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Abstract

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ULTRASTRUCTURE OF THE EXTENDED RIBONUCLEIC ACID MOLECULES FROM PURIFIED RIBOSOMES OF ROUS SARCOMA VIRUS-INDUCED MOUSE ASCITES SARCOMA CELLS

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Abstract: To clarify the ultrastructure of the extended ribosomal RNA molecules, electron microscopic observations were carried out on the RNA molecules extracted from purified ribosomes of mouse ascites sarcoma cells. By the treatment with ethylenediamine-tetra-acetate agglomerated rRNA molecules were elongated to thread-like structure by partial unfolding. The lengths of thread-like molecules were measured as less than 1μ. The strand of RNA molecules stained with uranyl acetate was observed approximately 15Å in width.

RNA extracted from the microsomal fraction of mouse ascites sarcoma cells was seen agglomerated into a globular structure with the electron microscopy using the protein monolayer technique (1). The electron microscopy of RNA isolated from the whole ascites sarcoma cells revealed linear structures mingled with the globular forms (1, 2). GRANBOULAN and SCHERRER reported that ribosomal RNA extended to a linear form by urea treatment in spreading solution (3). However, it was difficult to obtain the linear RNA from the microsomal fraction of mouse ascites sarcoma cells in the presence of urea under our experimental conditions (2). On the other hand, MATSUURA *et al.* (4) have revealed in *E. coli* that ribosomal RNAs in a free state appear as a thick threading structure in electron micrographs.

To clarify this discrepancy we isolated ribosomes from mouse ascites sarcoma cells and observed the conformational changes of RNA extracted from the free ribosomes without urea treatment. It was observed that ribosomal RNA changed to an extended form by treatment with ethylenediamine-tetraacetate (EDTA).

MATERIALS AND METHODS

Isolation of ribosomes from mouse ascites sarcoma cells: Cells from mouse ascites sarcoma induced by Schmidt-Ruppin strain of Rous sarcoma virus (SR-C3H cells) were originally supplied from Dr. Tadashi YAMAMOTO, Institute for

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Medical Science, University of Tokyo (5, 6), and maintained by intraperitoneal transplantation in C3H/He mice. On the sixth day, SR-C3H cells were harvested and washed by suspending in 0.25M sucrose, 0.01M tris-Cl, 0.001M EDTA, pH 7.6, until contaminated red cells were removed (1). The SR-C3H cells were then suspended in 0.025M KCl containing 0.004M $MgCl_2$, and 0.01M tris-Cl, pH 7.6 (KMT buffer), and homogenized by a homogenizer (Ganken type). Ribosomes were isolated from the homogenate by a modified method of MOLDAVE and SKOGERSON (7). All the procedures for isolation were carried out at 0-4°C. The homogenate was mixed immediately with an equal volume of 0.7M sucrose containing KMT buffer to make the molarity of sucrose to 0.35M and centrifuged at 12,000 g for 30 min. By discarding the residue, the supernatant was centrifuged at 105,000 g for 60 min. The sediment thus obtained was suspended in 0.35 M sucrose containing KMT buffer and gently homogenized with a teflon homogenizer, and then 0.1 M $MgCl_2$ was added to the homogenate in a final concentration of 0.009 M. Potassium deoxycholate, from a 10% solution, was added to obtain a final concentration of 0.26% and stirred for 15 min by a magnetic stirrer. The suspension was centrifuged at 105,000 g for 60 min. The pellets thus obtained were gently resuspended in 0.001 M $MgCl_2$, and $MgCl_2$ was added to make the final concentration to 0.05 M. Then the suspension was stirred for 15 min, and centrifuged at 21,000 g for 30 min. The precipitate was washed four times with 0.01 M $MgCl_2$. The final residue was suspended in 0.35 M sucrose containing KMT buffer and then dialyzed against the same solution overnight. The dialyzate was centrifuged at 21,000 g for 20 min, and the supernatant served as the whole ribosomal fraction. The sedimentation pattern of the ribosomal fraction after centrifugation in a 15-30% sucrose gradient containing 0.01 M tris-Cl, pH 7.6, at 44,000 rpm for 120 min are shown in Fig. 1. Free ribosomal fractions appeared at the main peak (Fraction 13). Ribosomes containing polyribosomes were observed in the peak of Fraction 10 and aggregated ribosomes in the bottom.

Extraction of RNA from free ribosomes: RNA was extracted by means of cold SDS-phenol from the free ribosomal fractions separated by sucrose density gradient centrifugation (2).

Electron microscopic observation: Specimens for electron microscopy were mounted directly on a carbon-coated collodion film by floating method (8) and the excess solution was removed with a piece of filter paper to the edge of the grid. Before staining the ribosomes the specimens were fixed with glutaraldehyde (2.5%), washed with distilled water, and stained either with 1% phosphotungstate (PTA, pH 7.4) or with 2% uranyl acetate.

The RNA dissolved in 0.01 M EDTA (pH 7.6) was mounted on the grid, and rinsed with ethanol. For positive staining the specimens were immediately stained with uranyl acetate in acetone (9) and were rinsed with ethanol to remove any excess uranyl solution. Rotaryshadow-casting with a platinum-palladium (Pt-Pd) alloy was performed by the previous method (1, 2).

The specimens were observed with an electron microscope, Hitachi HU-

11B-S, at a magnification of 40,000 for specimens stained negatively or positively and at 10,000X for the shadowed specimens.

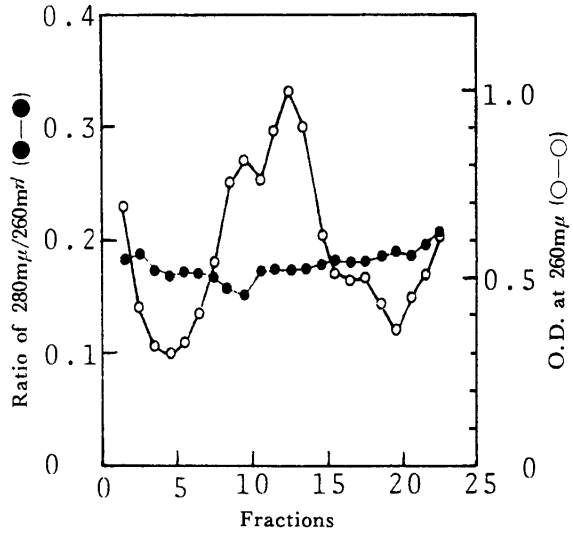

Fig. 1. Sedimentation pattern of ribosomes isolated from mouse ascites sarcoma cells in a sucrose density gradient. The centrifugation was carried out in a 15-30% linear sucrose gradient containing 0.01M tris-Cl, pH 7.6, at 4°C at 44,000 rpm for 120 min by an ultracentrifuge (Hitachi RSP-65TA).

RESULTS

Electron microscopic observation of ribosomes: The ribosomes isolated from SR-C3H cells and stained negatively or positively were mostly spherical as shown in Fig. 2. Free ribosomes were observed in the preparation, of which dimensions are as shown in Table 1. The negative staining with PTA caused

TABLE 1 SIZE OF THE RIBOSOMES ISOLATED FROM MOUSE ASCITES SARCOMA CELLS

Methods	Å in diameter
Uranyl acetate-staining	
Negative	227 ± 20 (± 8.5%)
Positive	211 ± 17 (± 8.0%)
Positive (core)	165 ± 19 (±11.4%)
PTA-staining	
Negative	263 ± 21 (± 8.0%)
Rotary shadowing	265 ± 25 (± 9.3%)

ribosomal swelling and then breaking to pieces; it required the prefixation with glutaraldehyde. These prefixed ribosomes were approximately 263Å in diameter after the staining with PTA (Fig. 2-A), which were slightly sudden compared with those stained by uranyl acetate, 227Å (Fig. 2-B).

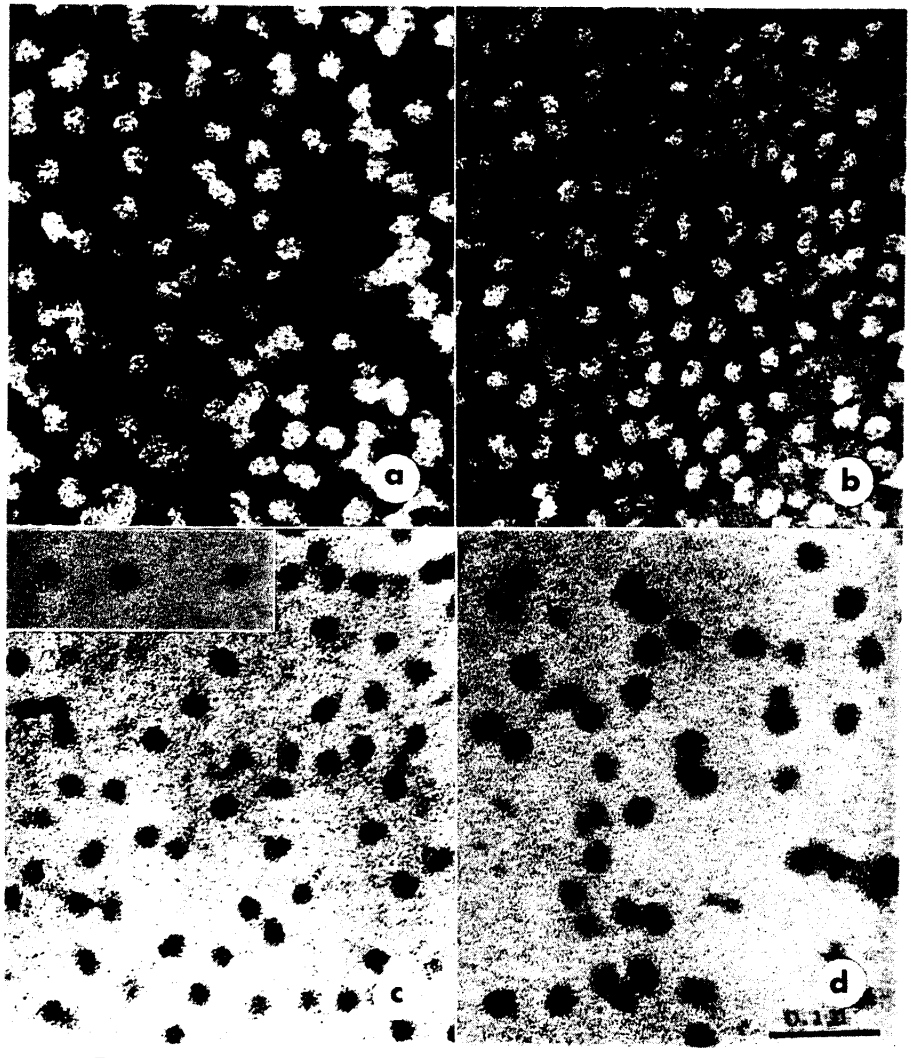

Fig. 2. Electron micrographs of ribosomes isolated from mouse ascites sarcoma cells.
A: Negatively stained with PTA
B: Negatively stained with uranyl acetate in water
C: Positively stained with uranyl acetate in acetone
D: Positively stained with uranyl acetate in water

On the other hand, the electron micrographs of specimens stained by uranyl acetate with or without prefixation showed two different images of

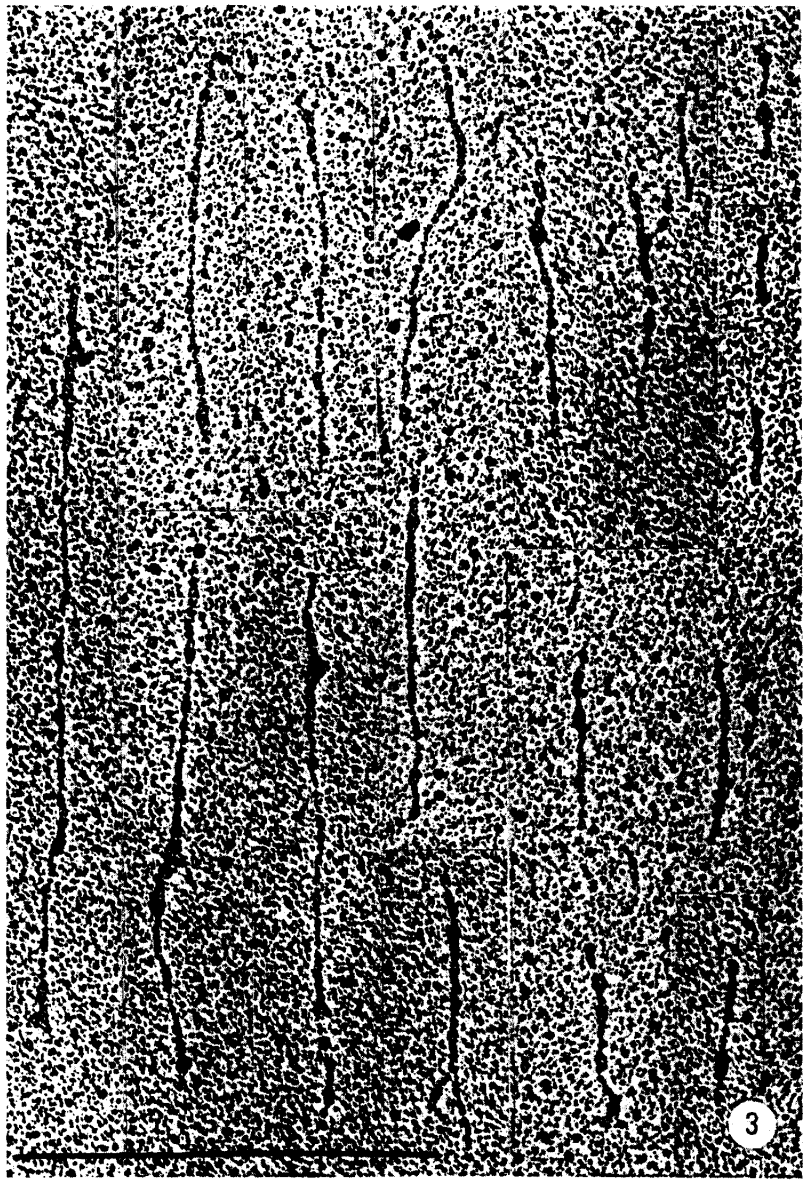

Fig. 3. Electron micrographs of ribosomal RNA in linear structure extracted from ribosomes of mouse ascites sarcoma cells. The specimen was prepared by the floating method and then rotationally shadowed with Pt-Pd. The bar represents 0.5μ.

ribosomes. The ribosomes positively stained with uranyl in acetone (Fig. 2-C) or in water (Fig. 2-D) showed the diameter of approximately 211Å, but the

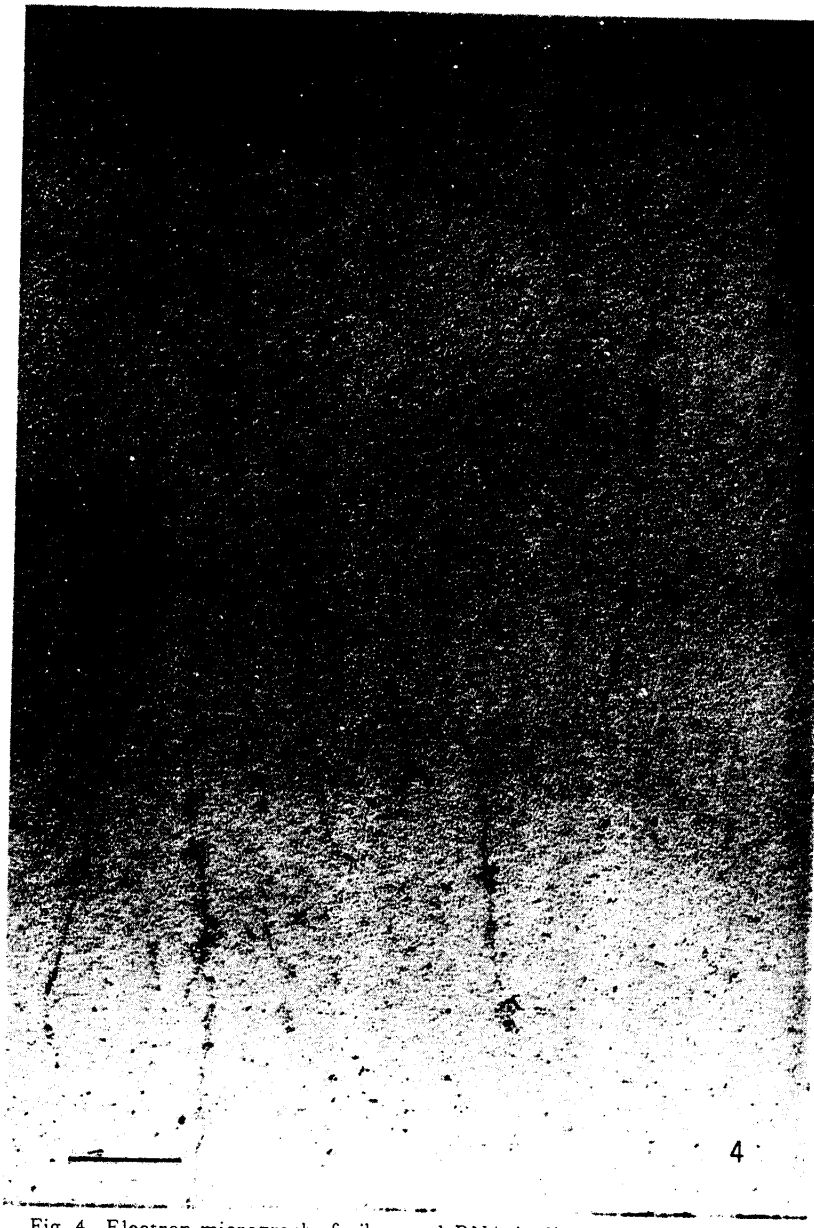

Fig. 4. Electron micrograph of ribosomal RNA in linear structure after treatment with EDTA and positively stained with uranyl acetate. 150,000X. The bar represents 0.1μ.

latter was stained more intensely in the central region in diameter of approximately 165Å. Since uranyl acetate stains the nucleic acids better in contrast than protein (9), the images stained positively imply the agglomeration of ribonucleic acid, which is composed of a strand with the width of approximately 15Å (Fig. 2-C).

Electron microscopic observation of RNA molecules: RNA extracted from practically free ribosomes was dissolved in EDTA solution and placed on the grid by the floating method. In the specimens shadowed rotationally, the ribosomal RNA appeared as a long rod-like structure. The electron micrographs of the selected molecules with various lengths in the specimens shadowed rotationally are shown in Fig. 3, and the histogram of their molecular lengths is shown with a mode of 0.1μ (Fig. 5). The majority of the molecular length was distributed up to 1.0μ.

The molecules of the ribosomal RNA stained positively showed an elongated, thread like conformation with a thick portion here and there, as shown in Fig. 4. The observations suggest that the thin parts of molecules observing approximately 15Å in width are extended parts of strands and the thick parts of the molecules are coiled more randomly in the strands. It was difficult to obtain the fully extended molecules by treatment with EDTA. This may be the reason why the length of the ribosomal RNA molecules was less than 1μ.

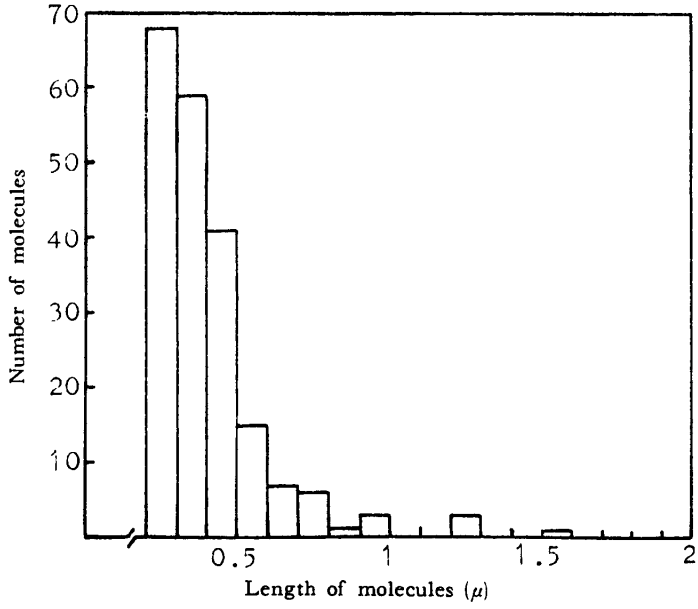

Fig. 5. Length distribution of RNA molecules in linear structure extracted from ribosomes of mouse ascites sarcoma cells.

DISCUSSION

The present method for the isolation and purification of the ribosomes from mouse ascites sarcoma cells by centrifugation is reproducible and sufficient to obtain a large amount of ribosomes. The isolated ribosomes are almost uniform in size and spherial in shape without contamination of membrane materials.

RNA was extracted from the isolated free ribosomes and dissolved in the EDTA solution. By these procedures it was possible to obtain the elongated and more linear RNAs similar to those observed in shadow-casted ribosomal RNAs separated by various authors (4, 10, 11). RNA shadowed rotationally represented the thread like structure with uneven width and occasionally with nodules and branches. The molecular lengths of elongated RNAs were observable less than 1μ, which were shorter than that caluculated from the molecular weight (3). It suggests that the molecular structure in the conditions employed here still contains some parts of coiled form of RNA. In fact, the RNA molecules stained positively with uranyl acetate showed thinner parts in unfolded region of RNA strands and thicker parts in folded region. A single strand of RNA stained with uranyl acetate represented the width of approximately 15Å, which coincides with the width of the strand observable on the original ribosomal surface stained positively with uranyl acetate. It is generally agreed that ribosomal surface consists chiefly of RNA with protein located internally (12, 13). It has been demonstrated that the connected strand of polysomes is approximately 10Å in width (14). The connected strand of polyribosomes in our experiment measured approximately 15Å in width (15), suggesting that the uranyl-stained structure in ribosome particles is RNA in ribonucleoprotein.

It is well known that magnesium ion is required for the maintenance of the structure of the ribosome particle and for the stabilization of the coiled structure of RNA (17, 18). RNA molecules were elongated by partial unfolding by chelation of metal ion with EDTA, suggesting that the molecules have a flexible part in conformational changes. It is considered that the remnant parts of RNA molecule which remains folded in the presence of EDTA have more electrostatically repulsive and attractive forces than the unfolded part. GRANBOULAN and SCHERRER (3) reported that these structures are capable of extending themselves by urea treatment, and the RNA strands extended to fully show the molecular length corresponding to the molecular weight.

Many facts are known of the morphology of the ribosomes as revealed by negative staining and shadow-casting methods, but little by positive staining

(12, 16). The present findings, in that the strand of RNA molecules stained positively with uranyl acetate is approximately 15Å in width, support the view that the positive staining method plays an important and useful role in the electron microscopic study related to the conformational changes in nucleic acid molecules.

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