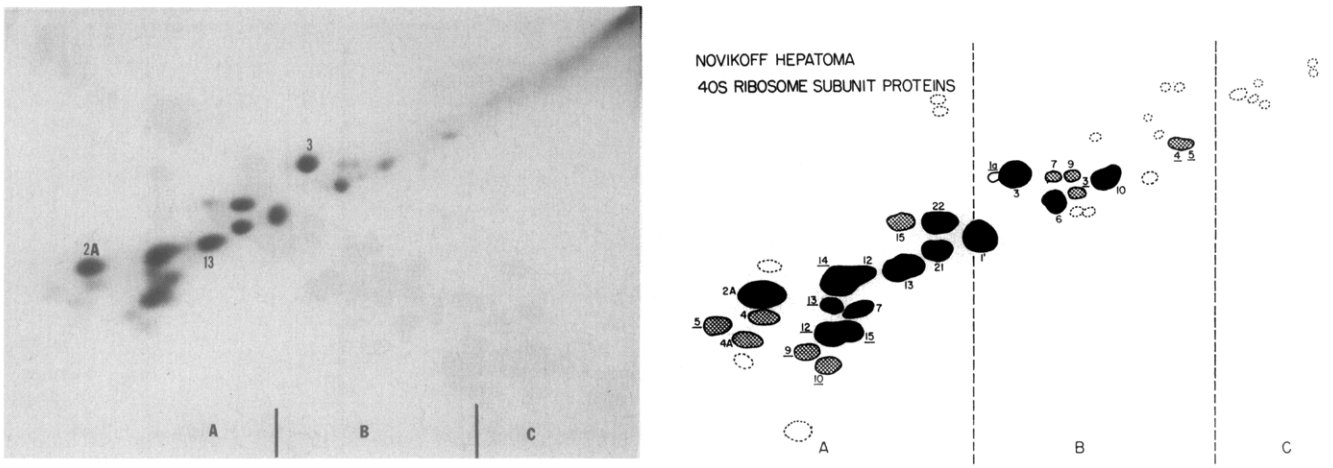

FIGURE 3: (a) Two-dimensional polyacrylamide gel electrophoresis of 250 μg of Novikoff hepatoma ribosomal small (40 S) subunit proteins (left diagram). See Figure 1a for conditions. (b) Diagrammatic representation of the electrophoretic pattern of Figure 3a (right diagram). See Figure 1b for legend.

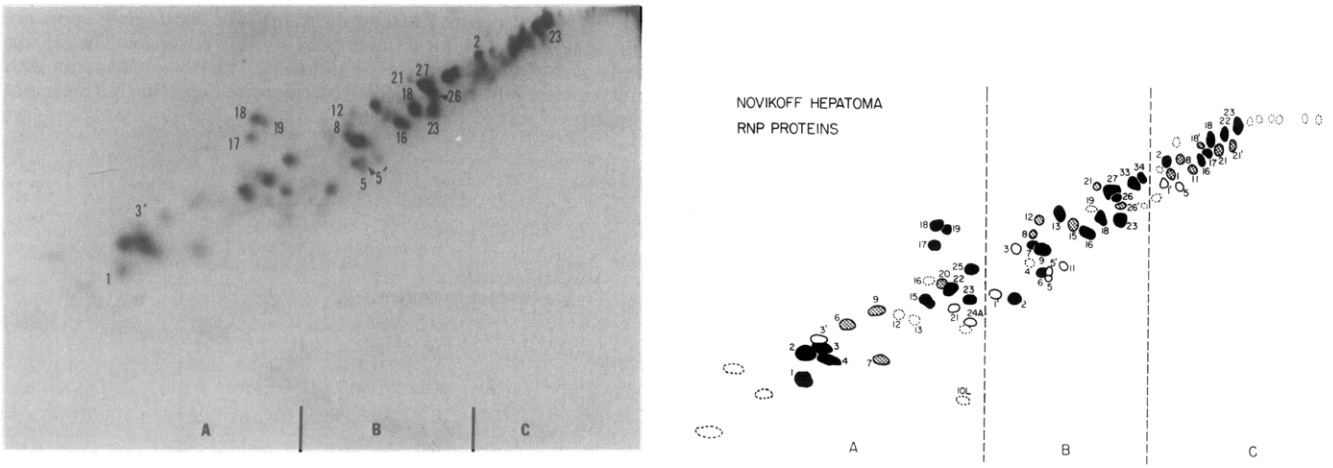

FIGURE 4: (a) Two-dimensional polyacrylamide gel electrophoresis of 250 μg of proteins from Novikoff hepatoma nucleolar preribosomal (nucleolar RNP) particles prepared by the EDTA method (left diagram). See Figure 1a for conditions. (b) Diagrammatic representation of the electrophoretic pattern of Figure 4a (right diagram). See Figure 1b for legend.

TABLE I: Proteins of Ribosomal Subunits.

Region	Small Ribosomal Subunit (40 S)	Large Ribosomal Subunit (60 S)
A	2A,4,4A,5,7,9,10, 12,12,13,13,14, 15,15,21,22	1,2,2,3,4,4A,5, 6,6,7,8,8,9,11, 15,15,16,16,17, 18,20,23,24A,25
B	1′,3,3,4,5,6,10	2,2,4,7,9,13,13′, 15,33,34
C		23

ribosomal subunits[2] (Figure 1b and Table I) except for proteins A 3, 4, and 12A; B 1 and 32.

Nucleolar Preribosomal Particle Proteins. A two-dimensional gel electrophoresis pattern of total nucleolar preribosomal particle proteins is shown in Figure 4a. A composite of this pattern (Figure 4b) indicates that a total of 60 distinct protein spots were resolved. Three small spots in the B region (1′,5′, and 26′) and three small spots in the C region (1′,18′, and 21′) were not detected in extracts of whole nucleoli and have been assigned prime numbers. All of the other proteins have been observed in extracts of whole nucleoli and have the corresponding spot number of Orrick *et al.* (1973).

A comparison of proteins of the nucleolar preribosomal particles and proteins of the ribosomal subunits is shown in Table II. For the most precise comparisons, subunit and nucleolar proteins were coelectrophoresed. Of the 60 spots of the nucleolar particles, 21 are present in the large ribosomal subunit and 10 in the small ribosomal subunit. Two of these in the A region (4 and 15) appear to be present in both subunits. The remaining proteins which are present only in the nucleolar particles are A 1, 3′, 17, 18, and 19; B 5, 5′, 8, 12, 16, 18, 19, 21, 23, 26, 26′, and 27; C, all spots in this region except 23.

Polyribosomal Proteins Absent from Nucleolar Preribosomal Particles. Although many of the proteins of the nucleolar preribosomal particles are present in the polyribosomes, a substantial number of proteins are apparently added after the nucleolar products leave the nucleolus and possibly the nucleus. These proteins include A, 1, 2, 2A, 3, 4, 4A, 5, 6, 7, 8, 9, 10, 11, 12, 12A, 13, 14, 15, 16, 17, 18; B, 1, 2, 3, 4, 5, 6.

Comparison of Proteins from Nucleolar Preribosomal Particles Isolated in the Presence of EDTA and Poly(vinyl sulfate). Preribosomal particles were extracted from Novikoff hepa-

[2] Polyribosomal proteins in the C region except spot 23 are not reproducibly seen on the two-dimensional gels. This may represent varying amounts of these proteins on polysomes in different preparations or may represent aggregates formed during isolation and electrophoresis of proteins.

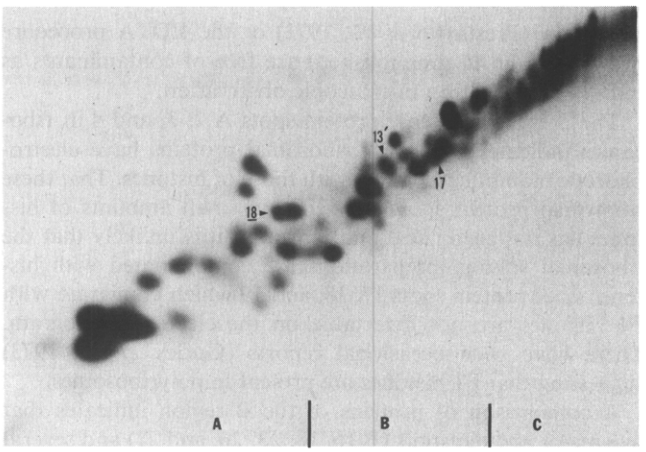

FIGURE 5: Two-dimensional polyacrylamide gel electrophoresis of proteins from Novikoff hepatoma nucleolar preribosomal particles prepared by the poly(vinyl sulfate) procedure.

toma nucleoli using poly(vinyl sulfate) instead of EDTA in the procedure (Prestayko *et al.*, 1972, 1973). Proteins were extracted with acetic acid as described in Materials and Methods and analyzed by two-dimensional polyacrylamide gel electrophoresis (Figure 5).

The spot pattern of Figure 5 compares well with that in Figure 4a with a few exceptions. A striking difference seen between the EDTA and poly(vinyl sulfate) in Figures 4a and 5 is the presence of three dense spots, A 18, B 13′, and B 17 in Figure 5 and the absence of these spots in Figure 4a. Two-dimensional polyacrylamide gel electrophoresis was carried out on protein released from ribosomes by EDTA treatment. Protein which remained near the top of the sucrose density gradient after centrifugation migrated as one major spot in the same position as spot B 13′. When protein markers of known electrophoretic mobility were electrophoresed in the second dimension, spot B 13′ was shown to have a molecular weight of about 40,000, which is very similar to that reported for the 5S RNA binding protein from rat liver (Petermann *et al.*, 1972).

TABLE II: Proteins from Nucleolar Preribosomal Particles[a] Which Are Present in Ribosomal Subunits.

Region	Small Ribosomal Subunit (40 S)	Large Ribosomal Subunit (60 S)
A	4,7,12,13,15,21,22	2,3,4,6,8,15,16, 20,23,24A,25
B	1′,3,6	2,4,7,9,11,13,15, 33,34
C		23

[a] These comparisons were made with preribosomal particles prepared by the EDTA method.

Discussion

Figure 6 shows a current view of the processing of nucleolar ribosomal precursor RNA and the maturation of preribosomal particles which is under continuing study (Perry, 1962; Scherrer and Darnell, 1963; Tamaoki, 1966; Warner and Soeiro, 1967; Darnell, 1968; Perry, 1962; Busch and Smetana, 1970; Mirault and Scherrer, 1971; Auger and Tiollais, 1973). The presence of low molecular weight RNAs in nucleolar preribosomal particles has also been reported (Warner and Soeiro, 1967; Prestayko *et al.*, 1970; Busch *et al.*, 1971). Ribosomal proteins are believed to be synthesized in the cytoplasm and are transported to the nucleolus where they

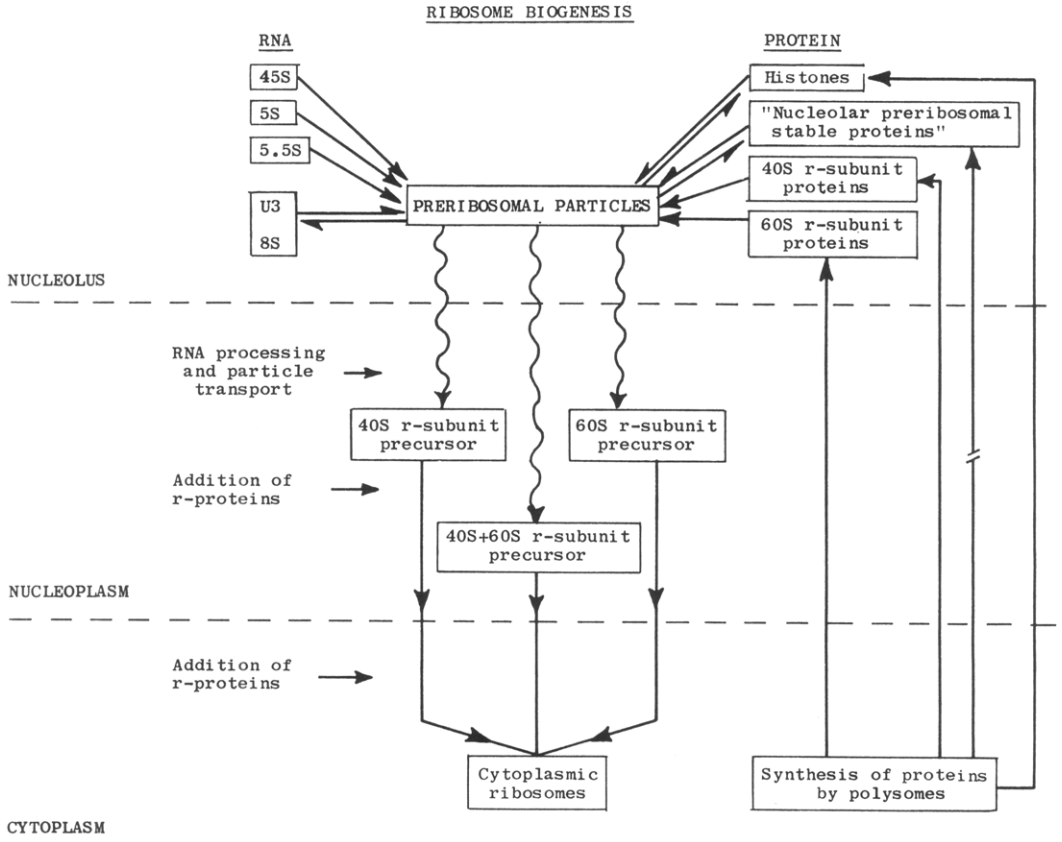

FIGURE 6: Schematic representation of events involved in the synthesis and transport of ribosomes in eukaryotic cells.

TABLE III: Number of Proteins Separated by Two-Dimensional Gel Electrophoresis.

Sample	Proteins in Regions of the Gel			Total Protein Spots
	A	B	C	
Nucleolar particles	22	24	14	60
Polyribosomes	38	19	7	64
60S ribosomal subunit	24	10	1	35 37[a]
40S ribosomal subunit	16	7		23 26[a]

[a] These are the number of proteins reported by Martini and Gould (1971) to be present in the large and small ribosomal subunits from rabbit reticulocytes using a very similar electrophoresis procedure.

accumulate and form a complex with newly synthesized ribosomal precursor RNA (Craig and Perry, 1971; Maizel and McConkey, 1971; Kumar and Warner, 1972; Soeiro and Basile, 1973). These preribosomal proteins vary with respect to turnover, *i.e.*, those nucleolar proteins that become associated with cytoplasmic ribosomes turn over more rapidly than the "nucleolar stable" proteins which were found to remain in the nucleolus for much of the cell generation time. A recent study showed that in contrast to ribosomal proteins, the accumulation of "nucleolar stable" proteins in nucleoli is unaffected by actinomycin D (Soeiro and Basile, 1973). Furthermore, the demonstration of protein synthesis in isolated nucleoli (Lamkin *et al.*, 1973) may be related to intranucleolar synthesis of these proteins. This group of "nucleolar stable" proteins has been suggested to be involved in the processing mechanism of ribosome precursors.

This study was undertaken to more clearly define the nucleolar stable proteins of preribosomal nucleolar particles. The increased resolving power of the two-dimensional polyacrylamide electrophoresis technique (Kaltschmidt and Wittman, 1970) has made possible a more precise determination of the similarities and differences of proteins of ribosomal subunits and nucleolar preribosomal particles. The EDTA extraction method (Warner and Soeiro, 1967) for isolation of nucleolar particles was chosen for this comparison since the dissociation of polyribosomes into ribosomal subunits was effected by EDTA.

Many of the proteins common to nucleolar preribosomal particles and ribosomal subunits were shown to migrate in the A and B regions of the gel (Figures 2–4). Table III summarizes the number of proteins in each of the fractions studied. Approximately 21 proteins of the large ribosomal subunit and 10 proteins of the small ribosomal subunit are present in nucleolar preribosmal particles.

Nonribosomal proteins which are present in nucleolar preribosomal particles include proteins A 1, 3′, 17, 18, and 19 of the A region of the gel. Recently it has been reported (Goldknopf and Busch, 1973) that proteins A 1, 2, 4, 17, 18, and 19 comigrate on two-dimensional gel electrophoresis with Novikoff hepatoma nuclear histones. Results of this study suggest that histones or histone-like proteins are associated with nucleolar preribosomal particles and are not present because of contamination of the preparation with nucleolar or nuclear elements. Previous studies demonstrated that nucleolar preribosomal particles isolated by the poly(vinyl sulfate) procedure (Prestayko *et al.*, 1972) or the EDTA procedure (Y. Daskal *et al.*, unpublished) are free of contaminants as indicated by electron microscopic observation.

The presence of dense protein spots A 2, 3, and 4 in ribosomes indicates that these ribosomal proteins have electrophoretic mobilities identical with those of histones. That these ribosomal protein spots may contain small amounts of histones has not been ruled out. However, it is unlikely that the ribosomal subunit preparations are contaminated with histones since protein spots 17, 18, and 19 which comigrate with F1 histones are not detectable on the electrophoretogram. There have been occasional reports (Gurley *et al.*, 1973) suggesting that F1 histones are present in polyribosomes.

A comparison of proteins of the B region indicates that five major protein spots (B 16, 18, 23, 26, and 27) and several minor spots (B 5, 5′, 8, 12, 19, 21, and 26′) are present only in nucleolar preribosomal particles. Tsurugi *et al.* (1973) reported that four major proteins and several minor proteins were present in nucleolar 60S particles which were not found in the cytoplasmic large ribosomal subunit.

Proteins in the C region of the gels, except for spot C 23, appear to be uniquely present in nucleolar particles. These proteins have a high molecular weight and hence a low migration into the 12% sodium dodecyl sulfate gel. The presence of a larger number of nucleolar preribosomal specific proteins in this study as compared to that of Tsurugi *et al.* (1973) may reflect a different composition of nucleolar stable proteins for nucleolar particles which contain proteins common to both large and small ribosomal subunits than for those nucleolar particles containing proteins common to only the large ribosomal subunit.

The patterns of the proteins of nucleolar preribosomal particles appeared to be similar when isolated either by the poly(vinyl sulfate) or the EDTA procedure. However, protein spots A 18, B 13′, and B 17 were not present when the particles were isolated by the EDTA procedure. These results suggest that these three proteins are probably bound to nucleolar particles by divalent cations, particularly magnesium, which is included in the isolation media containing poly(vinyl sulfate).

The presence of spot B 13′ in Figure 5 and the absence of this spot in Figure 4 suggests that this protein is removed by the EDTA procedure. Several reports have established that 5S RNA is found in close association with a specific protein in ribosomes of mammalian cells and this complex is removed from the large ribosomal subunit after treatment with EDTA[3] (Bloebel, 1971; Lebleu *et al.*, 1971; Petermann *et al.*, 1972). The nucleolar spot B 13′ comigrates with a major protein spot released from Novikoff hepatoma ribosomes by the EDTA procedure. Its molecular weight on the basis of migration in the second dimension sodium dodecyl sulfate gel is about 40,000, which is similar to that reported for the 5S RNA binding protein from rat liver (Petermann *et al.*, 1972). Accordingly, this complex is apparently present in the nucleolar preribosomal particles. Our previous studies (Busch *et al.*, 1971) and those of Warner and Soeiro (1967) showed that 5S ribosomal RNA was associated with nucleolar preribosomal particles prepared by either the poly(vinyl sulfate) or EDTA method, respectively. The dissociation from ribosomes by EDTA of the complex of 5S RNA and bound protein as opposed to the apparent dissociation from nucleo-

[3] A faint spot of B 13′ can be seen in the pattern of large ribosomal subunits (Figure 2a) and may represent incomplete removal of this protein from ribosomes by EDTA.

lar particles of only the 5S RNA binding protein by EDTA may suggest a different binding of 5S RNA to protein in nucleolar particles.

The results of these and other studies demonstrate that individual ribosomal proteins of eukaryotes can be separated and identified. Studies on the structural organization of proteins and RNA in eukaryote ribosomes analogous to those reviewed by Kurland (1972) and Garrett and Wittman (1973) in prokaryotes are now feasible. The availability of methods for isolation and characterization of ribosome precursors from nucleoli now permits further studies on ribosome biogenesis. Studies of the binding of "core" or "structural" ribosomal proteins to specific regions of ribosomal precursor RNA should provide further information on the mechanism of processing of nucleolar ribosomal precursor RNA and the maturation of preribosomal particles.

Acknowledgment

The authors thank Dr. Miklos Kellermayer for making molecular weight estimations of proteins in the sodium dodecyl sulfate gel and Mrs. Rose K. Busch for supplying the transplanted tumors.

References

Auger, M. A., and Tiollais, P. (1973), *Biochimie 55*, 163.
Ballal, N. R., and Busch, H. (1973), *Cancer Res. 33*, 2737.
Bloebel, G. (1971), *Proc. Nat. Acad. Sci. U. S. 68*, 1881.
Bloebel, G., and Sabatini, D. (1971), *Proc. Nat. Acad. Sci. U. S. 68*, 390.
Busch, G. I., Yeoman, L. C., Taylor, C. W., and Busch, H. (1974), *Physiol. Chem. Phys.* (in press).
Busch, H. (1967), *Methods Enzymol. A 12*, 448.
Busch, H., Ro-Choi, T. S., Prestayko, A. W., Shibata, H., Crooke, S. T., El-Khatib, S. M., Choi, Y. C., and Mauritzen, C. M. (1971), *Perspect. Biol. Med. 15*, 117.
Busch, H., and Smetana, K. (1970), The Nucleolus, New York, N. Y., Academic Press.
Craig, N. C., and Perry, R. P. (1971), *Nature (London), New Biol. 229*, 75.
Darnell, J. E. (1968), *Bacteriol. Rev. 32*, 262.
Das, N. K., Micou-Eastwood, J., Ramamurthy, G., and Alfert, M. (1970), *Proc. Nat. Acad. Sci. U. S. 67*, 968.
Delaunay, J., Mathiew, C., and Schapira, G. (1972), *Eur. J. Biochem. 31*, 561.
Delaunay, J., and Schapira, G. (1972), *Biochim. Biophys. Acta 259*, 243.
Garrett, R. A., and Wittman, H. G. (1973), *Endeavour 32*, 8.
Goldknopf, I. L., and Busch, H. (1973), *Physiol. Chem. Phys. 5*, 131.
Gurley, L. R., Enger, M. D., and Walters, R. A. (1973), *Biochemistry 12*, 237.
Hamilton, M. G., Pavlovec, A., and Petermann, M. L. (1971), *Biochemistry 10*, 3424.
Hardy, S. J. S., Kurland, C. G., Voynow, P., and Mora, G. (1969), *Biochemistry 8*, 2906.
Hulten, T., and Sjoquist, A. (1972), *Anal. Biochem. 46*, 342.
Kaltschmidt, E., and Wittman, H. G. (1970), *Proc. Nat. Acad. Sci. U. S. 67*, 1276.
Koshiba, K., Thirumalachary, C., Daskal, Y., and Busch, H. (1971), *Exp. Cell Res. 68*, 235.
Kumar, A., and Warner, J. R. (1972), *J. Mol. Biol. 63*, 233.
Kurland, C. G. (1972), *Annu. Rev. Biochem. 41*, 377.
Lamkin, A. F., Smith, D. W., and Hurlbert, R. B. (1973), *Biochemistry 12*, 4137.
Lebleu, B., Marbaix, G., Huez, G., Temmerman, J., Burny, A., and Chantrenne, H. (1971), *Eur. J. Biochem. 19*, 264.
Liau, M. C., and Perry, R. P. (1969), *J. Cell Biol. 42*, 272.
Maizel, J. C., and McConkey, E. H. (1971), *J. Mol. Biol. 61*, 251.
Martini, O. H. W., and Gould, H. J. (1971), *J. Mol. Biol. 62*, 403.
Mirault, M. E., and Scherrer, K. (1971), *Eur. J. Biochem. 23*, 372.
Orrick, L. R., Olson, M. O. J., and Busch, H. (1973), *Proc. Nat. Acad. Sci. U. S. 70*, 1316.
Perry, R. P. (1962), *Proc. Nat. Acad. Sci. U. S. 48*, 2179.
Petermann, M. L., Hamilton, M. G., and Pavlovec, A. (1972), *Biochemistry 11*, 2323.
Prestayko, A. W., Lewis, B. C., and Busch, H. (1972), *Biochim. Biophys. Acta 269*, 90.
Prestayko, A. W., Lewis, B. C., and Busch, H. (1973), *Biochim. Biophys. Acta 319*, 232.
Prestayko, A. W., Tonato, M., and Busch, H. (1970), *J. Mol. Biol. 47*, 505.
Rodgers, A. (1973), *Biochim. Biophys. Acta 294*, 292.
Scherrer, K., and Darnell, J. E. (1963), *Proc. Nat. Acad. Sci. U. S. 49*, 240.
Shankarnarayan, K., and Birnstiel, M. L. (1969), *Biochim. Biophys. Acta 190*, 470.
Shepherd, J., and Maden, B. E. H. (1972), *Nature (London) 236*, 211.
Sherton, C. C., and Wool, I. G. (1972), *J. Biol. Chem. 247*, 4660.
Soeiro, R., and Basile, C. (1973), *J. Mol. Biol. 79*, 507.
Steele, W. J., and Busch, H. (1966), *Biochim. Biophys. Acta 119*, 501.
Tamaoki, T. (1966), *J. Mol. Biol. 15*, 624.
Tsurugi, K., Morita, T., and Ogata, K. (1973), *Eur. J. Biochem. 32*, 555.
Warner, J. R., and Soeiro, R. (1967), *Proc. Nat. Acad. Sci. U. S. 58*, 1984.
Welfe, H., Stahl, J., and Bielka, H. (1971), *Biochim. Biophys. Acta 243*, 416.
Welfle, H., Stahl, J., and Bielka, H. (1972), *FEBS (Fed. Eur. Biochem. Soc.) Lett. 26*, 228.
Yoeman, L. C., Taylor, C. W., and Busch, H. (1973), *Biochem. Biophys. Res. Commun. 51*, 956.