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Abstract

The RNA extracted from Rous sarcoma virus (RSY)induced mouse ascites sarooma cells (SR·C3H, N. P.) by means of the cold SDS-phenol was examined by the electron microscopy on the specimens spread wi th or wi thout urea according to the protein mono· layer technique. The majority of RNA molecules was found in a collapsed agglomerated form, derived from matured ribosomal RNA. Using sucrose gradient, linear molecules of RNA were observed in the interspace of the agglomerated form of RNA at the region of high molecular weight of the band sedimentation. The histogram of the distribution in length of the linear molecules involved up to 6 /1 in length wi th a modal length of 2. 28 f1 and 2.0 to 2. 2 f1 in a pro. minent peak; longer molecules up to 18 f1 in length were scarcely observed. Species of the linear RNA molecules is not exactly known, although this is not mature ribosomal RNA and likely to be messenger RNA or nascent RNA molecules, some of which might associate with RSY·RNA.

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ELECTRON MICROSCOPIC OBSERVATIONS ON THE RIBONUCLEIC ACID MOLECULES OF LINEAR STRUCTURE IN ROUS SARCOMA VIRUS-INDUCED MOUSE ASCITES SARCOMA CELLS

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Abstract: The RNA extracted from Rous sarcoma virus (RSV)-induced mouse ascites sarcoma cells (SR-C3H, N. P.) by means of the cold SDS-phenol was examined by the electron microscopy on the specimens spread with or without urea according to the protein monolayer technique. The majority of RNA molecules was found in a collapsed agglomerated form, derived from matured ribosomal RNA. Using sucrose gradient, linear molecules of RNA were observed in the interspace of the agglomerated form of RNA at the region of high molecular weight of the band sedimentation. The histogram of the distribution in length of the linear molecules involved up to 6 μ in length with a modal length of 2.28 μ and 2.0 to 2.2 μ in a prominent peak; longer molecules up to 18 μ in length were scarcely observed. Species of the linear RNA molecules is not exactly known, although this is not mature ribosomal RNA and likely to be messenger RNA or nascent RNA molecules, some of which might associate with RSV-RNA.

The protein monolayer technique (1) allows the direct visualization of nucleic acids with aid of electron microoscopy. Double-stranded DNA, single-stranded preribosomal RNA (pre-rRNA) and messenger-like RNA (ml-RNA) from animal cells have been shown to be an extended linear form, and ribosomal RNA(rRNA) as a collapsed agglomerated structure (1, 2). GRANBOURAN and SCHERRER (2) have demonstrated that the correlation of apparent lengths and molecular weight of RNA exists in various species of RNA which is spread in an extended form using urea treatment. Oncogenic viral RNA molecules of about 10^7 daltons in the molecular weight with sedimentation analysis have been observed in length of about 8 to 10 μ (2, 3). It is unlikely that much longer molecules of RNA could be found in normal cells (2, 4). To find out viral genome or its subunits, it would be of interest to study linear RNA molecules in RNA virus-induced tumor consisting of virus-

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nonproductive cells.

In the present study, we have carried out electron microscopic observations on linear molecules of RNA isolated from Rous sarcoma virus (RSV)-induced mouse ascites sarcoma cells (virus-nonproductive), and found RNA molecules in an extended linear form of varying lengths under the conditions where ribosomal RNA appeared in collapsed structure.

MATERIALS AND METHODS

Mouse ascites sarcoma (SR-C3H) cells, transformed by Schmidt-Ruppin strain of RSV and originally supplied from Dr. Tadashi YAMAMOTO (Institute for Medical Science, University of Tokyo) (5, 6), were maintained in our laboratory and prepared by the methods as described in the previous report (7).

RNA extraction from the cells was performed by means of SDS-phenol as previously described (7) except for all the procedures carried at 0-4°C. The RNA samples were treated with 10μg/ml of DNase (Sigma) in STE (0.14M NaCl, 0.01M tris-Cl, 0.001M EDTA, pH 7.6) solution containing 0.005M $MgCl_2$ for 60 min in an ice-bath, and phenol extraction was repeated. Two volumes of ice-cold ethanol were added to the aqueous solution, and after storing in a freezer the RNA precipitate was centrifuged to a pellet. The RNA pellet was resuspended in the STE solution and layered onto 5 to 20% sucrose gradient containing the STE solution. The gradient was centrifuged at 4°C in a swinging bucket rotor (SW-39) at 37,000 rpm for 210 min. The fractions were harvested from the bottom of the tubes and were assayed for absorbance at 260 mμ.

The samples of RNA with or without dilution in 8M urea (purchased from Katayama Kagaku Co.) in final concentration were spread according to the method of FREIFELDER and KLEINSCHMIDT (8). Other procedures for electron microscopy were performed as described previously (7). The lengths of the molecules were measured by tracing magnified electron micrographs with a map measurer.

RESULTS

Rotary shadowing with platinum-palladium was used on the spread sample by the protein monolayer technique under the conditions described previously (7). Electron microscopically, double-stranded DNA appears a filament and single-stranded rRNA as a collapsed, aggregated particle or globular form. It has been reported, however, that the agglomeration of RNA can be avoided using 8 M urea in the solution forming monolayer (2). Ribosomal RNA extracted from SR-C3H cells and suspended in the STE solution was diluted to 8 M urea in final molarity, and served for electron microscopy according to GRANBOURAN and SCHERRER (2). Figs. 1 (A) and 1 (B) show the molecules spread without or with urea, respectively. RNA molecules of

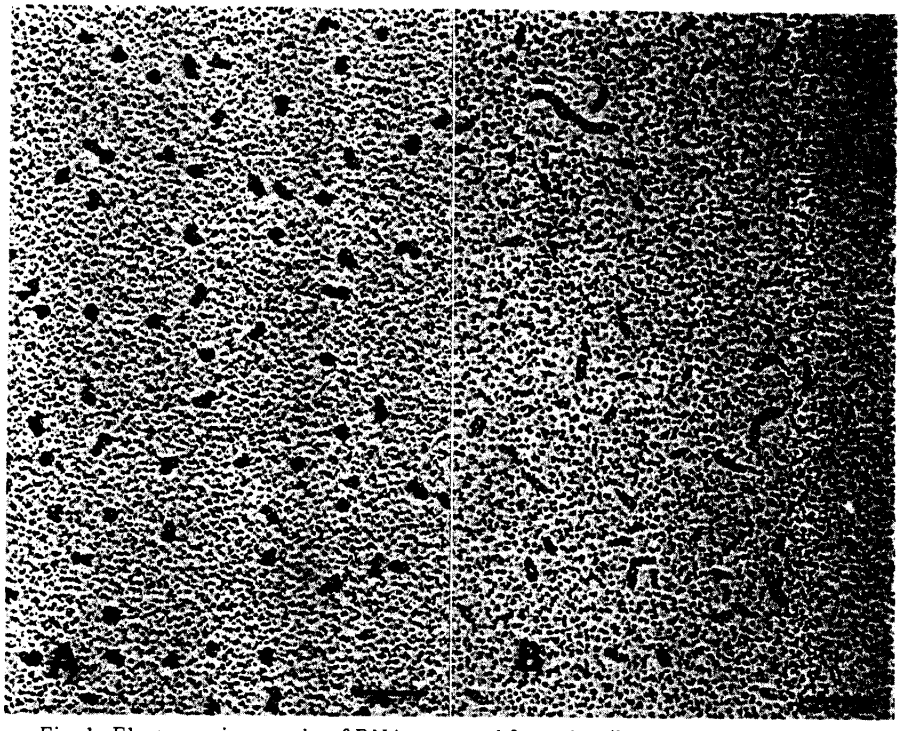

Fig. 1. Electron micrographs of RNA extracted from the ribosomal fraction of SR-C3H cells. Rotary shadow-casted specimens spread with(B) or without(A) urea. Bar is expressed as 0.1 μ in length.

the ribosome spread in the presence of urea showed no definite configuration varying from the particle-like to slightly elongated forms, and it was difficult to observe any filamentous form of the rRNA. On the other hand, addition of formamide (50% in final concentration) to the rRNA did not bring about any noticeable change in the configuration.

Fig. 2 represents the sedimentation pattern of the RNA extracted from the SR-C3H cells on the sucrose gradient centrifugation. Careful observations on all the fractions of RNA revealed the linear RNA molecules to be dispersed in the interspace of the globular RNA molecules. The treatment of the fractions with RNase resulted in a complete disappearance of the both molecules, suggesting the filaments to be RNA molecules. The filaments were not digested by treatment with RNase-free DNase. However, the length distribution of linear RNA molecules in the selected fractions of the sedimentation gradient did not show any prominent peak corresponding to their sedimentation pattern. The shadow-casted molecules of linear RNA, clear distinguishable from the globular, appear smoother in profile and con-

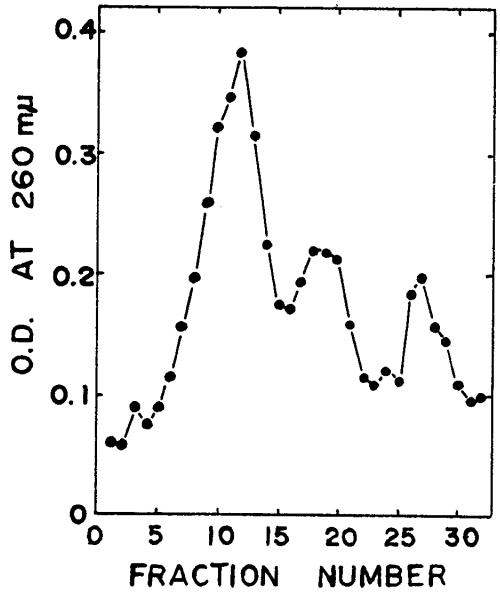

Fig. 2. Sucrose gradient sedimentation of the RNA extracted from SR-C3H cells. 0.5 ml of RNA in the solution (0.14 M NaCl, 0.001 M EDTA, 0.01 M tris-Cl, pH 7.6) was layered on the top of a 4.5 ml linear sucrose gradient, 5 to 20% (w/v) in the same buffer, and centrifuged for 210 min at 37,000 rpm in a Spinco SW-39 rotor at 4°C.

siderably thinner than the native DNA preparation in electron micrographs. In some cases, the curly or tangled region was observed in the linear RNA molecules. The filamets were located far more in the region of high molecular weight of the sedimentation gradient, and were often observable in the RNA molecules treated with urea.

The molecules shorter than 0.4 μ were always observed but they were not subjected to the analysis. The distribution pattern is heterogenous. The majority of the molecules (97%) was up to 6 μ in length with modal length of 2.28 μ. The longer molecules ranging up to 18 μ were scarcely observed in this instance. A histogram of the length distribution in a total of 198 linear molecules of RNA, in particular in the high molecular weight region, is presented in Fig. 3. The histogram was based on grouping the RNA molecules by a unit 0.2 μ in length ; this resulted in a major peak of 2.0 to 2.2 μ. The electron micrographs of the linear molecules of RNA in various lengths are shown in Fig. 4.

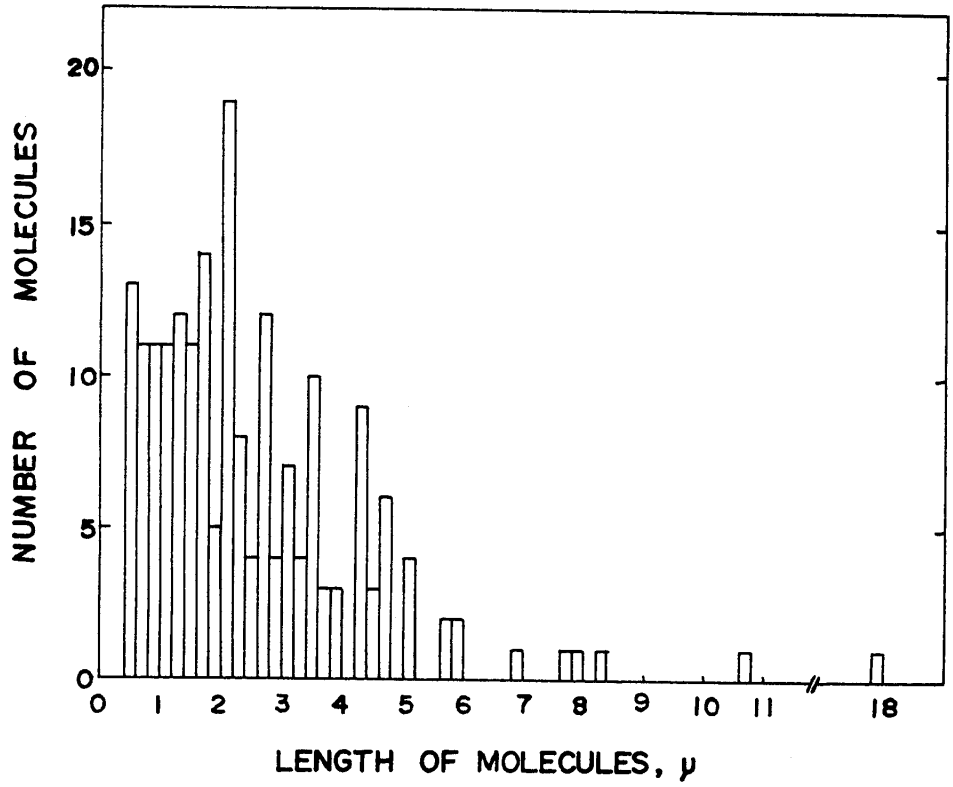

Fig. 3. Length distribution of linear molecules in high molecular weight region of RNA separated in a sucrose density gradient centrifugation from SR-C3H cell RNA. 194 Molecules were analyzed with a mode of 0.2 μ in length.

DISCUSSION

In electron microscopy using the protein monolayer technique under the conditions described in the present experiment, the majority of RNA molecules from SR-C3H cells appeared in an unextended globular form. But the well-extended linear structures were noticed on more careful observation with or without treatment by urea in the high molecular weight RNA region after the band sedimentation. Measured molecular length showed a wide variation in histogram with a prominent peak in the length of 2.0-2.2 μ and a few number of much longer molecules. It has been reported that the use of 8 M urea, which suppresses hydrogen bonds to a large extent, eliminates the agglomerations occurring in RNA spreading by the protein monolayer technique (2). It was, however, impossible to observe the extended molecules of the rRNA in SR-C3H cells under the present conditions of urea. Subsequent experiment (9) indicated that one of the effective treatments for

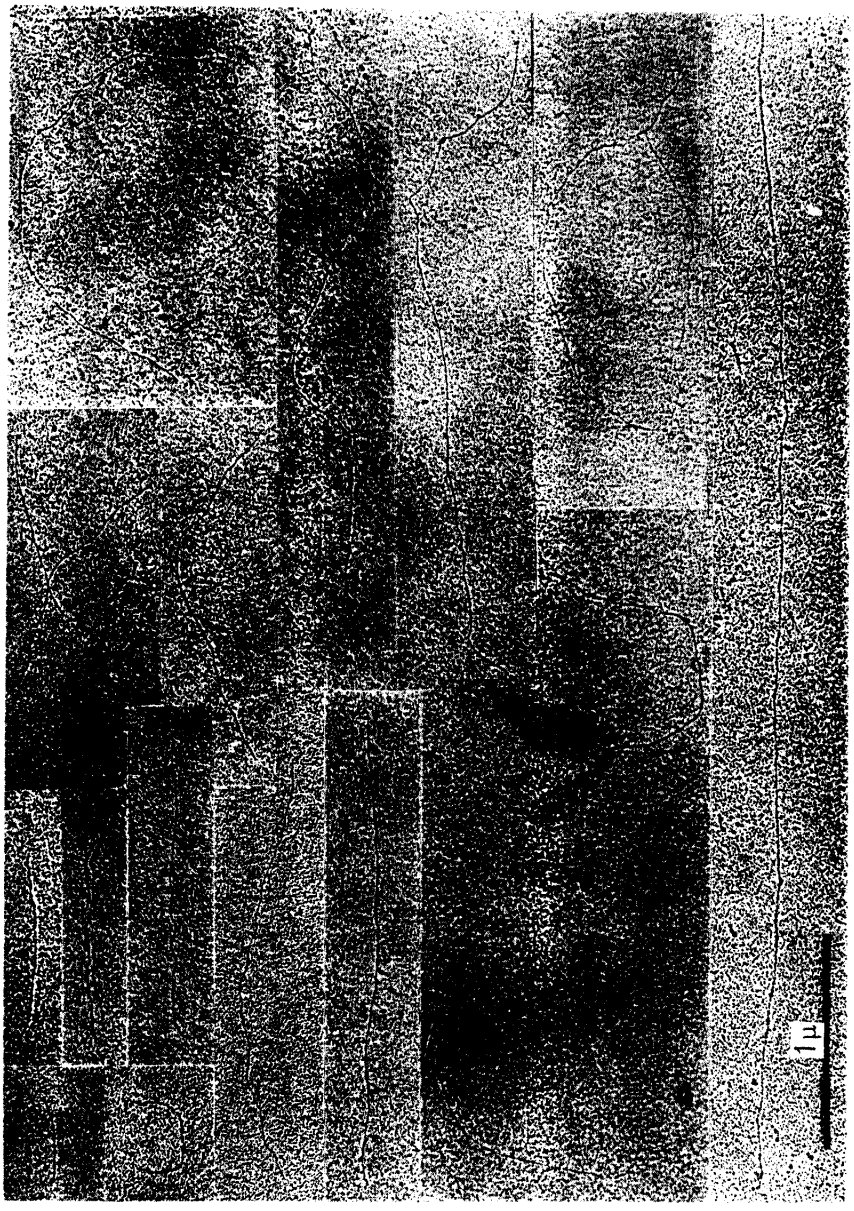

Fig. 4. Electron micrographs of linear molecules of RNA extracted from SR-C3H cells. The specimens were spread by the protein monolayer technique and then rotationally shadowed with Pt-Pd.

spreading the RNA molecules to extended linear form was EDTA treatment which removes magnesium ions.

The linear RNAs measuring 0.4-18 μ in length gave a modal length of 2.28 μ, which corresponds approximately to 3.2×10^6 or 2.4×10^6 daltons in the molecular weight according to a spacing of 2.45Å of 3.17Å betweem bases respectively, as described by GRANBOURAN and SCHERRER (2).

It had been reported that the RNAs of murine leukemia virus (MLV) and RSV were found in an extended linear form measuring 8 to 11 μ long after isolation in the presence or absence of urea, but the rRNA of rat liver had the collapsed structure of tangled complexes (3). On the other hand, OGURA *et al.* (10) reported that RSV-RNA, prepared from the primary culture of chicken tumor induced by transplanting SR-C3H cells to a wing-web of young chicken, was found in an linear form; its length distribution was at about 2.5 to 3 μ in the most frequent group and a few number of much longer RNA molecules were observed. SR-C3H cells contain a complete copy of the genetic information for producing the RSV (11) and presumably viral specific RNA, but further alterative studies are required for clarifying the correlation between the viral RNA molecules and the filamentous RNA of SR-C3H cells. Attempts are being made to analyze the filamentous RNA by molecular hybridization with the ^{3}H-labeled complementary DNA which is synthesized *in vitro* by reverse transcriptase with RSV-RNA as a template. At any rate, there is no doubt that the linear RNA molecules observed in the present study are not mature rRNA molecules and are likely to be composed of mRNA or nascent RNA molecules (2), some of which might be associated with RSV-RNA.

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