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Low-Molecular-Weight RNAs of Murine Sarcoma Virus: Comparative Studies of Free and 70S RNA-Associated Components

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Several low-molecular-weight RNAs were released from mouse leukemia sarcoma virus 70S RNA under conditions of thermal denaturation. One of them, the 70S-associated 8S RNA, exhibits a number of structural properties characteristic of a previously described free viral 8S RNA. This similarity was revealed by polyacrylamide gel electrophoresis and nucleotide composition.

Several investigators observed that, upon heating, the avian oncornavirus high-molecular-weight 70S RNA (HMW-RNA) releases a broad spectrum of molecules which can be classified into two categories according to their size. The first consists of large molecules with an average sedimentation coefficient of 35S; the second contains the small RNA species, among which tRNA was recently identified (4, 8).

In this communication, we describe the presence of several 70S-associated low-molecular-weight RNAs in a murine oncornavirus, the Moloney sarcoma virus [MSV-M(MLV)]. Among them, special attention was given to one component presenting the same electrophoretic mobility and the same nucleotide composition as the free viral 8S RNA previously described (6, 7).

Mouse sarcoma virus (Moloney strain) was prepared from growth fluids of 78 A_1, a chronically infected cell line (2). The cells were grown as monolayers in Eagle minimal essential medium supplemented with 10% calf serum.

The cells were labeled with ^{32}P- or ^{3}H-uridine as described (7). Cell supernatants were harvested usually 18 h after labeling. No difference in the results was noted when the supernatants were harvested after a labeling period of 4 h.

The growth fluids were clarified by centrifugation at 21,000 rpm in the Spinco rotor 21 for 100 min at 0 C. Pellets were homogenized in NTE buffer (0.01 M Tris, HCl [pH 7.4], 0.1 M NaCl, 0.001 EDTA) and purified by isopycnic centrifugation in a sucrose gradient (15 to 50% [wt/vol] in NTE), in a Spinco SW65 rotor for 75 min at 50,000 rpm. The virus band, located at a buoyant density of 1.16 g/cm^3, was concentrated by centrifugation at 40,000 rpm for 60 min in the Spinco rotor 40. RNA was extracted from the purified virus by means of the cold phenol sodium dodecyl sulfate procedure already described (5). All the operations were performed in the presence of potassium polyvinyl sulfate (20 μg/ml). HMW-RNA was separated from the low-molecular-weight RNAs by velocity centrifugation, 5 to 20% wt/vol (sucrose

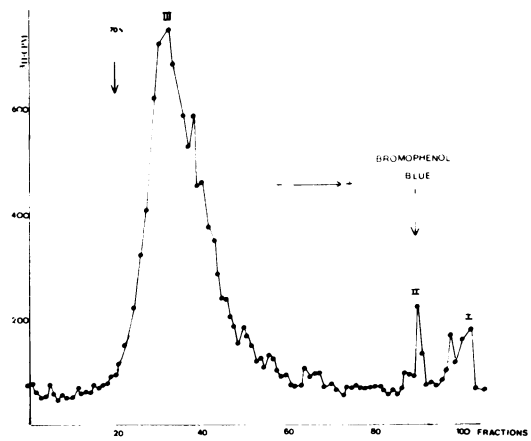

FIG. 1. *Electrophoresis of heated 70S murine sarcoma virus, in 1.7% polyacrylamide-0.5% agarose gel. The ^{32}P-labeled 70S RNA was dissolved in NTE buffer (pH 7.4) (0.01 M Tris, HCl, 0.01 M NaCl, 0.001 M EDTA) heated 3 min at 80 C, rapidly chilled, and layered directly on a 1.7% polyacrylamide-0.5% agarose gel. Migration was performed at 5 mA per gel for 3.5 h, following the procedure of Tiollais et al. (9). Slices were cut 1.5-mm thick, hydrolyzed for 1 h at 60 C in 1 ml of water, and counted with Bray solution in a Packard scintillation counter.*

gradient Spinco rotor SW41, 65 min, at 40,000 rpm). Nucleic acid was precipitated with 2 volumes of cold ethanol in the presence of 0.1 M NaCl and 200 μg of unlabeled RNA as carrier and analyzed by polyacrylamide gel electrophoresis according to Tiollais et al. (9).

Purified 70*S* RNA was heated under conditions known to entirely dissociate its subunits (0.1 M NaCl, 80 C, 3 min) (1). Components obtained by this treatment were separated by electrophoresis in a composite polyacrylamide-agarose gel (1.7 to 0.5%) as illustrated in Fig. 1. Peak III contained the large 35 to 40*S* subunits. In the region of the small RNA species, two peaks were clearly resolved. Peak I migrated to the position of 4*S* RNA. Material of peak II had the same electrophoretic mobility as the free viral 8*S* RNA recently described in this virus (6). As 8*S* RNA was previously found to be composed of two species $8S_A$ and $8S_B$, which can be separated from each other by electrophoresis in a 10% acrylamide gel, comparative analysis of free and 70*S*-associated RNAs under the same conditions was deemed of interest. This was done by comigration of ^{3}H-labeled 70*S*-heated RNA and ^{32}P-labeled light viral free RNA recovered from the top of a 5 to 20% sucrose gradient (Fig. 2).

The ^{32}P profile (free viral RNA species) shows the presence of $8S_A$ and $8S_B$ components, 5*S* and 4*S* RNA. The ^{3}H profile of 70*S*-associated RNA species shows the presence of four monodisperse species named I, II, III, and IV. By analogy with avian viruses, we assume that peak I contains transfer RNA and molecular species which could act as primer of the reverse transcriptase (3). It can be seen that this RNA fraction migrates slightly slower than the host cell or the free viral 4*S* RNA and that peaks II and III comigrate at the same position as cellular 5*S* and 5.5*S* RNAs, respectively. Recently, 5*S* cellular RNA associated with 70*S* RNA was identified in purified Rous sarcoma virus (A. C. Garapin, personal communication). It is likely that a similar situation prevails in murine oncornaviruses.

Special attention was given to the material of peak IV. Since peak IV clearly had the same

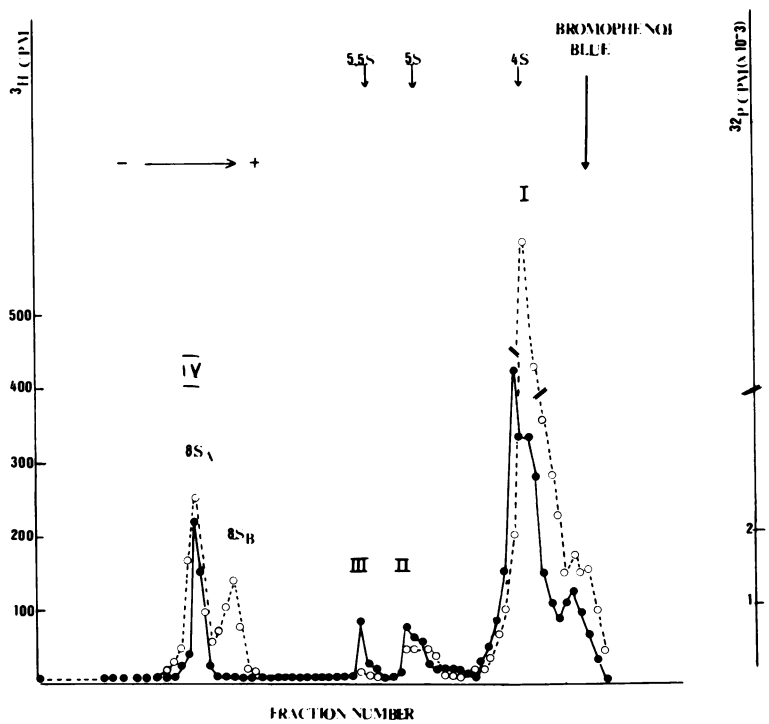

FIG. 2. *Electrophoresis of heated 70S RNA and low-molecular-weight "free" viral RNA in composite gels of 2.5% and 10% polyacrylamide. ^{3}H-labeled heated 70S RNA (same conditions as Fig. 1) and ^{32}P-labeled "free" low-molecular-weight RNA are mixed and layered directly onto the gel. The gels consisted of 12 cm of 10% polyacrylamide overlaid with 4.5 cm of 2.5% polyacrylamide. The 2.5% overlayer is not shown in the figure. Migration was performed overnight at 4 mA/gel. Symbols: ●, ^{3}H-70S associated RNAs; ○, ^{32}P-free viral low-molecular-weight RNAs. Arrows indicate the positions of cellular 5S, 5.5S, and 4S RNA markers.*

TABLE 1. *Nucleotide composition of MSV-M(MLV) RNAs*[a]

RNA	Nucleotide				
	C	A	G	U	G + C
8S (70S-associated)	24.7 ± 0.6	21.7 ± 0.5	37.0 ± 0.9	16.4 ± 0.4	61.7 ± 1.5
8S A (free)	24.7 ± 0.6	21.5 ± 0.5	35.0 ± 0.8	18.7 ± 0.4	59.7 ± 1.4
8S B (free)	24.1 ± 0.6	24.8 ± 0.6	39.1 ± 0.9	11.8 ± 0.2	63.2 ± 1.5
8S[b] (A + B) (free)	24.6 ± 0.6	22.3 ± 0.6	36.0 ± 0.9	16.9 ± 0.4	60.6 ± 1.5
70S	30.8 ± 0.3	25.9 ± 0.3	21.8 ± 0.2	21.6 ± 0.2	52.6 ± 0.5

[a] 70S-associated small RNA components were isolated by running 70S-heated RNA in 10% polyacrylamide gels. Slices corresponding to free and 70S-associated 8S RNAs were broken in a glass Teflon homogenizer in the presence of 2×10^{-2} M, pH 5.1 acetate buffer. Polyacrylamide was discarded by sedimentation, and RNA was precipated by adding 2 volumes of ethanol and 0.1 M NaCl. Precipitates were hydrolyzed overnight by 0.5 M NaOH at 37 C. The hydrolysate was applied to a Whatmann no. 1 paper and subjected to high-voltage electrophoresis in a solvent consisting of pyridine, acetic acid, and water (5:5:90), pH 3.5, at 3,000 V for 1 h. Radioactive spots were located by autoradiography, cut out, and eluted in scintillation vials by 0.1 N HCl. A 10-ml amount of Bray solution was added, and counting was done in a Packard scintillation counter.

[b] Relative proportion of the 8S RNA species (A = 75% and B = 25%) were taken in account for calculation.

electrophoretic mobility as the $8S_A$, it was called 70S-associated 8S RNA. We have recently found that the free $8S_A$, and $8S_B$ RNAs can be converted by heating into a single species moving to the position of the $8S_A$ peak (to be published). It is therefore difficult to decide whether the 70S-associated 8S RNA, also released by heat, is a single species A or a mixture of A + B.

The similarity in nucleotide composition of free and 70S-associated 8S RNAs might be in agreement with the second hypothesis (Table 1). Moreover, the base ratios of both low-molecular-weight RNA species differ from that of MSV-M(MLV) 70S RNA, giving support to the hypothesis that these components are not degradation products of 70S RNA.

The relative proportions of the 70S-associated RNAs were determined from several experiments. 8S RNA was found to represent 1 to 1.5% of the 70S RNA, whereas the amounts of 5S and 5.5S species did not exceed 1%. 4S RNA represented about 4% of the HMW-RNA.

The present data indicate a close relationship between murine and avian oncornaviruses in their ability to release light RNA components by denaturation of the 70S RNA. The presence of 4S RNA seems to be a common feature of these viruses. However, 70S-associated 8S RNA is not known to be present in the avian viruses. As judged by its electrophoretic mobility in polyacrylamide gels and nucleotide composition, this MSV-M(MLV) component is similar to the free viral 8S RNA. Oligonucleotide mapping after enzymatic digestion should provide further detail concerning the similarity of free and 8S-associated RNAs.

The function of all the free and associated small RNAs is not yet known. Rous sarcoma virus 70S-associated 4S RNA constitutes an exception since E. Canaani and P. Duesberg have shown that a fraction thereof act as a primer for reverse transcriptase. As for the 70S-associated 8S RNA, it could be a linker in the substructure of the murine viral genome.

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