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Abstract

Native and heat-treated RNAs from the purified Schmidt-Ruppin strain of Rous sarcoma virus (RSV) were fractionated by sucrose density gradients in the presence of ribonuclease inhibitor diethyl-pyrocabonate and observed by electron microscopy. The structure of native 60-70S RNA was classified into two forms: tanglefolded type and linear type. In the tangle-folded type double stranded portions were observed in several sites. A high frequency of 60-70S RNA were 1.0 μm and 3-3.5 μm in length. Molecules with length about 9 μm were of the tangle-folded type while molecules shorter than 6 μm were of the linear form. The structure of heat-treated RNA(30-40S) was linear with the most frequent length being 1-1.5 μm . These results indicate that native 60-70S RNA is folded with the total molecular length being in the order of 6 to 9 μm . Molecules about 3 μm long are likely to be the main subunits of 60-70S RNA, and they are fragmented further into smaller subunits of about 1 μm length.

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ELECTRON MICROSCOPY OF ROUS SARCOMA VIRUS GENOME RNA AND ITS HEAT-DISSOCIATED SUBUNITS

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Abstract: Native and heat-treated RNAs from the purified Schmidt-Ruppin strain of Rous sarcoma virus (RSV) were fractionated by sucrose density gradients in the presence of ribonuclease inhibitor diethyl-pyrocabonate and observed by electron microscopy. The structure of native 60-70S RNA was classified into two forms: tangled type and linear type. In the tangled type double stranded portions were observed in several sites. A high frequency of 60-70S RNA were 1.0 μm and 3-3.5 μm in length. Molecules with length about 9 μm were of the tangled type while molecules shorter than 6 μm were of the linear form. The structure of heat-treated RNA (30-40S) was linear with the most frequent length being 1-1.5 μm . These results indicate that native 60-70S RNA is folded with the total molecular length being in the order of 6 to 9 μm . Molecules about 3 μm long are likely to be the main subunits of 60-70S RNA, and they are fragmented further into smaller subunits of about 1 μm length.

The RNA tumor virus contains single stranded RNA as the principal nucleic acid in its nucleoid which appears electron microscopically dense in the mature virion and tubular in the immature virion (1). Extraction of the nucleic acid from virions followed by sedimentation analysis reveals a major RNA species with a sedimentation coefficient of 60-70S and minor RNA fractions of 4-7S (2-5), but the conformation of these RNAs and their structural arrangements within the virions have not yet been elucidated. The large molecular weight 60-70S RNA can be converted to 30-40S subunits and other small species by treatment with reagents or heating which dissociates the hydrogen bonding (6, 7). The molecular weight of 30-40S RNA corresponds to about $3-4 \times 10^6$ daltons and that of 60-70S RNA to about 10^7 daltons when calculated from S values and other evidence (8-11). The 60-70S RNA may consist of three to four subunits linked with hydrogen bonding (12).

The other group communicates a different mechanism of S value conversion after hydrogen bond dissociation; native 60-70S RNA maintains a globular configuration with many hydrogen bonds spreading over the whole RNA molecule, and if the hydrogen bonds are dissociated, the peculiar

secondary and tertiary structure are modulated, which may reflect changes in the S value (7). Therefore, it is extremely important to determine the actual structure and length of the 60-70S and 30-40S RNAs by electron microscopy.

In the present investigation the RNA molecules extracted from SR-RSV were purified by sucrose density gradient centrifugation in the presence of the ribonuclease inhibitor diethylpyrocarbonate (13). The purified RNA was observed directly with the electron microscope using the protein monolayer technique. Heat-dissociated subunits were also purified and observed in a similar manner. The molecular length and structure of these RNAs were analyzed and the implications of these data for changes in S value were discussed. A preliminary report of the findings appeared earlier (14).

MATERIALS AND METHODS

Preparation of virus I: The Schmidt-Ruppin strain of Rous sarcoma virus (SR-RSV) was kindly supplied by Dr. K. Toyoshima of the Research Institute for Microbial Diseases, Osaka University, in the form of cultured medium suspension.

Cultivation of chick cells was carried out as follows. Primary culture of 10-day old chick embryo fibroblasts (white leghorn, Kimber-137) which were gs-antigen negative (15) was performed by the method of Rubin (16). Cells from the embryos were suspended in Eagle's minimum essential medium (MEM) supplemented with 10% bovine serum and plated in the culture bottles (11 × 5 cm). After 5 days cultivation the cells were removed from the bottles by trypsinization, resuspended in the above medium containing virus of which the infectivity titer was 2×10^3 FFU/ml, and transferred to 9 cm glass petri dishes at a concentration of 10^6 cells per plate. Transformed foci were found on the fourth day after infection. From the seventh day after infection the culture medium was replaced by fresh medium containing ^3H -uridine ($1 \mu\text{Ci/ml}$) at intervals of 4 hours to label the viral RNA. All cultures were incubated in 5% CO_2 atmosphere at 40°C .

About 1.5 l of the cultured medium was pooled and temporarily stored at -20°C . The virus-containing medium was centrifuged at $1,000 \times g$ for 15 min to remove the cell debris. The supernatant was centrifuged at $105,000 \times g$ (Rmax) for 1 hour. The pellet was suspended in STE medium (0.1 M NaCl, 10 mM Tris-Cl, and 1 mM EDTA, pH 7.2) and purified by centrifugation through a 45 ml linear density gradient of 4-40% sodium tartrate in SSC (0.15 M NaCl, 0.015 M sodium citrate, pH 7.2) at $105,000 \times g$ (Rmax) for 1 hour at 4°C (17). After centrifugation the samples were fractionated to 2.5 ml each. A 0.1 ml aliquot of each fraction was added to 7.5 ml of Bray's solution, and the radioactivity was counted by a liquid scintillation counter (Model 3326 Tri-Carb Spectrometer, Packard) (18). The peak fractions were monitored and the viral fractions were collected.

Preparation of virus II: RSV-induced mouse ascites sarcoma (SR-C3H/He)

established from SR-RSV induced C3H/He mouse solid sarcoma (19) was kindly supplied by Prof. T. Yamamoto of the Institute of Medical Science, University of Tokyo. This ascites sarcoma cell contains no RSV but induces RSV-producing fibrosarcoma in chicks inoculated in the wing web (20). Therefore, ascites sarcoma cells were inoculated into the chick wing web. The tumor thus induced was treated with 0.25% trypsin, and cells suspended in the culture medium were plated on 9 cm glass petri dishes. After 4-5 days cultivation the cells were removed with 0.25% trypsin and mixed at the rate of 10^5 cells to 10^6 of normal chick embryo fibroblasts. The mixed cells were suspended in MEM supplemented with 10% heat inactivated bovine serum, plated on 9 cm glass petri dishes (1.1×10^6 cells per plate), and cultured. Transformed foci were confirmed at the seventh day after virus inoculation, and the virus was labeled with ^3H -uridine, as described previously, at intervals of 12 hours. Purification of the virus was carried out from 500 ml of the cultured medium, as previously described.

Extraction of RNA from purified virus: Extraction of RNA was performed separately for virus preparation I and II according to the SDS-phenol method (21). The radioactive peak fractions in the sodium tartrate density gradients were diluted with the STE containing diethylpyrocarbonate at a final concentration 0.6% (STED), and centrifuged at $105,000 \times g$ (R_{max}) for 1 hour at 4°C . Virus pellets were suspended in 1.0 ml of STED medium containing 0.5% sodium dodecyl sulfate (SDS), and the suspension was shaken for 10 min at room temperature. Two volumes of phenol saturated with STED containing 0.5% SDS were added to the above suspension; the extraction mixture was shaken gently for 10 min, centrifuged at $2,000 \times g$ for 20 min to accelerate the phase separation, and the aqueous and intermediate phases were collected. The above phenol extraction procedure was repeated twice. Finally, the aqueous phase was collected in a test tube, and sodium acetate was added at a final concentration 3%. RNA was precipitated by adding two volumes of cold ethanol and stocked in a freezer at -20°C .

Purification of viral RNA labeled for 4 hours: The precipitated RNA in ethanol was centrifuged at $10,000 \times g$ for 20 min, and the pellet was suspended in 0.5 ml of STED. The RNA solution was layered on top of 5-20% linear sucrose density gradients, and centrifuged at $120,000 \times g$ (R_{max}) for 2 hours at 4°C . After centrifugation, fractions (8 drops) were collected from the bottom of the centrifuged tube, and 0.01 ml aliquots from each fraction was added to 7.5 ml of Bray's solution, and the radioactivity was counted by a Packard liquid scintillation counter. The peak fraction was used for electron microscopy.

Purification of heat treated and untreated RNA labeled for 12 hours: Ethanol-precipitated RNA from virus harvested at intervals of 12 hours was suspended in 1.0 ml of SSC, and 0.5 ml of RNA solution was transferred to another test tube; one tube was heated in boiling water for 1 min and cooled rapidly in ice water, and the other tube was not heat treated. Each was layered on top of 5-20% linear density gradients in STED. Centrifugation and fractionation were performed, as described previously. Marker 28S RNA was obtained as follows.

Rat liver ribosomal RNA was supplied by Dr. K. Inaba in the author's laboratory (22). The RNA in SSC was centrifuged in the same density gradient at $160,000 \times g$ (Rmax) for 4 hours. Fractionation was carried out as described previously. Half a ml of SSC was added to each fraction, and the optical density was measured at a 260 nm wave length by Hitachi EPS-3T spectrophotometer, and 28S RNA was collected. The purified 28S RNA was recentrifuged and fractionated with viral RNA in the same manner.

Electron microscopy: Electron micrographs of RNA were obtained using a modified procedure of the protein monolayer technique of Kleinschmidt *et al.*

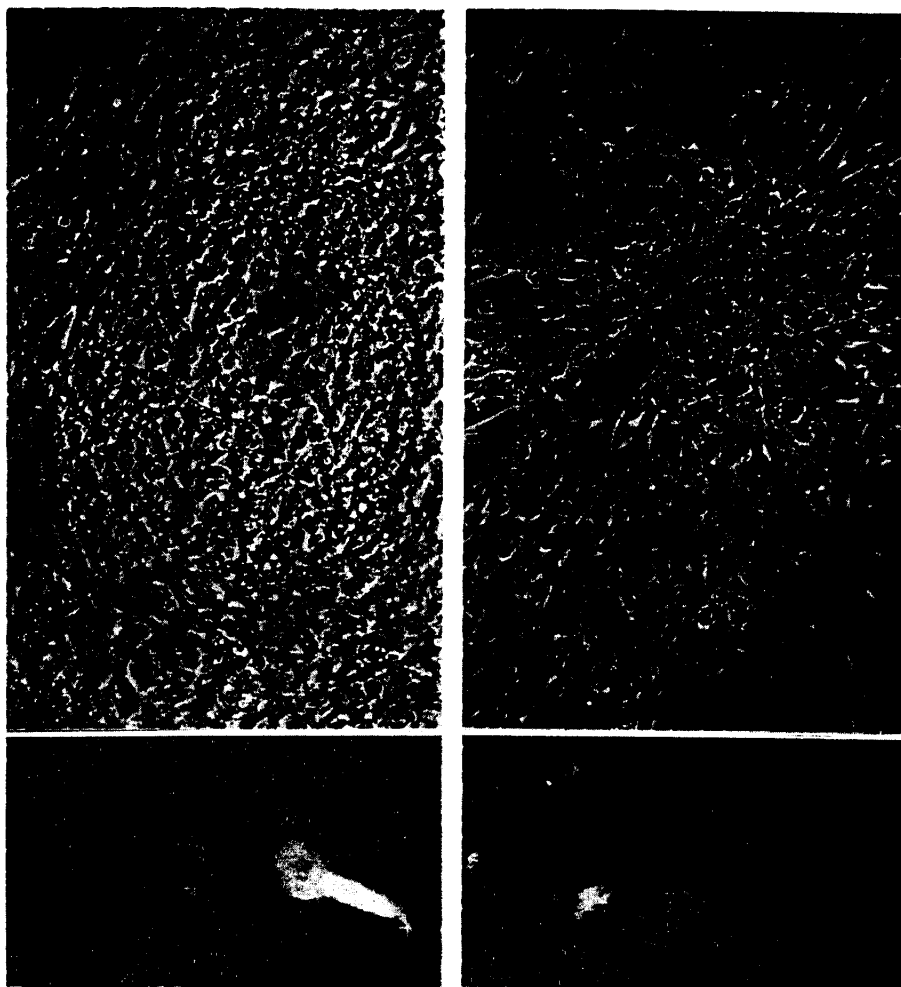


Fig. 1a. Transformed focus in monolayer culture of chick embryo fibroblasts induced by RSV. Fig. 1b, Transformed focus in mixed culture of chick embryo fibroblasts and SR-C3H/He cell-induced chick fibrosarcoma cells. Figs. 1c and 1d. Electron micrographs of negatively stained virions obtained from the above culture cells ($\times 80,000$).

(23). The spreading solution contained viral RNA in 1M ammonium acetate, 0.01% cytochrome *c*, and 0.5% formalin. A drop of the hypophase solution was placed on parafilm and the spreading solution in the micropipette was placed on the surface of the hypophase. After spreading, RNA in the cytochrome monolayer was picked up on carbon-coated grids. The liquid on the grid was dehydrated by touching it to the surface of absolute alcohol and dried on filter paper. The sample picked up on the grid was rotary shadowed with platinum and palladium. The shadowed grids were examined by a Hitachi HU-11-C electron microscope. Measurement of the length of RNA was carried out by tracing these molecules with a calibrated map ruler. The virus was negatively stained with 2% phosphotungstic acid (PTA) neutralized with KOH and examined in the same instrument.

RESULTS

Transformation of chick embryo fibroblasts by RSV: Transformed foci were confirmed microscopically before harvesting the virus. Round cells were observed in the foci, as shown in Figs. 1a and 1b.

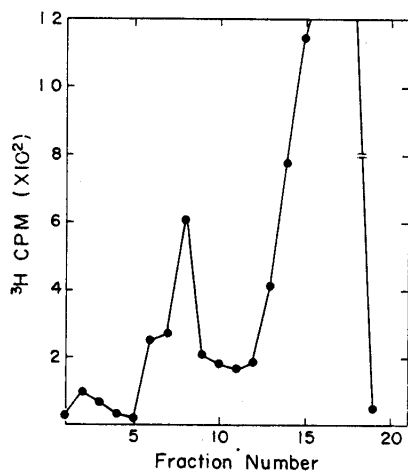


Fig. 2. Centrifugation pattern of RSV labeled for 4 hours in a 4-40% sodium tartrate linear density gradient.

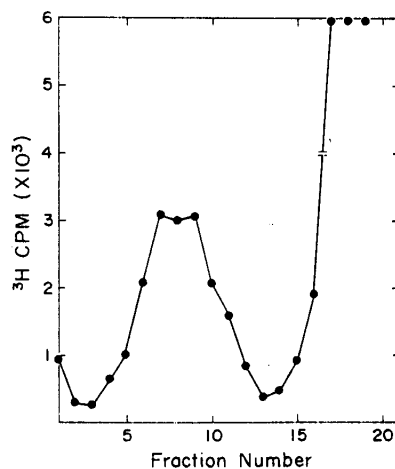


Fig. 3. Centrifugation pattern of RSV labeled for 12 hours in a 4-40% sodium tartrate linear density gradient.

Purity of virus: The purification of nucleic acid from viruses requires a purified virus preparation. In the present study RSV were purified as described under the section on Materials and Methods. Figs. 2 and 3 show the profiles of the labeled RSV in the sodium tartrate gradient. The viral peak was localized in the middle fractions. The samples in the peak fractions were negatively stained with phosphotungstic acid and observed under the electron

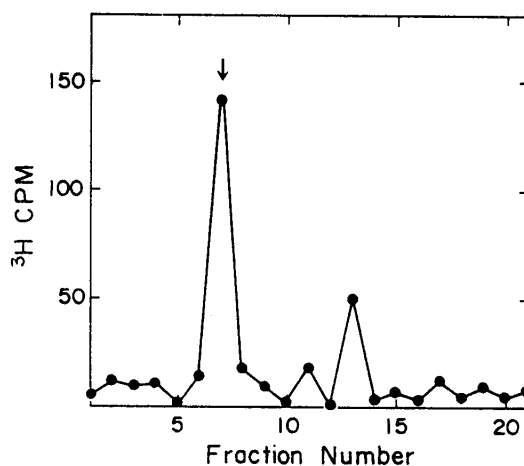


Fig. 4. Sedimentation pattern of RSV-RNA labeled for 4 hours. Half ml of RNA solution was layered on 5-20% sucrose density gradients in STED medium. Centrifugation and fractionation were carried out as described in Materials and Methods. The fraction indicated by the arrow was used for electron microscopy.

microscope. Tadpole and spherical forms which are typical features of RNA tumor virus were observed (24, 25) (Figs. 1c and 1d). Contaminated materials were hardly observable. The peak fractions in Figs. 2 and 3 were centrifuged, and the pellets were used for RNA extraction.

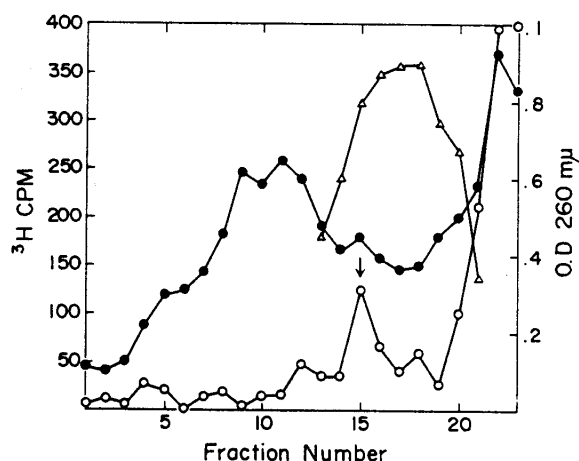


Fig. 5. Sedimentation patterns of untreated and heat treated RSV-RNAs labeled for 12 hours. Each 0.5ml of untreated and heat treated RNA in SSC was layered on 5-20% sucrose density gradients in STED. Centrifugation, fractionation, and radioactivity counting were carried out as described in Materials and Methods. ●, untreated RNA; ○, heat treated RNA; △, 28S rat liver ribosomal RNA. The peak fraction (arrow) was used for electron microscopy.

Purification of untreated viral RNA labeled for 4 hours: Fig. 4 shows the sucrose density gradient profile of extracted viral RNA. The main peak was observed at the high molecular weight region (60–70S), and a smaller peak at the lighter region. The peak fraction of the high molecular weight region was pooled and used for electron microscopy.

Purification of heat treated and untreated RNAs labeled for 12 hours: Both RNAs were simultaneously centrifuged under the same conditions to compare the heat treated RNA and untreated RNA in the density gradient pattern. Fig. 5 shows the sedimentation pattern of both RNAs and rat liver ribosomal 28S RNA centrifuged under the same condition.

The high molecular weight (60–70S) RNA was converted by heat treatment to low molecular weight (30–40S) RNA, which was slightly heavier than 28S ribosomal RNA. The peak of the untreated RNA labeled for

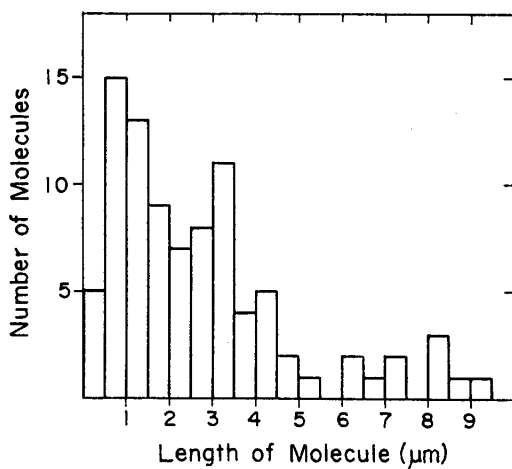


Fig. 6. Histogram of length distribution of untreated 60–70S viral RNA purified by 5–20% sucrose density gradient centrifugation. Ninety molecules were analysed.

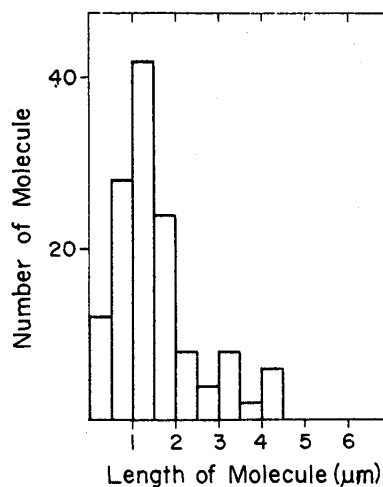


Fig. 7. Histogram of length distribution of heat treated 30–40S RNA purified by 5–20% sucrose gradient centrifugation. Analyses were made on 134 molecules.

12 hours was wider than that of 4 hour labeled RNA. This may indicate that intravirion degradation occurs during long culture incubation. The heat treated low molecular weight fraction (arrow in Fig. 5) was pooled and used for electron microscopy.

Electron microscopy of the purified RNA: Fig. 6 shows the molecular size distribution of high molecular weight RNA labeled for 4 hours. The molecular length was heterogeneous. RNA molecules about 1 μm and 3.0–3.5 μm

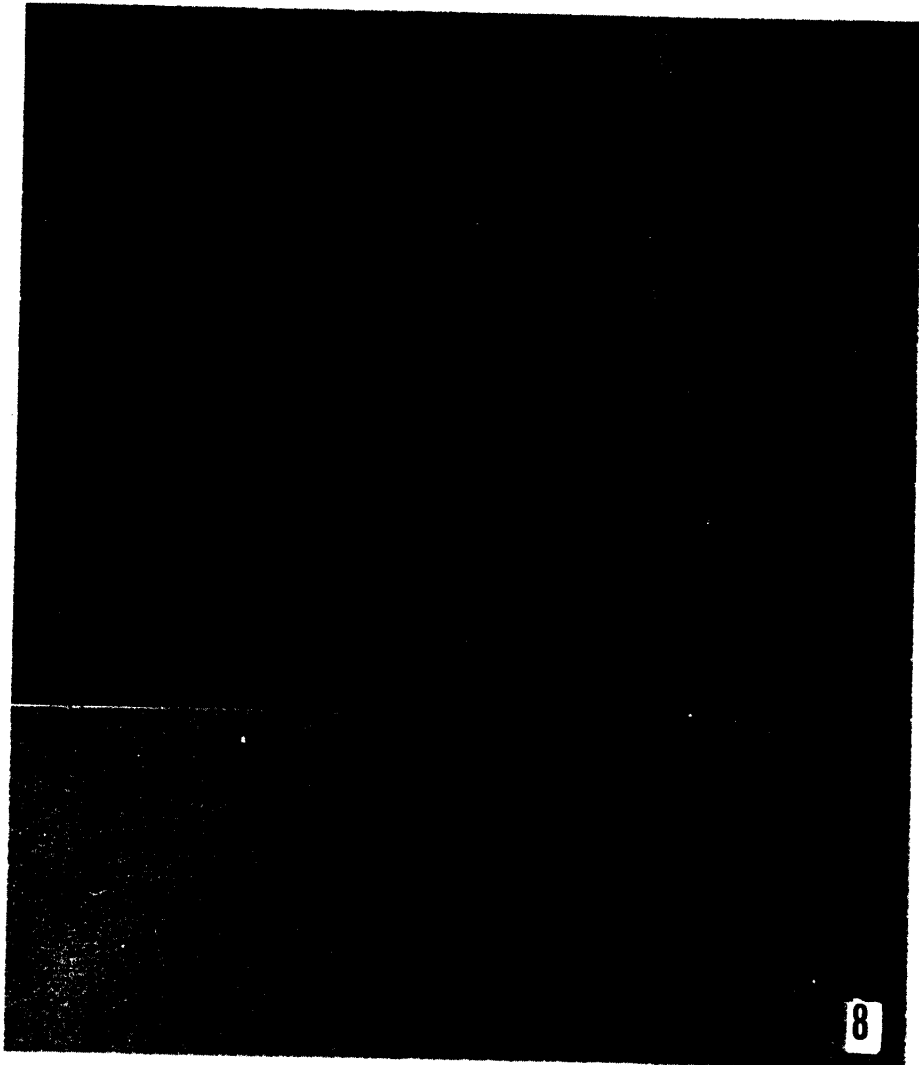


Fig. 8. Electron micrographs of molecules of 60-70S RNA in 60-70S fractions extracted from RSV labeled for 4 hours ($\times 30,000$).

long were found in high frequency. Long molecules ($>6.5 \mu\text{m}$) were found coiled and folded (Fig. 8). Twisted portions which appeared to be double stranded were measured as two strands, but accurate measurements could not be determined. The shorter molecules were linear and their molecular lengths were easily measured. Longer size molecules ($>10 \mu\text{m}$) exhibiting coiled feature were occasionally observed in the same grid (Fig. 9), but more detailed analysis of these molecules was not carried out. Fig. 7 shows

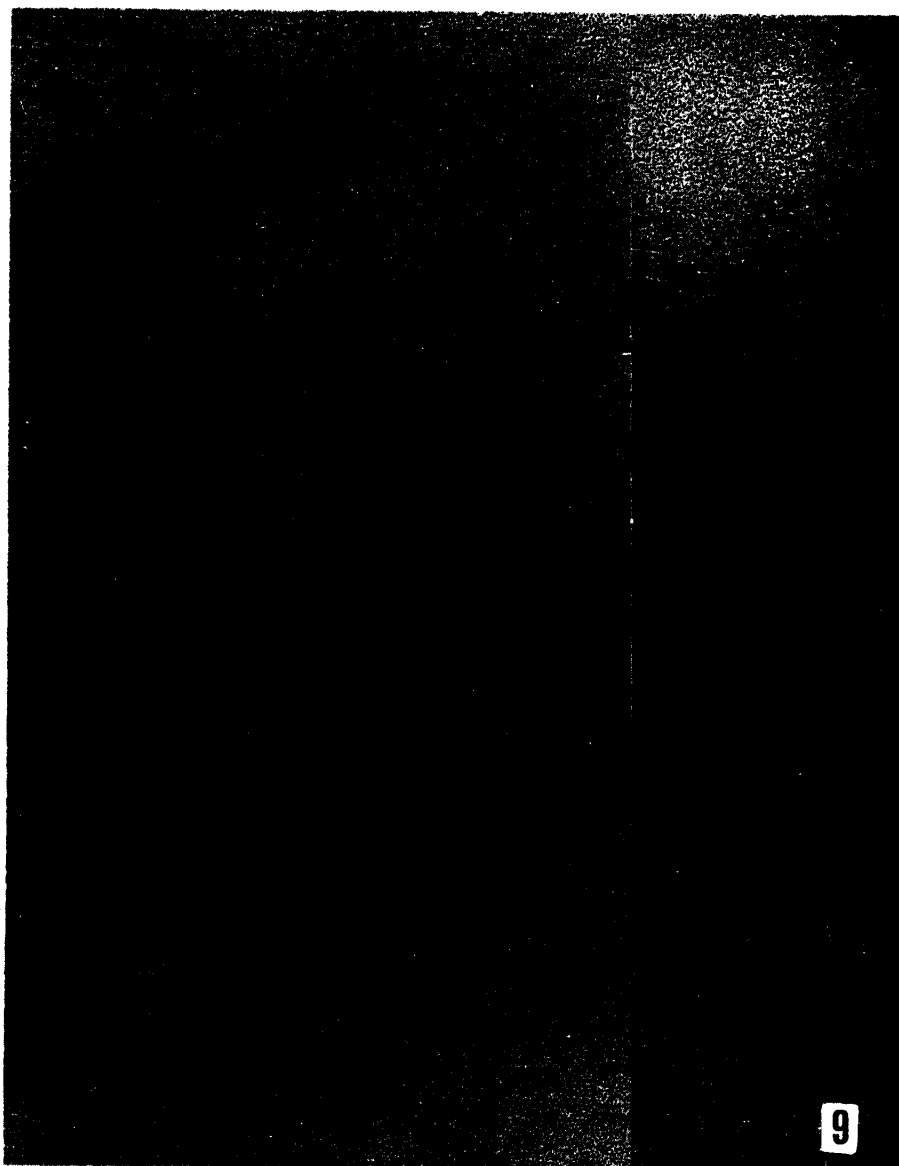


Fig. 9. Electron micrograph of long size RNA in 60-70S fraction extracted from RSV labeled for 4 hours. The arrows indicate the continuation ($\times 27,000$).

the histogram of RNA in the heat treated peak fraction: (30-40S). Short molecules of 1-1.5 μ m long were observed in high frequency. All molecules were in linear form (Fig. 10). Globular-form RNA, such as ribosomal RNA was not found to be contaminated in all grids.

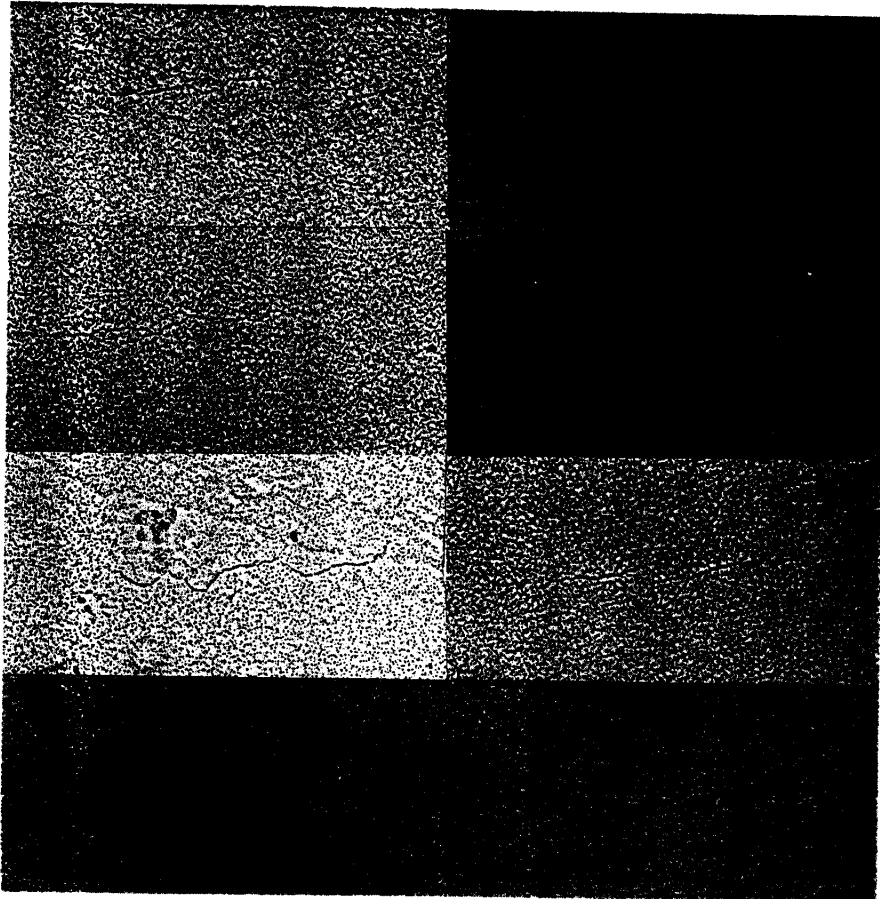


Fig. 10. Electron micrographs of linear molecules of 30-40S RNA obtained by heat treatment of RNA extracted from 12 hour labeled RSV ($\times 30,000$).

DISCUSSION

The 60-70S RNA, the largest species RNA in B and C type tumor viruses, are believed to be a viral genome. The molecular weight of the 60-70S RNA has been estimated to be about 10^7 daltons (8-11, 26, 27) by comparing the sedimentation constants and molecular weight of single stranded RNA derived from the tobacco mosaic virus and from other evidence. When this RNA was heated or treated with agents that disrupt intramolecular hydrogen bonds, it was converted to 30-40S RNA and analysed by density gradient centrifugation and gel electrophoresis (6-11). The molecular weight of 30-40S subunits is estimated to be $3-4 \times 10^6$ daltons; therefore, 60-70S RNA has been postulated to consist of three or four 30-40S subunits (10, 12).

It was also suggested that these subunits were electrophoretically different in tumorigenic and leukemogenic viruses in the avian tumor virus (10, 12).

The alternative explanation of these results is that 60-70S RNA undergoes an extreme conformational change in the denaturing condition from compact to amorphous configuration, as described previously (7). To obtain more direct evidence on the native structure of 60-70S RNA and the subunit-binding mechanism, it would be useful to study the heat treated and untreated viral RNA by electron microscopy. If the native RNA is treated with RNA-extending reagents, such as dimethylsulfoxide or formamide, RNA structure is modified (28, 29). Native form may be observed directly without treatment with the above reagents.

According to Granboulan *et al.* (28) and Kakefuda and Bader (29), the molecular length of 60-70S RNA in avian myeloblastosis virus was in the order of 8-9 μm and that of murine leukemia virus was in the order of 14 μm . The present study revealed that 60-70S RNA of RSV was heterogeneous in molecular size and was usually shorter than 9.5 μm , and molecules of about 1 μm and 3.0-3.5 μm long existed in high frequency. Molecules longer than 6.5 μm were found to be folded, while the shorter subunits were linear. The folded molecules had double stranded portions, which were ribonuclease resistant, as shown by Travnické and Riman (30). If native 60-70S RNA is about 9 μm in total length, the molecular weight calculated from the chain length would be about 10^7 daltons; this is consistent with the data of other researchers (28, 31). Molecules of 3.0-3.5 μm would correspond to 30-40S subunits and three or four subunit molecules would produce 9 μm genome. But if 1.0-1.5 μm strands are the true subunits, 6-9 molecules would be necessary to complete the 9 μm RNA chain; the molecular weight of 1.0-1.5 μm is in the order of $1.0-1.5 \times 10^6$ daltons, which is too small to satisfy 10^7 dalton molecule with 3-4 subunits. Several explanations are possible for the heterogeneous distribution of the molecular length in non-heat-treated 70S RNA and the higher incidence of 1.0-1.5 μm RNA than 3.0-3.5 μm RNA in the heat treated 30-40S fraction.

First, there is the possibility that the 30-40S RNA strands have labile sites broken by heat treatment and the shortened strands may attach to each other, and 30-40S RNA peak contains mixed population of the native subunits and pseudo-bound fragments. Second, procedures of specimen preparation for electron microscopy might dissociate or artificially shorten the RNA strands to 1.0-1.5 μm length. Weber *et al.* (32) also reported that the most common length of the dimethylsulfoxide-treated RNA from avian myeloblastosis virus was in 1.0-1.5 μm . The present data are consistent with the data of Travnické *et al.* (30) suggesting that the binding strength between

each subunit is different and that subunits are present that are smaller than 30-40S RNA (33).

From the present observations and discussion, it may be concluded that native RNA of SR-RSV has a folded structure measuring about 6-9 μm in total molecular length and that 3.0-3.5 μm RNA is the principal subunit. This subunit is further fragmented into much smaller subunits of 1.0-1.5 μm length by drastic treatment such as heat and other unknown conditions encountered in the processes of specimen preparation for electron microscopy.

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REFERENCES

1. Sarkar, N.H., Moore, D.H. and Nowinski, R.S.: Symmetry of the nucleocapsid of the oncornavirus. In *RNA Viruses and Host Genome in Oncogenesis*, ed. P. Emmelot and P. Bentvelzen, North-Holland, Amsterdam, p. 71, 1972.
2. Robinson, W.S., Pitkanen, A. and Rubin, H.: The nucleic acid of the Bryan strain of Rous sarcoma virus: Purification of the virus and isolation of the nucleic acid. *Proc. Natl. Acad. Sci. USA* 54, 137, 1965.
3. Robinson, W.S. and Baluda, M.A.: The nucleic acid from avian myeloblastosis virus compared with the RNA from the Bryan strain of Rous sarcoma virus. *Proc. Natl. Acad. Sci. USA* 54, 1686, 1965.
4. Duesberg, P.H. and Robinson, W.S.: Nucleic acid and protein isolated from the Rauscher mouse leukemia virus. *Proc. Natl. Acad. Sci. USA* 55, 219, 1966.
5. Watson, J.D.: The structure and assembly of murine leukemia virus: Intracellular viral RNA. *Virology* 45, 586, 1971.
6. Duesberg, P.H.: Physical properties of Rous sarcoma virus RNA. *Proc. Natl. Acad. Sci. USA* 60, 1511, 1968.
7. Bader, J.P. and Steck, T.L.: Analysis of the ribonucleic acid of murine leukemia virus. *J. Virol.* 4, 454, 1969.
8. Erikson, R.L.: Studies on the RNA from avian myeloblastosis virus. *Virology*, 37, 124, 1969.
9. Montagnier, L., Golde, A. and Vigier, P.: A possible subunit structure of Rous sarcoma virus RNA. *J. Gen. Virol.* 4, 449, 1969.
10. Duesberg, P.H. and Vogt, P.K.: Differences between the ribonucleic acids of transforming and nontransforming avian tumor viruses, *Proc. Natl. Acad. Sci. USA* 67, 1673, 1970.
11. Weinberg, R.A. and Penman, S.: Processing of 45S nucleolar RNA. *J. Mol. Biol.* 47, 169, 1970.
12. Duesberg, P.H., Vogt, P.K. and Canaani, E.: Structure and replication of avian tumor virus RNA. *Lepetit Colloq. Biol. Med.*, North-Holland, Amsterdam, 2, p. 154, 1971.
13. Solymosy, F., Fedorcsak, I., Gulyas, A., Farkas, G.L. and Ehrenberg, L.: A new method based on the use of the diethyl pyrocarbonate as a nucleases inhibitor for the extraction of undegraded nucleic acid from plant tissues. *Eur. J. Biochem.* 5, 520, 1968.
14. Kanzaki, Y., Hatase, O., Ishii, H. and Oda, T.: Electron microscopic observation on

- Rous sarcoma virus (SR-strain) genome RNA. *Proc. Jap. Cancer Assoc.* 32nd Annual Meeting, Tokyo, p. 12, 1973.
15. Ogura, H.: Personal communication.
 16. Rubin, H.: An analysis of the assay of Rous sarcoma cells *in vitro* by the infective center technique. *Virology* 10, 29, 1960.
 17. Temin, H.M.: Participation of deoxyribonucleic acid (DNA) in Rous sarcoma virus production. *Virology* 22, 486, 1964.
 18. Kusama, K.: On the measurement of biological substances by liquid scintillation counter. *Biophysics* 7, 32, 1967 (in Japanese).
 19. Yamamoto, T. and Takeuchi, M.: Studies on Rous sarcoma virus in mice. I. Establishment of an ascites sarcoma induced by Schmidt-Ruppin strain of Rous sarcoma virus in C3H/He mouse. *Jpn. J. Exp. Med.* 37, 37, 1967.
 20. Yamaguchi, N., Takeuchi, M. and Yamamoto, T.: Rous sarcoma virus production in mixed cultures of mammalian Rous sarcoma cells and chick embryo cells. *Int. J. Cancer* 4, 678, 1969.
 21. Kirby, K.S.: Isolation of nucleic acids with phenolic solvents. In *Methods in Enzymology*, XII, ed. L. Grossman and K. Moldave, Academic Press, New York, p. 87, 1968.
 22. Inaba, K., Misumi, M. and Oda, T.: Molecular forms and size determination of 18S RNA from rat liver microsomes by electron microscopy. *J. Electron Microsc.* 23, 45, 1974.
 23. Kleinschmidt, A.K., Lang, D., Jacherts, D. and Zahn, R.K.: Preparation and length measurements of the total deoxyribonucleic acid (DNA) content of T2 bacteriophages. *Biochim. Biophys. Acta* 51, 875, 1962.
 24. Zeigel, R.F. and Rauscher, F.J.: Electron microscopic and bioassay studies on a murine leukemia virus (Rauscher). I. Effects of physicochemical treatments on the morphology and biological activity of the virus. *J. Natl. Cancer Inst.* 32, 1277, 1968.
 25. Bonar, R.A., Heine, U. and Beard, J.W.: Structure of BAI strain A (myeloblastosis) avian tumor virus. *Natl. Cancer Inst. Monograph*, 17, 589, 1968.
 26. Spirin, A.S.: Some problems concerning macromolecular structure of RNA. In *Prog. Nucleic Acid Res.*, Vol. 1, ed. J.N. Davidson and W.F. Cohn, Academic Press, New York, p. 301, 1963.
 27. Crawford, L.V. and Crawford, E.M.: The properties of Rous sarcoma virus purified by density gradient centrifugation. *Virology* 13, 227, 1961.
 28. Granboulan, N., Huppert, J. and Lacour, F.: Examen au microscope electronique du RNA virus du la myeloblastose aviare. *J. Mol. Biol.* 16, 571, 1968.
 29. Kakefuda, T. and Bader, J.P.: Electron microscopic observations on the ribonucleic acid of murine leukemia virus. *J. Virol.* 4, 460, 1969.
 30. Travnick, M. and Riman, J.: Subunits of oncornavirus high-molecular-weight RNA. II. Detection of double-stranded-regions in 60S AMV (avian myeloblastosis virus) RNA. *Biochem. Biophys. Res. Commun.* 54, 1347, 1973.
 31. Granboulan, N. and Franklin, R.M.: Electron microscopy of viral RNA, replicative form and replicative intermediate, of bacteriophage R 17. *J. Mol. Biol.* 22, 173, 1966.
 32. Weber, G.H., Heine, U., Cottler-Fox, M. and Beaudrea, G.S.: Visualization of single-stranded nucleic acid of RNA tumor virus with electron microscope. *Proc. Natl. Acad. Sci. USA* 71, 1887, 1974.
 33. Travnick, M. and Riman, J.: Subunits of oncornavirus high-molecular-weight RNA, I. Stepwise conversion of 60S AMV (avian myeloblastosis virus) RNA to subunit. *Biochem. Biophys. Res. Commun.* 53, 217, 1973.