ULTRASTRUCTURAL AND BIOCHEMICAL STUDIES ON THE PRECURSOR RIBOSOMAL PARTICLES ISOLATED FROM RAT LIVER NUCLEOLI

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ABSTRACT

Sucrose density gradient analyses of pH 5.5 and pH 7.4 extracts from rat liver nucleoli revealed the presence of two broad peaks of approximately 60S and 80S, and 60S and 80–100S, respectively. Ribonucleoprotein (RNP) particles containing precursor ribosomal RNA in these peaks have been characterized by electron microscopy and RNA analyses. Spherical particles only were found in the 60S peak of the pH 5.5 extract, from which 28S RNA and smaller RNA (23S and 18S RNA) exclusively were extracted. In the broad 80S peak of the pH 5.5 extract, about 60% of the particles were spherical while 30% were rodlike. In the RNA species present there were 28S plus smaller RNA (80%) and 35S RNA (20%). The 60Speak of the pH 7.4 extract contained mainly spherical particles (84%), and the RNA species present was mostly 28S plus smaller RNA (89%). In addition to spherical particles (43%), a number of rodlike (31%) and filamentous molecules (26%) were observed in the heavier side of the 80–100S peak of the pH 7.4 extract, from which 45S (14%), 35S (26%), and 28S and smaller RNA (60%) were extracted. Thus the precursor ribosomal particles containing 45S RNA and 35S RNA appear to be filamentous and rodlike molecules, respectively. Folding of loose ribonucleoprotein filaments into compact, spherical, large subparticles may be part of the maturation process of ribosomal large subparticles, in addition to the so-called sequential cleavage of RNA.

It has been well established that eukaryotic ribosomal RNA is synthesized in the form of a large common precursor RNA (45S RNA) in the nucleolus and then, through a number of steps, is processed to mature 28S and 18S RNA (see reviews by Darnell (1), Maden (2), and Perry (3, 4). These precursor rRNAs are present as ribonucleoprotein (RNP) complexes, such as have been isolated from HeLa cells (5, 6, 7), L cells (8, 9, 10), ascites tumor cells (11), and liver cells (12, 13, 14). The buoyant density of the RNP particles was definitely less than that of mature ribosomes, and it has been proposed that during the maturation of ribosomes in the nucleoli the reduction in RNA size is accompanied by a progressive diminution in this protein-to-RNA ratio (10). Recently Kumar and Warner (6) have shown that nucleolar precursor ribosomal particles have extra proteins in

addition to the majority of the ribosomal proteins fround on the cytoplasmic large ribosomal subparticles.

Ultrastructural and autoradiographic studies of nucleoli *in situ* or of isolated nucleoli have shown that nucleoli are composed of a fibrillar component, a granular component, and nucleolus-associated chromatin (15, 16, 17), and that the fibrillar component is a precursor of the granular component; [³H]uridine is first incorporated into the fibrillar component and then transferred to the granular component (18-21).

Narayan et al. (12) and Koshiba et al. (13) isolated RNP particles from rat liver nucleoli and observed them. Their RNP particles, however, contained mainly 30-32S and 28S RNA.

In this report, ribosomal precursor particles containing 45S and 35S RNA were extracted from rat liver nucleoli in an attempt to clarify the biosynthesis and maturation of ribosomes in nucleoli.

MATERIALS AND METHODS
Isolation of Nuclei and Nucleoli

Male albino rats (Donryu strain) weighing 200-300 g were fed laboratory chow ad libitum, and fasted overnight before sacrifice. Nuclei and nucleoli were isolated from the perfused livers in the presence of magnesium ion, according to the procedure of Higashinakagawa et al. (22). The recovery of nuclei from the liver homogenate was about 60%, whereas recovery from the nuclear suspension was 30%.

Extraction of RNP Particles from Isolated Nucleoli

RNP particles were extracted from nucleoli according to the procedures of Muramatsu et al. (14). The nucleolar pellet obtained from 40 g of liver was mixed with 50 μl of 0.1% DNase solution (Worthington Biochemical Corp., Freehold, N.J., electrophoretically purified, RNase-free), and then homogenized by 10 strokes of a motor-driven Potter-Elvehjem homogenizer with a Teflon pestle, in 2.0 ml of pH 5.5 extraction medium (50 mM sodium acetate buffer (pH 5.5), 25 mM KCl, 0.1% polyvinylsulfate (PVS), and 1% Brij 98). After being left standing at 0°C for 10 min, the suspension was centrifuged at 12,000 g for 10 min and the supernate was stored as the pH 5.5 extract. The pellet was resuspended in 2.0 ml of pH 7.4 extraction medium (50 mM Tris-HCl buffer [pH 7.4], 25 mM KCl, 0.1% PVS, and 1% Brij 98), and after 10 min in an ice-bath the suspension was centrifuged at 12,000 g for 10 min, and the supernate was stored as the pH 7.4 extract for further use.

Fractionation of the Nucleolar Extracts by Sucrose Density Gradient Centrifugation

A 2-ml portion from each extract was layered onto 16 ml, 10-30% (wt/wt) sucrose gradients in 50 mM sodium acetate buffer (pH 5.5), 25 mM KCl, and 5 mM MgCl₂. After centrifugation at 23,000 rpm for 12-15 h in a Hitachi RPS 25-3 rotor, the gradients were monitored at 260 nm from the top to the bottom and fractionated into about 30 tubes by an Auto Densiflow (Buchler Instruments, Fort Lee, N. J.) coupled to a Hitachi 124 spectrophotometer and a fraction collector.

Preparation of Rat Liver Ribosomal Large Subparticles and Subsequent Treatment with pH 5.5 Extraction Medium

Cytoplasmic large ribosomal subparticles were prepared from rat liver free polyribosomes according to the methods of Blobel and Sabatini (23), except that 750 mM KCl was used instead of 500 mM as control for electron microscope studies of the precursor particles.

In this experiment, the nucleolar RNP particles were prepared in the presence of 0.1% PVS and 1% Brij 98. As it is feasible that exposure to these surface-active agents may induce conformational change in the spherical large subparticles to rodlike or filamentous molecules, an aliquot of the large subparticle solution was treated with the pH 5.5 extraction medium. After several hours the medium was replaced by the conventional TKM buffer (50 mM Tris-HCl buffer, pH 7.4, 25 mM KCl, 5 mM MgCl₂), either by dialysis or by centrifugation, after which the particles were observed under the electron microscope.

Analyses of RNA by Sucrose Density Gradient Centrifugation and by Polyacrylamide Gel Electrophoresis

RNA was extracted from nucleolar RNP particles by the hot phenol-SDS method (24) and analyzed either by sucrose density gradient centrifugation or by polyacrylamide gel electrophoresis, with a composite gel of 2.25% acrylamide-0.5% agarose (25). The molecular weight was estimated from the relationship between electrophoretic mobility and molecular weight, with 28S (1.7 × 10⁶ daltons) and 18S rRNA (0.7 × 10⁶ daltons) as a standard.

Chemical Determination and Counting of Nuclei and Nucleoli

Nucleic acid and protein were extracted from precursor particles or from nucleoli by Siekevitz's procedure (26). RNA was determined by the orcinol reaction with rat liver RNA as a standard (27), DNA by Burton's modification of the diphenylamine method (28), and

protein by Lowry's method (29), with bovine serum albumin as a standard.

Isolated nuclei and nucleoli were stained with 0.1% (wt/vol) azur C in 0.25 M sucrose and 0.03% azur C in 7% sodium citrate containing 0.25 M sucrose, respectively, and were counted in a hemocytometer.

Electron Microscopy

Specimens for electron microscopy were first dialyzed against a buffer solution to remove sucrose and then were mounted directly onto a carbon-coated collodion film with a micropipette, or were sprayed from a low-pressure nebulizer.

Shadow casting with platinum-carbon, and negative staining with uranyl acetate solution were done as described previously (30). Specimens were observed in a Hitachi HU-12 electron microscope. The photographic plates were printed with the emulsion side of the plate facing the surface of the bromide paper.

RESULTS

Biochemical Studies

CHEMICAL COMPOSITION OF NUCLEOLI: Chemical composition of the nucleolar fraction (Table I) paralleled that reported by other authors (31–34), except for a higher DNA content. This is presumably due to the fact that repeated washing of the nucleolar fraction with the extraction medium was not carried out.

Nucleolar RNA was effectively extracted at pH 5.5 and pH 7.4, and the total recovery of RNA was approximately 90%. Such being the case, it is considered that the RNP particles in the extracts were indeed nucleolar RNP particles.

SUCROSE DENSITY GRADIENT ANALYSES OF RNA EXTRACTED FROM THE NUCLEOLAR

EXTRACTS: RNA was extracted by the hot phenol-SDS method and then was analyzed by sucrose density gradient centrifugation in order to characterize the species in the nucleolar extracts. Although both extracts contained 45S, 35S, 28S, 23S, and 18S RNA, the relative amounts were quite different. In the pH 5.5 extract, 28S and 35S RNA were the major RNAs, comprising 38% and 37% of the total RNA in the extract, respectively. In the pH 7.4 extract, 45S and 35S RNA were found in a larger amount (22 and 29%) in addition to 28S, 23S, and 18S RNA (18, 21, and 6%).

RNA from the nucleolar extracts was also analyzed by polyacrylamide gel electrophoresis, and bands corresponding to 45S, 35S, 32S, 28S, 23S, and 18S were identified. The molecular weights of 45S, 35S, 32S, and 23S RNA were estimated to be 4.1, 3.0, 2.1, and 0.97 × 10⁶ daltons, respectively. These values are in good agreement with the molecular weights of precursor RNAs extracted from HeLa cell nucleoli (35). Thus, the numbers of 45S and 35S RNA molecules per nucleolus were calculated to be 1.37×10^4 and 4×10^4 molecules, respectively. As these values paralleled the corresponding data of 1×10^4 and 4 $\times 10^4$ molecules for the HeLa cell nucleolus reported previously (36), serious degradation of the extracted precursor particles was ruled out.

SUCROSE DENSITY GRADIENT ANALYSES OF THE NUCLEOLAR EXTRACT: The above results show that although partial fractionation of the nucleolar RNP particles is possible by successive extraction at pH 5.5 and 7.4, these extracts were still quite heterogeneous with respect to the presence of the RNA species. Fractionation of the

TABLE I

Chemical Composition of Nucleolar and Subnucleolar Fractions

Fraction	Protein	RNA	DNA
Nucleolar fraction	0.123 (100)	0.0349 (100)	0.0640 (100)
pH 5.5 extract	0.0615 (50)	0.0168 (48)	0.0603* (94)
pH 7.4 extract	0.0425 (35)	0.0142 (41)	0.00455 (7.1)
Residue	0.0072 (5.9)	0.00278 (8)	0.00203 (3.2)

Fractions are expressed by yield in milligrams of protein, RNA, and DNA per gram of liver. The values in parentheses indicate distribution of protein, RNA, and DNA in the various subnuclear fractions (nucleolar fraction 100%).
* Although nucleoli were extracted in the presence of DNase, most DNA was recovered as acid-insoluble DNA in the pH 5.5 extract. This is attributed to the incomplete digestion of DNA. Incubation with DNase was carried out in the absence of MgCl₂ and in the presence of PVS, a potent inhibitor of DNase.

RNP particles from both extracts by sucrose density gradient centrifugation was thus attempted.

Sedimentation profiles of the pH 5.5 and pH 7.4 extracts are shown in Fig. 1 A and B, respectively. In Fig. 1 A, two broad components can be seen approximately at the 60S and 80S regions, respectively. Similarly, two broad components are present approximately at the 60S region and the 80–100S region as shown in Fig. 1 B.
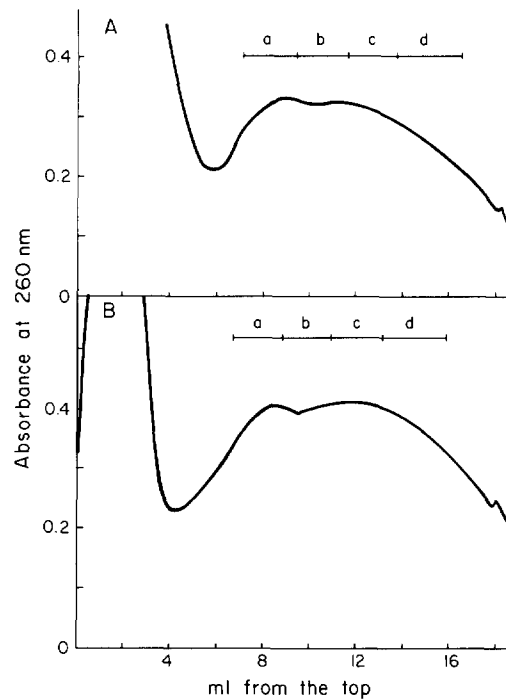

FIGURE 1 Sucrose density gradient profile of the nucleolar RNP particles extracted using pH 5.5 (A) and pH 7.4 (B) medium. Small letters above the profiles indicate the RNP particle fractions used for further analyses.

CHARACTERIZATION OF THE RNP PARTICLE FRACTIONS BY SUCROSE DENSITY GRADIENT CENTRIFUGATION: In order to characterize the RNP particles in the gradients, the 60–100S region was divided into four fractions as shown in Fig. 1: fraction a contained the 60S component; fraction b the heavier side of the 60S component and the lighter side of the 80S (pH 5.5 extract) or the 80–100S component (pH 7.4 extract); fraction c the broad peak of the 80S or the 80–100S component; and fraction d the heavier side of the 80S or the 80–100S component, respectively. The homogeneity of each fraction and the sensitivity of the RNP particles to RNase treatment were re-examined by sucrose density gradient centrifugation.

The sedimentation patterns showed fraction 5 a to be rather homogeneous and composed mainly of the 60S component, whereas the other fractions were heterogeneous and composed of at least two of the 60S and 80S or the 80–100S components. The heavier component was more dominating in the faster-sedimenting fractions. The incubation of these fractions at 0°C for 15 min with 2 μg of RNase markedly reduced the area of the 80S or 80–100S component. The effect of RNase digestion was, therefore, more clearly demonstrated in fraction d than in fraction a.

The homogeneity of the RNP particle fractions was also examined by sucrose density gradient centrifugation. As shown in Table II, all the fractions contained more than one species of RNA, and it was not possible to isolate precursor particles containing a single species of 45S or 35S RNA. It was clear, however, that the faster-sedimenting fractions (fractions c and d), especially from the pH 7.4 extract, contained more 35S and 45S RNA than the slower-sedimenting fractions fractions a and b).

TABLE II

Distribution of Various Species of RNA in Fractions 5 a–5 d and 7 a–7 d

RNA	pH 5.5 extract				pH 7.4 extract			
Fraction	5a	5b	5c	5d	7a	7b	7c	7d
45S	0 (0)	0 (0)	0 (0)	8 (4)	0 (0)	9 (3)	19 (8)	30 (14)
35S	0 (0)	23 (12)	38 (20)	48 (30)	23 (9)	40 (20)	44 (26)	40 (26)
28S	65 (49)	52 (46)	36 (33)	28 (31)	21 (15)	12 (11)	13 (14)	11 (13)
23S	30 (42)	15 (23)	17 (27)	10 (19)	46 (58)	29 (46)	18 (34)	13 (27)
18S	5 (10)	9 (19)	9 (20)	6 (16)	10 (17)	9 (20)	7 (18)	7 (20)

The figures with and without parentheses indicate the relative numbers and relative amounts of each species of RNA in the fraction, respectively. The former values were calculatd by dividing the relative amount of each RNA by the corresponding molecular weight.
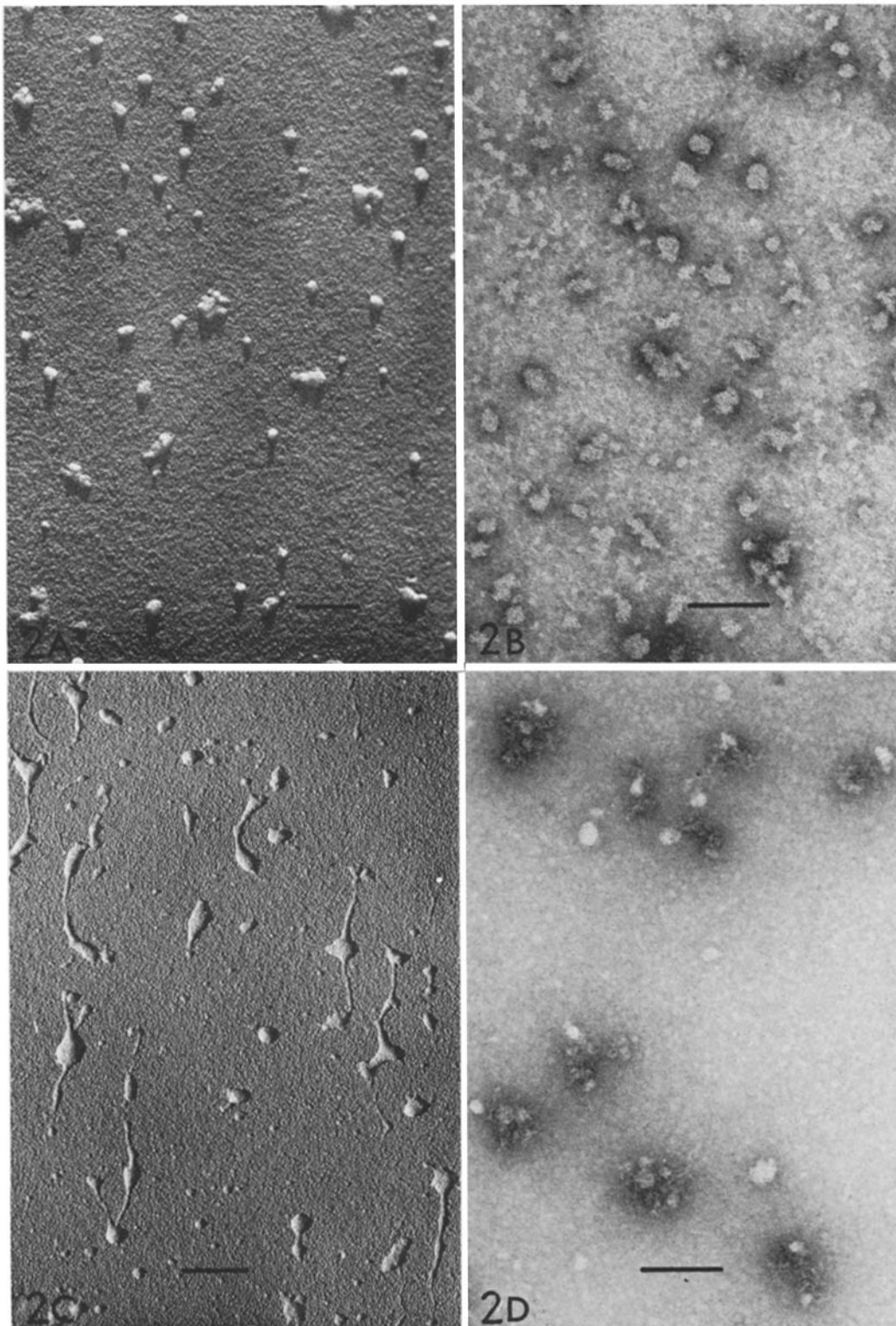
FIGURE 2 General views of precursor RNP particles in fraction 7a (2 A, 2 B) and 7d (2 C, 2 D). Fig. 2 A, Shadow-cast image of fraction 7 a. × 50,000. Bar, 2,000 Å. Fig. 2 B, Negatively stained image of fraction 7 a. × 125,000. Bar, 1,000 Å. Fig. 2 C, Shadow-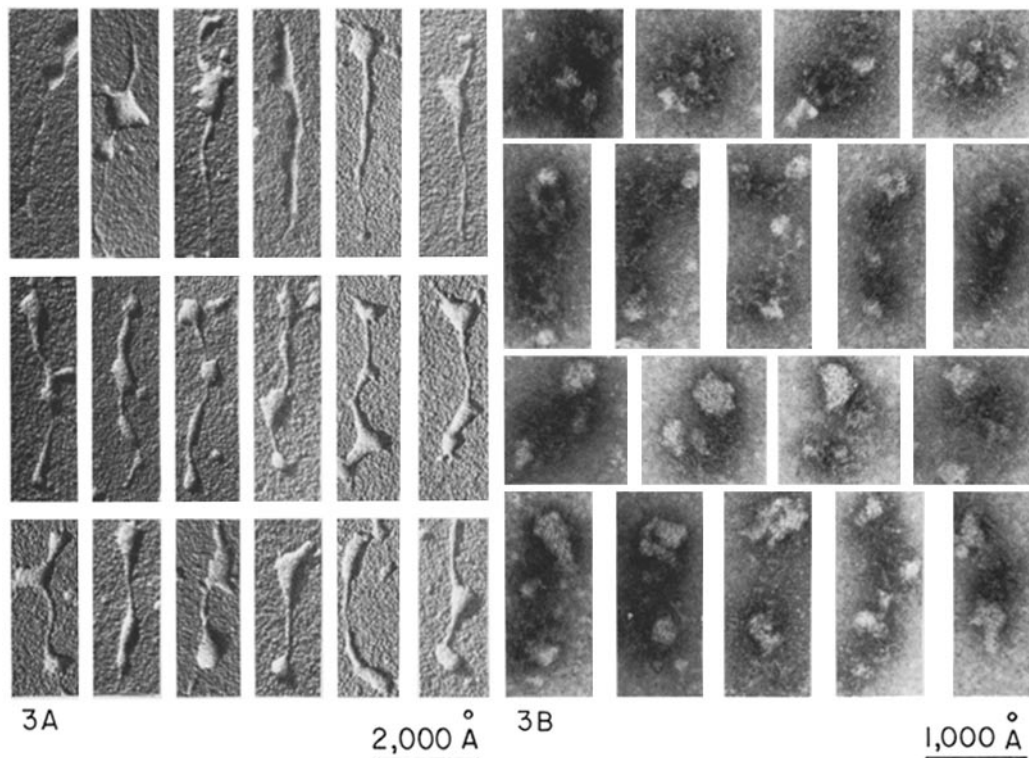cast image of fraction 7 d. × 50,000. Bar, 2,000 Å. Fig. 2 D, Negatively stained image of fraction 7 d. × 125,000. Bar, 1,000 Å.

FIGURE 3 Gallery of selected shadow-cast images (3 A) and negatively stained images (3 B) of filamentous or Group 1 precursor RNP particles. Fig. 3 A, × 60,000. Fig. 3 B, × 150,000.

Electron Microscope Studies

GENERAL VIEWS OF THE VARIOUS FRAC-
TIONS FROM THE PH 7.4 EXTRACT: Nucleolar
extracts at pH 5.5 and 7.4 were fractionated into
four: 5 a–d and 7 a–d, respectively, and the
specimens from all fractions were examined in the
electron microscope.

Fig. 2 A, B, and Fig. 2 C, D are the electron
micrographs of fraction 7 a and fraction 7 d,
respectively. It is evident that the RNP particles in
fraction 7 a are either spherical or polygonal and
appear compact as judged from the shadow length.
There are also rodlike particles, as well as aggre-
gates of the spherical particles. The particles in
fraction 7 d are much more heterogeneous than
those in fraction 7 a as shown in Fig. 2 C and D; in
addition to spherical particles similar to those in
fraction 7 a, there are a number of filamentous and
rodlike molecules (Fig. 2 C) which are pleo-
morphic and quite loose as judged from the
shadow length. In the negatively stained images
(Fig. 2 D), these molecules appear as loosely
coiled, threadlike molecules with no compact

configuration. Fractions 7 b and 7 c revealed an
image intermediate between that of fraction 7 a
and that of fraction 7 d; filamentous molecules
were more frequently observed in fraction 7 c than
in fraction 7 b, and the particles in fractions 7 b
and 7 c were looser and more heterogeneous than
those in fraction 7 a.

GENERAL VIEWS OF THE VARIOUS FRAC-
TIONS FROM THE PH 5.5 EXTRACT: The im-
ages of the particles in fraction 5 a were similar to
those in the corresponding fraction 7 a of the pH
7.4 extract, i.e., usually spherical and quite com-
pact. In fraction 5 d, rodlike and filamentous
molecules with nodular thickenings were also
observed in the shadow-casting image, and these
were quite pleomorphic and loose as in Fig. 2 C. In
the negatively stained images, these were usually
round and looked much less compact than the
mature large subparticles. Fractions 5 b and 5 c
showed an electron microscope image intermediate
between that of fraction 5 a and that of fraction 5
d, as in the case of fractions 7 b and 7 c; for
example, the rodlike particles were more fre-
quently observed in the faster-sedimenting frac-

tions (7 c, 7 d) than in the slower-sedimenting fractions (7 a, 7 b).

CYTOPLASMIC MATURE LARGE SUBPAR-TICLES AND THE EFFECT OF PVS TREAT-MENT: The shadow-cast and negatively stained images of mature large ribosomal subparticles were compact and spherical and it is possible to identify rounded and skiff-shaped configurations of the 60S ribosomal subparticles in the negatively stained images, such as were observed and reported earlier (37).

Electron microscope observations of large subparticles treated with PVS and Brij 98 revealed

that such treatment induces little or no detectable change in the images of the large subparticles. Thus it is concluded that rodlike or filamentous molecules are not artifacts produced by unfolding of the mature large subparticles in vitro.

CLASSIFIED IMAGES OF THE RNP PAR-TICLES FROM THE PH 7.4 EXTRACTS: As the RNP particles in the various fractions from the pH 7.4 and 5.5 extracts were usually heterogeneous in shape and size, we attempted to classify these particles according to axial ratio, assuming them to be prolate ellipsoids.
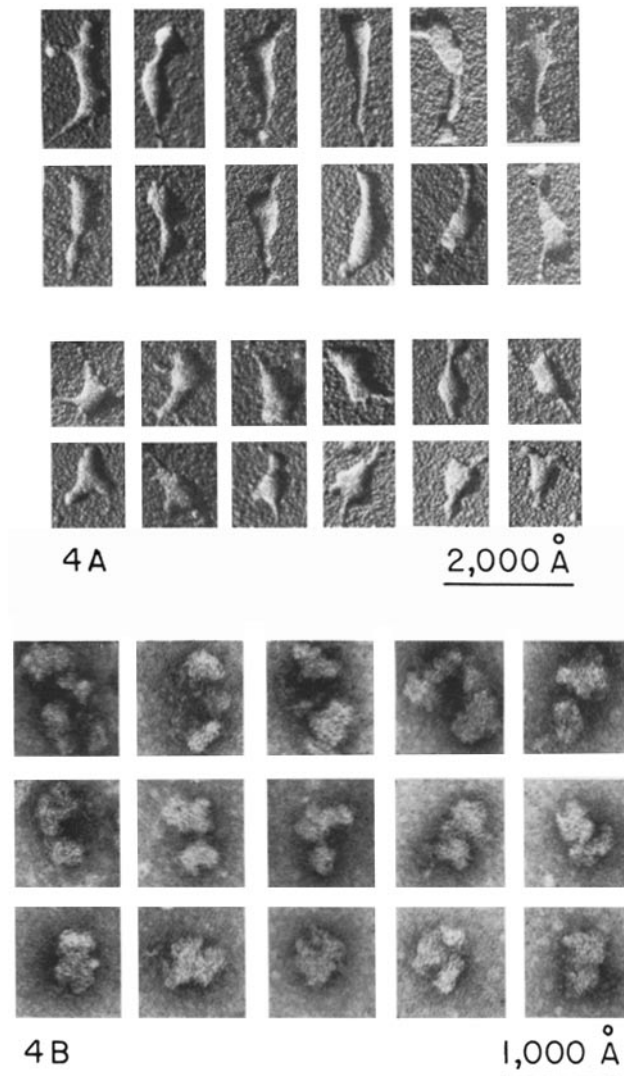
Group 1 (Fig. 3 A) consists of filamentous RNP

FIGURE 4   Gallery of selected shadow-cast images (4 A) and negatively stained images (4 B) of rodlike or Group 2 precursor RNP particles. Fig. 4 A, × 60,000. Fig. 4 B, × 150,000.

molecules with an axial ratio of about 10 or more. These molecules are sometimes several hundred nm in length (about 500 nm on the average), and their width is variable. Their appearance is, therefore, partly filamentous and partly nodular and quite irregular. Negatively stained profiles of RNP particles resembling the filamentous, loosely coiled, threadlike molecules are shown in Fig. 3 B.

Group 2 has rodlike RNP particles of which the axial ratio was about five, as shown in Fig. 4 A and B. The thickness of the molecules was not always uniform and the configuration of the particles was quite irregular. Occasionally observed were several filamentous processes resembling carrot roots. Fig. 4 B shows negatively stained images of particles in Group 2. These individual particles were loose in structure and irregular in shape, but were more compact and spherical, however, than those in Group 1.

Group 3 includes the RNP particles of which the axial ratio was about one (Fig. 5). These were divided into two subgroups: 3 a and 3 b. The particles of Group 3 b (Fig. 5 B) were compact and spherical molecules, and were not distinguishable from the mature large subparticles, while those of subgroup 3 a (Fig. 5 A) were spherical but slightly polygonal, sometimes possessing one or two processes. The negatively stained profiles corresponding to the former (3 b) and the latter (3 a) are shown in Figs. 5 D and C, respectively. The upper and lower series of Fig. 5 D show RNP particles with shapes that are very similar to the respective skiff and rounded shapes of the mature large subparticles. The RNP particles in Fig. 5 C were slightly larger and more irregular in shape than the mature subparticles.

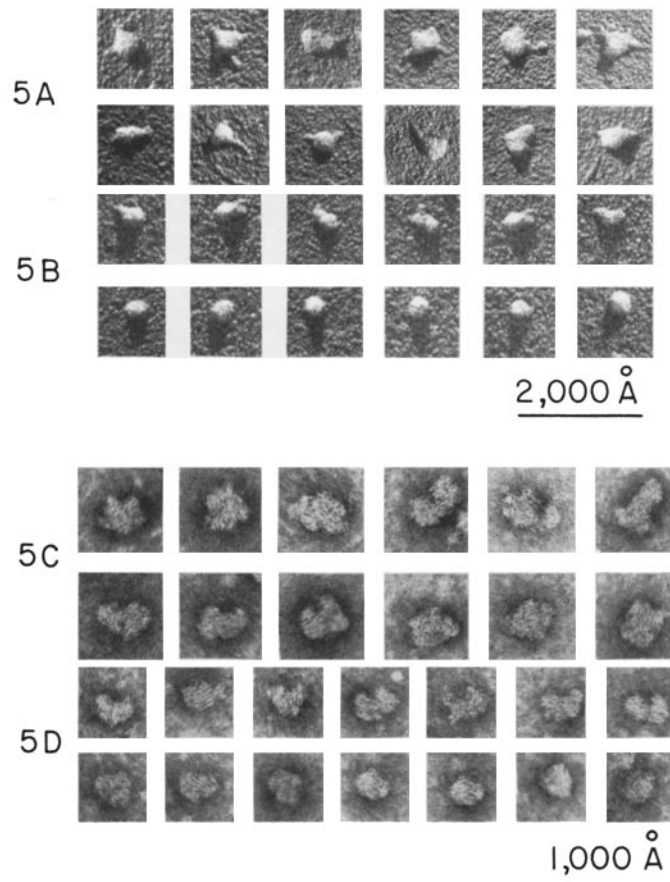In addition to these particles, there were a number of spherical particles which were definitely

FIGURE 5   Gallery of selected shadow-cast images (5 A) and negatively stained images (5 B) of spherical or Group 3 precursor RNP particles. The electron micrographs of 5 A, 5 C, and 5 B, 5 D show RNP particles of subgroups 3 a and 3 b, respectively. Fig. 5 A and 5 B, × 60,000. Fig. 5 C and 5 D, × 150,000.
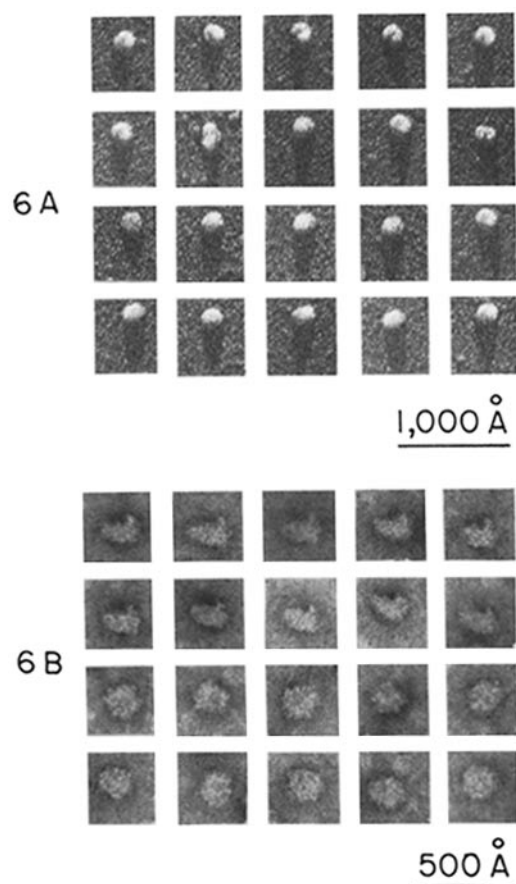
FIGURE 6   Gallery of selected shadow-cast images (6 A) and negatively stained images (6 B) of mature large ribosomal subparticles. Fig. 6 A, × 100,000. Fig. 6 B, × 200,000.

smaller than the large subparticles. These may be mature small subparticles or their precursor particles.

Fig. 6 shows a gallery of mature large subparticles observed by shadow casting (Fig. 6 A) and negative staining (Fig. 6 B). The skiff shape (the upper two rows of Fig. 6 B), and rounded shape (the lower two rows of Fig. 6 B), are readily observed. The rounded, negatively stained particles have a round opening or eyelike structure which is characteristic of large subparticles, while the skiff-shaped particles have a point at the right side of the subparticles, as described by Nonomura et al. (37).

The above grouping of the RNP particles according to axial ratio was quite arbitrary and there were a number of intermediate particles among these three groups; nevertheless, an attempt was made to classify the RNP particles in the various

fractions of the pH 7.4 and 5.5 extracts according to the axial ratio. At least 500 particles were examined in each fraction, and the results are summarized in Table III.

It was clearly demonstrated that spherical particles are present mainly in 60S region of both extracts, whereas rodlike and filamentous molecules are more numerous in the faster-sedimenting fractions, and that filamentous molecules are more frequently found in the pH 7.4 extract than in the pH 5.5 extract.

DISCUSSION

Identification of Various Forms of Precursor Particles

Isolation of RNP particles containing a single species of RNA is said to facilitate characteriza-

tion of ribosomal precursor particles. However, despite several trials, including CsCl density gradient centrifugation, we were unsuccessful.

An attempt was then made to correlate the distribution of the various species of ribosomal RNA (Table II) with the distribution of the classified images of the RNP particles (Table III) in the various fractions of the pH 5.5 and pH 7.4 extracts. For this purpose, specimens from each fraction were utilized for simultaneous electron microscopy and RNA analyses, after which the results were compared.

The relative numbers of various species of RNA in each fraction were calculated by dividing the relative amount of each RNA species by the corresponding molecular weight (bracketed in Table II).

Fraction 5 a contained 28S RNA (about 50%) and smaller RNA (about 50%). Approximately 50% of the particles found in this fraction were spherical and similar in appearance and size to mature large subparticles. Since Group 3 b particles are probably mature large subparticles (see Results), Group 3 a particles are considered nucleolar 60S particles containing 30S RNA, a direct precursor of mature 28S RNA (38, 39). The other 50% of the particles were smaller in size and flatter in shape than mature large subparticles. These may be either mature small subparticles or their precursor particles which contain 23S RNA, as proposed by Egawa et al. (40).

In fractions 5 c and 5 d, another type of particle appeared. These were rodlike or Group 2 particles which constituted about 30% of the population. More than 20% of RNA in these fractions was 35S RNA, and it would appear that these rodlike particles are the precursor particles containing 35S RNA.

In fraction 7 a, spherical particles were predominant (84%), and the RNA species observed was mainly 28S plus smaller RNA (91%).

In fraction 7 d, numerous filamentous (26%) and rodlike (31%) molecules were observed. The relative amounts of 45S, 35S, and 28S plus smaller RNA found in this fraction were 14%, 26%, and 60%, respectively. From these results it is proposed that the filamentous molecules are the precursor particles containing 45S RNA. This suggestion is supported by the results of RNase digestion studies which revealed that the precursor particles containing 45S and 35S RNA were quite sensitive to RNase digestion (7, 41).

Possible Ultrastructural Change of Precursor Particles in the Maturation Process of Mature Large Subparticles

It has been demonstrated that ribosomal precursor RNA is synthesized in the nucleolus and is readily taken up by protein synthesized in the cytoplasm (see reviews by Darnell (1) and Perry (3, 4)). The ribosomal precursor particles undergo all subsequent maturation in the form of nucleoprotein particles, and it is evident that the precursor particles possess a relatively higher proportion of protein to RNA and that ribosome maturation involves, in addition to a decrease in the size of the RNA molecules, a sequential decrease in the proportion of associated proteins.

Filamentous molecules and rodlike molecules appear to be precursor particles containing 45S and 35S RNA, respectively, and if such is the case, the ribosome maturation process may also involve gradual conformational changes from loose filamentous structure to compact spherical molecules as schematized in Fig. 7.

This postulation is also supported by ultrastructural and electron microscope autoradiographic studies of mammalian nucleoli in situ (15–21). The authors of those studies have reported that there are two basic components in nucleoli, one fibrillar and the other granular, and that ^{3}H-labeled uridine

TABLE III

Distribution of Various Precursor RNP Particles in the Various Fractions of the pH 5.5 and pH 7.4 Nucleolar Extracts

Particles \ Fraction	pH 5.5 extract				pH 7.4 extract			
	5a	5b	5c	5d	7a	7b	7c	7d
Filamentous	0	2	8	15	0	5	11	26
Rodlike	0	27	29	31	16	32	38	31
Spherical	100	71	63	54	84	63	51	43

The figures indicate the relative numbers of various RNP particles in each fraction.
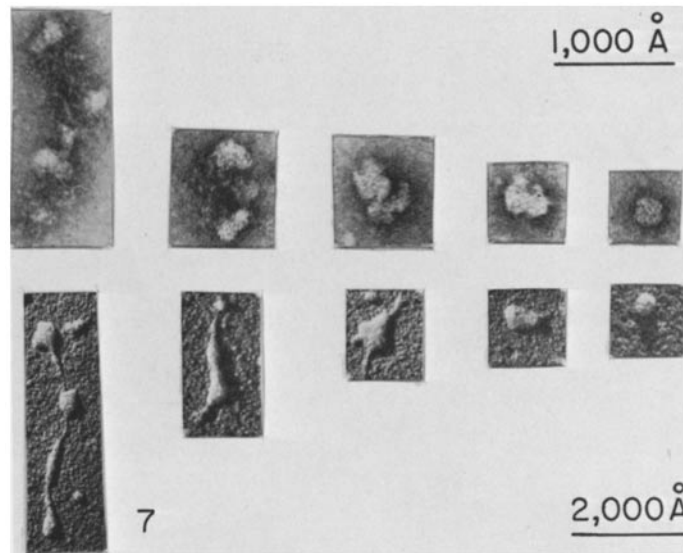
FIGURE 7   Ultrastructural change of precursor RNP particles in the process of becoming mature, large ribosomal subparticles (L → R). Upper row, negatively stained images, × 150,000. Lower row, shadow-cast images, × 60,000.

is first incorporated into the fibrillar component before being transported to the granular component. It is very probable that filamentous or rodlike molecules are derived from the fibrillar component, whereas spherical particles are derived from the granular component of the nucleoli.

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