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# NOTE

# Association of 4S Ribonucleic Acid with Oncornavirus Ribonucleic Acids

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Oncornavirus 60 to 70S ribonucleic acids (RNA), such as those from avian myeloblastosis virus, Schmidt-Ruppin virus, or mouse sarcoma-mouse leukemia viruses, isolated by conventional techniques, contain 4S transferlike RNA molecules that are released upon dissociation of the 60 to 70S RNA with heat. The 4S RNA represents 2.5 to 3.0% of the RNA in the 65S aggregate or 4 to 5 molecules per molecule of 35S RNA formed.

A number of investigators have consistently observed that the high-molecular-weight (60 to 70S) ribonucleic acid (RNA) isolated from several oncornaviruses is converted, upon denaturation, to a form with a sedimentation coefficient of 35S (1-3, 6). We have initiated experiments to investigate the chemical homogeneity of avian myeloblastosis virus (AMV) RNA (R. L. Erikson et al., Virology, *in press*) and have noted that conversion of the high-molecular-weight form to the 35S form also results in the release of a 4S RNA molecule. This observation has important implications concerning the chemical study of viral RNA and we, therefore, present the experimental results in this communication.

Growth and purification of $^{32}$P-labeled AMV has been described previously (3). Schmidt-Ruppin virus (SRV) was prepared from the supernatant medium from infected secondary chick embryo cells. Mouse sarcoma-mouse leukemia virus mixtures (MSV-MLV) were prepared from the supernatant medium from infected 3T3 cells. Although some virus preparations were precipitated with polyethylene glycol (4) instead of ammonium sulfate and, in some cases, a second sucrose equilibrium gradient was run during the purification of the viruses, neither step made a difference in the results described here. RNA was extracted from each virus preparation and the high-molecular-weight RNA was separated from low-molecular-weight viral nucleic acid by sucrose gradient centrifugation.

The release of 4S RNA from 65S AMV RNA is illustrated in Fig. 1a. AMV 65S RNA was heated to 70 C in $10^{-3}$ M ethylenediaminetetraacetic acid plus $10^{-2}$ M tris(hydroxymethyl)-aminomethane (*p*H 7.2) for 3 min, chilled, and placed directly on an acrylamide gel (5) of which the top 1 cm consisted of 2.5% acrylamide plus 0.5% agarose and the bottom 10 cm consisted of 6% acrylamide. The 35S AMV RNA remains at the top of the 6% acrylamide. The RNA which was released by the heat treatment migrated between 7.5 and 8.0 cm. No such RNA is observed upon electrophoresis of an unheated sample (Fig. 1a). To establish the size of the RNA released, it was extracted from the gel, precipitated, and analyzed witn low-molecular-weight cytoplasmic RNA prepared from myeloblasts. Figure 1b shows that the released RNA (termed 65S-associated RNA) migrated with myeloblast 4S RNA (7.5 to 8.2 cm) and faster than myeloblast 5S RNA (6.2 to 6.5 cm).

The same results are observed upon analysis of SRV or MSV-MLV RNA mixtures.

We believe the conditions used here probably release all of the 4S RNA in the 65S RNA aggregate because, by the same treatment, all 28S-associated ribosomal RNA described by Pene et al. (7) is released from the large ribosomal RNA of chick cells (T. A. Walker et al., *unpublished data*). The 65S-associated RNA from AMV or SRV represents between 2.5 and 3.0% of the total radioactivity applied to the gel. Assuming a molecular weight of $3 \times 10^6$ g mole$^{-1}$ for 35S RNA, this would amount to about 5 molecules of 4S RNA per molecule of 35S.

The base ratios of AMV 65S-associated RNA (35%G, 26%C, 17%C, and 22%U) are different from those of 35S RNA (3), and this fact plus the homogeneity of 65S-associated RNA sug-

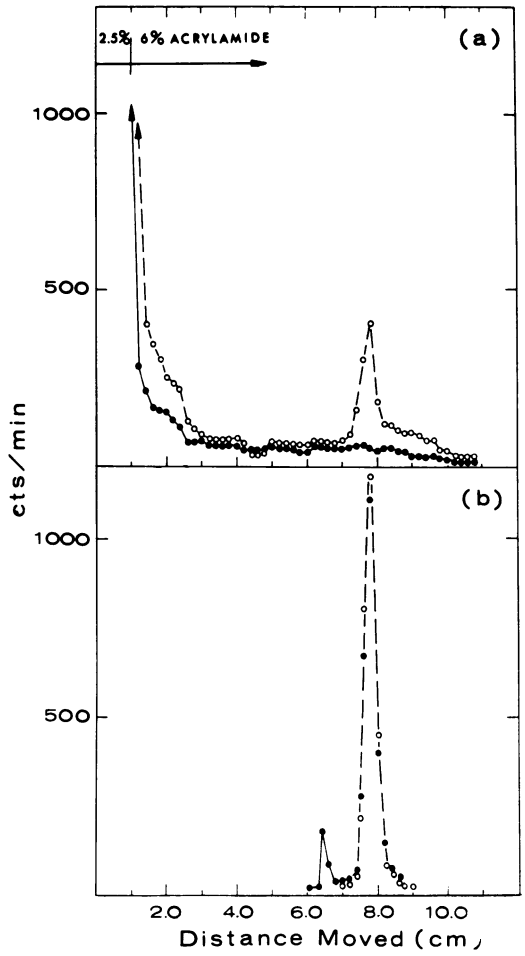


FIG. 1. *(a) Electrophoresis of heated and unheated 65S avian myeloblastosis virus RNA. 65S avian myeloblastosis virus RNA was dissolved in ethylenediaminetetraacetic acid-tris(hydroxymethyl)aminomethane (pH 7.2), heated for 3 min at 70 C, chilled, and layered directly on the gel (○). An unheated preparation was layered on a parallel gel (●) and electrophoresis was carried out for 2 hr at 110 v on both gels. The total recovery from the gels is 85%. There is a total of 30,000 counts/min of 35S RNA at the top of the 6% gel. (b) Size analysis of $^{32}P$-labeled 65S-associated RNA. The RNA released as described in Fig. 1a was extracted from the gel, precipitated with ethanol, and redissolved in electrophoresis buffer with $^{3}H$-labeled 4 and 5S RNA from myeloblasts. Electrophoresis conditions are the same as described in Fig. 1a. $^{3}H$ (●), $^{32}P$ (○). The regions of the gel for which no points are given displayed only background radioactivity.*

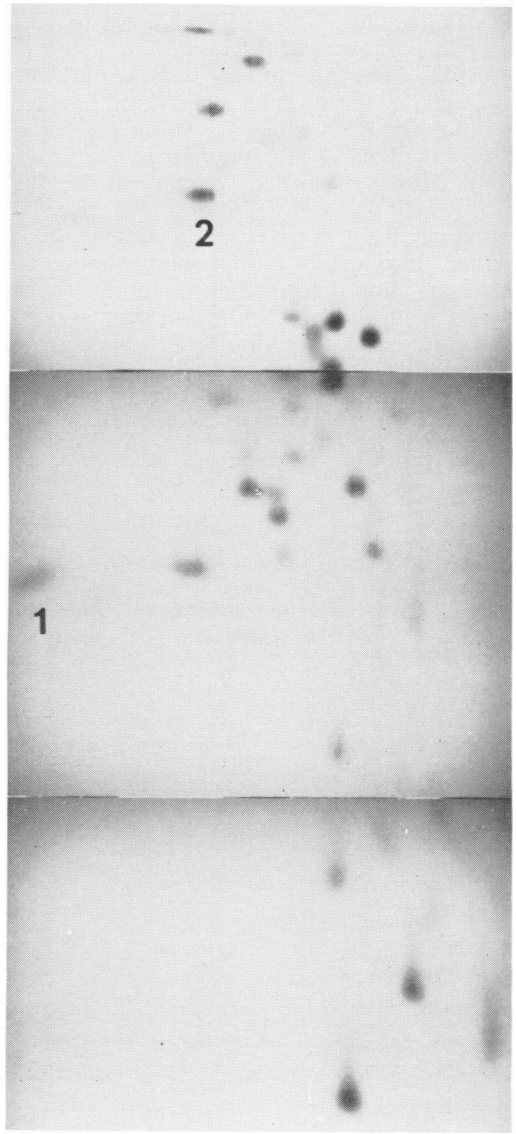


FIG. 2. *Radioautograph of a two-dimensional fractionation of a ribonuclease T1 digest of 65S-associated avian myeloblastosis virus RNA prepared as described in Fig. 1. Spot 1 is pGp and spot 2 is in the region of TψCG (8).*

gests that it is not a degradation product of viral RNA. To characterize the RNA more directly, the 65S-associated AMV RNA was digested with ribonuclease T1 and analyzed by the two-dimensional electrophoresis procedures described by Sanger et al. (8). The radioautograph of the oligonucleotides (Fig. 2) showed similarities to that of host cell 4S RNA and to that of the free 4S RNA in the virion. Two prominent spots were found, one of which was pGp and the other was in the region of TΨCG, which is an oligonucleotide characteristic of many transfer RNA species

(8). In particular, 2.0% of the $^{32}P$ was found in the pGp position and 5.0% in the TΨCG position. This result strongly suggests that the 65*S* RNA aggregate isolated from the virion contains transfer-like RNA molecules which are not released until the RNA is converted to the 35*S* form. There are no data available at this time that would suggest a function for the 65*S*-associated RNA as far as viral replication is concerned. Its presence is important, however, as more attention is focused on the primary structure of tumor virus RNA species. For example, end-group analysis of viral RNA will be greatly influenced by the presence of this 4*S* RNA.

Recently a 4*S* RNA associated with 69*S* MSV-MLV RNA has been reported (B. McCain et al., Bacteriol. Proc., p. 221, 1971). In all probability, it is similar to the transfer-like RNA molecule described here.

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