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Abstract

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ULTRASTRUCTURE OF THE RIBONUCLEOPROTEIN AND MESSENGER-LIKE RIBONUCLEIC ACID OF THE POLYRIBOSOMES ISOLATED FROM ROUS SARCOMA VIRUS-INDUCED MOUSE ASCITES SARCOMA CELLS

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Abstract: Electron microscopic observation was made on the length distibution of messenger RNA molecules in polyribosome preparation isolated from mouse ascites sarcoma cells, which was destroyed by ethylenediamine tetraacetate treatment in hypotonic solution. The ribosomes appeared first to be a hollowed structure by swelling and then were destroyed to a rod-like structure consisting of ribonucleoprotein strand, which was clearly distinguishable from the linear structure of messenger RNA released from the polyribosomes. The length of messenger RNA was poly-dispersed measuring from 0.02 up to 6 μ, the majority (92%) of which was in the length less than 3 μ with a prominent peak between 0.6 to 0.8 μ.

Ultrastructures of the ribosomes have been investigated from physical, biochemical and morphological view points (1-3). Conformation of ribosomal RNA (rRNA) in solution has been studied in some details (4-7), whereas little advance has been made electron-microscopically as regards the organization of RNA and protein. SPIRIN *et al.* (4) have postulated a model of ribonucleoprotein. A series of electron microscopic studies was carried out on the nucleic acids of Rous sarcoma virus-induced mouse ascites sarcoma cells (SR-C3H cells) (8-10) and revealed some results that (a) maturated rRNA is observable in an agglomerated globular-like form by protein monolayer technique (8); (b) RNA extracted from the isolated free ribosomes is a somewhat elongated thread-like form by EDTA treatment, but the length is not comparable to that caluculated from molecular weight (9); and (c) the linearly extended RNA, however, is detectable in RNA from the whole cells (10).

The linear structure of RNA from SR-C3H cells can be observed without the urea treatment (10) which induces the extension of RNA molecules (11). It has been demonstrated that the conformation of rRNA in ribosomes

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is similar to that isolated from ribosomes (3, 16). It suggests that the linear form of RNA molecules from SR-C3H cells may be virus-associated RNA (12) or nascent RNA (11, 13) and/or messenger RNA (mRNA) (14, 15).

The paper intendes to clarify whether mRNA of polyribosome is observable in a linear form without urea treatment and to analyze the morphological interaction of rRNA and ribonucleoprotein strand by electron microscopic observation of the destroyed ribosomes from EDTA-treated polyribosomes. The work suggests that RNA molecules of a linear structure in various lengths correspond to mRNA and those with flat ribbon-like structure (4) to ribonucleoprotein strand.

MATERIALS AND METHODS

Ribosomes and polyribosomes were isolated from the Schmidt-Ruppin strain of Rous sarcoma virus-induced mouse ascites sarcoma cells (SR-C3H cells) by the differential and the sucrose density gradient centrifugation methods as described in the previous paper (9), and the fractions were suspended in the sucrose-buffur (0.35M sucrose, 0.025M KCl, 0.004M $MgCl_2$ and 0.01M tris-Cl, pH 7.6).

Destruction of ribosomes was carried out as follows. The ribosomes in the sucrose-buffer were layered on a EDTA solution (0.01M EDTA and 0.01M Tris-Cl, pH 7.6), and after osmotic shock the sample was picked up on the grid by touching the surface of the solution with the lapse of time. In another case, the ribosomes were dialyzed against a large amount of EDTA solution in a cold room. The redialysis of the broken ribosome segments was carried out against the sucrose-buffer in a cold room.

Surcose density gradient sedimentation of the samples was carried out in the condition of 15-30% sucrose with 0.01 M tris-Cl (pH 7.6) at 4°C for 120 min at 44,000 rpm by an ultracentrifuge (Hitachi RSP-65TA) (9). The fractions were collected from the bottom of the tubes.

Chromatography on Sephadex G-100 was conducted at 1-4°C as follows: The column, 1×30 cm, was conditioned with 0.05M ammonium acetate (pH 6.9), and one ml sample suspension was layered on the top of the column. The sample eluted with 0.05M ammonium acetate was fractionated to each one ml. Absorbance of the fractions at 260 mμ was measured by a spectrophotometer (Hitachi, model 3T3). Protein was measured by Lowry's method (17).

Electron microscopic observations were done on the specimens rotationally shadowed with platinum-palladium (Pt-Pd) and positively stained with uranyl acetate with an electron microscope, Hitachi HU-11BS. These staining procedures and other details were described in the previous papers (8, 9). The double staining with rotaryshadowing after positive staining was also performed.

RESULTS

Electron microscopic observation: Photographs of ribosomes and their broken fragments shadowed rotationally are shown in Fig. 1. The size of

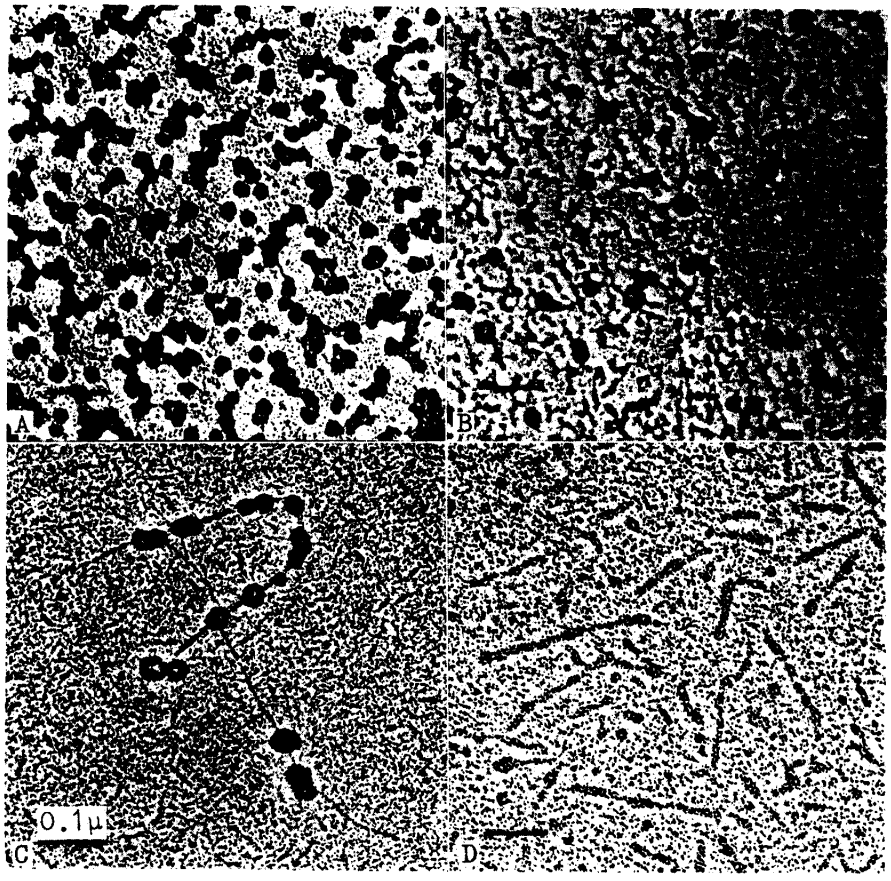

Fig. 1. Electron micrographs of ribosomes isolated from SR-C3H cells. Rotationally shadowed specimens with Pt-Pd; (A) ribosomes, (B) osmotically shocked ribosomes and (C) polyribosomes, (D) dialyzed ribosomes against a EDTA solution.

spherical ribosomes shadowed rotationally was approximately 265Å in diameter, details in the previous paper (9). The strand connecting the array of ribosomes was also seen. After the osmotic shock for a few minutes the ribosomes swelled to a hollow cup-like structure and then went to pieces. The connected strand in some preparations consisted of two types; namely single stranded-like part and thick part as shown in Fig. 1-C. The ribosome components after the dialysis against EDTA solution over-night were observed as a rod-like or flat ribbon-like structure (Fig. 1-D). Some of these structure were measured 0.14 μ and 0.28 μ in length. The photographs of the redialyzed ribosome samples against the sucrose-buffer showed the aggregated feature and some spherical structure reconstructed from brocken fragments.

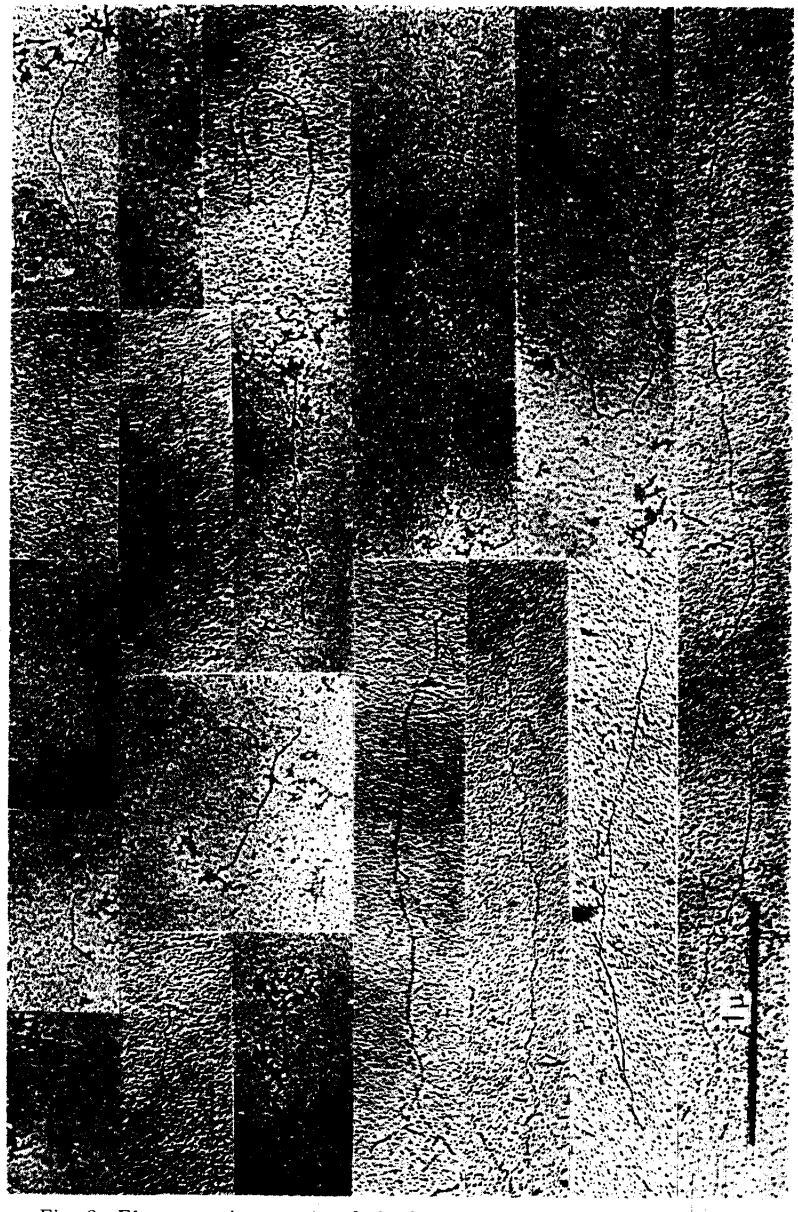

Fig. 2. Electron micrographs of the broken polyribosomes obtained by osmotic shock. Areas containing the filamentous structure were collected. The specimens were stained with uranyl acetate in acetone and then rotationally shadowed slightly with Pt-Pd.

The polyribosomes in the fraction separated by sucrose density gradient method (9) were destroyed osmotically by EDTA hypotonic solution. As shown in Fig. 2, filamentous structures were observed together with a short rod-like structure and aggregated form. It suggests that the filamentous forms are associated with mRNA molecules, and the rod-like ones are with ribonucleoprotein strands. A histogram of length distribution of the linear molecules is shown in Fig. 3. A total of 146 molecules from 0.02 μ up to 6 μ in length were analyzed with a mode of 0.2 μ. The majority of molecules (92% of total molecules) were less than 3 μ in length, and the prominent peak was found in length between 0.6 to 0.8 μ fractions.

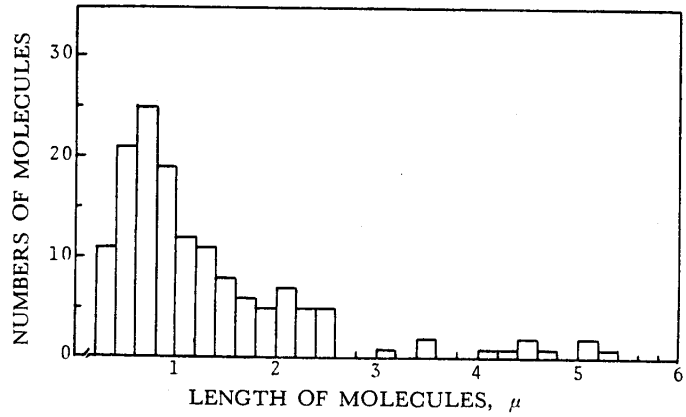

Fig. 3. Histogram of length distribution of the filamentous structure obtained from polyribosomes of SR-C3H cells.

The filamentous structure stained positively with uranyl acetate exhibited a more coiling and unfolding pattern, while the rod-like structure showed a coiling and twisting of two strands.

Biochemical analysis of the broken ribosomes: Patterns in sucrose gradient sedimentation of the dialyzed and redialyzed ribosomes were compared with that of the RNA extracted from the ribosomes (Fig. 4). The destroyed ribosomes revealed the sedimentation pattern similar to that of RNA in the top of the tubes, and the redialyzed ribosomes revealed the pattern in the top, middle and bottom of the tubes. The pattern seen in the middle fraction appears to be a slightly reconstructed form and that in the bottom fraction to be a more aggregated form, coinciding with the observations by electron microscopy. Another sedimentation pattern of broken ribosomes of a low density revealed that two main peaks appeared to be associated forms with large and small subunits of RNAs, but slightly lighter in density than those of RNA.

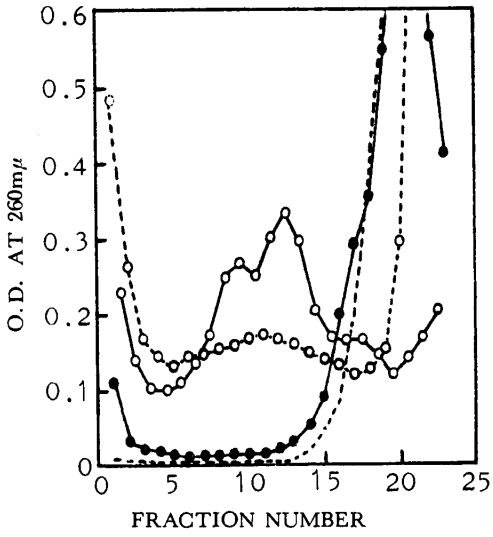

Fig. 4. Comparison of sedimentation patterns in sucrose density gradient centrifugation. Ribosomes (○-○), dialyzed ones (●-●), redialyzed ones (○ - - ○), and rRNA (- - -) were centrifuged in 15-30% linear sucrose density containing 0.01M tric-Cl, pH 7.6 at 4°C at 44,000 rmp for 120 min by Hitachi RPS-65TA centifuge.

The ratio of A_{260}/A_{280} of the sedimentation pattern did not clarify any releases of RNA and protein by the treatment. Hence, they were analyzed chromatographically on Sephadex G-100 column (Fig. 5). The pattern of the destroyed ribosomes (the main fraction, No. 9) was similar to those of ribosomes and rRNA, though the light fraction of destroyed ribosomes which was not obtainable from native ribosomes was richer in protein than in nucleic acid. The findings suggest that the rRNA-dependent ribonucleoprotein is not likely to be fragmented form.

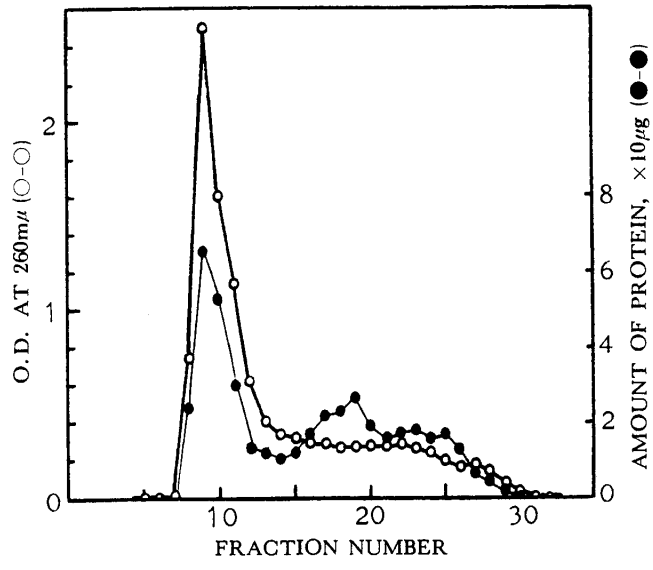

Fig. 5. Sephadex G-100 column chromatography of the broken ribosomes. Details are in the text.

DISCUSSION

The results presented in this paper indicate the linear RNA molecules of various lengths were produced by the destruction of polyribosomes by the EDTA treatment. Since the mRNA is released by EDTA treatment from polyribosomes (19), linear molecules are likely to be mRNA. On the other hand, the strand connecting the arrays of ribosomes was observed in two types ; one is a thin strand and the another a thick. The former seemed to be the single stranded mRNA, but the nature of the latter is obscure. It has been reported that polysomes associated with cytoplasmic membrane which is distinct from free polyribosomes are obtained in myeloma cells (20) and in HeLa cells (21). Hence the thick strand in the present case might be a segment of microsomal membrane.

The ribosomes appeared as a hollow, cup-like structure by osmotic destructions. BEER *et al.* (18) have reported the cup-like structure of ribosome of *E. coli* and they concluded that the structure is caused from the loss of its RNA. In the present experiment, however, the cup-like structure seems to be associated with ribonucleoprotein of swelled ribosome.

The structure of ribonucleoprotein strands gave a rod-like image in shadow-cast photographs in noticeable length of 0.14 μ and 0.28 μ. These may be derived from ribonucleoproteins associated with small and large subunits of ribosomes. In a possible principle of ribonucleoprotein postulated by SPIRIN *et al.* (4), a flat ribbon-like array is the structure consisting of ten parallel rows packed closely and formed by folding the continuous strand. The observations of rod-like structure in the present experiment by positive staining did not disclose such a structure.

The length distribution of mRNA observed by electron microscopy were little known. Concerning the length distribution of the heavy messenger-like RNA ($>$45s) isolated from nuclei of HeLa cells, GRANBOULAN and SCHERRER reported prominent peak to be about 3 μ (11). The prominent peak of length distribution of polyribosomal mRNA in our observation (0.6–0.8 μ) was shorter than that of the nuclear heavy menssenger-like RNA. The linear RNA molecules from the whole SR-C3H cells, which contained nuclear heavy RNA molecules, were longer in dispersed distribution than the polyribosomal mRNA (10).

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