Murine Oncornavirus High-Molecular-Weight RNA Structure: Thermal Stepwise Dissociation of 70S Murine Leukemia-Sarcoma Virus to Subunits and Low-Molecular-Weight Associated RNAs

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The thermal dissociation into subunits and low-molecular-weight (LMW) associated RNAs of the aggregate structure of 70S RNA of a murine leukemia sarcoma viral complex was studied. By polyacrylamide-agarose gel electrophoresis, it was found that at low temperature a fraction of the genome was converted into an intermediate population of RNA (Im.P) with an apparent molecular weight of $6.6 \times 10^4$. At higher temperature, the 70S RNA and the Im.P RNA were successively dissociated into two RNA subunits called "I" and "II" and 70S-associated LMW RNAs. The apparent molecular weight of subunit I was about $5 \times 10^4$ and that of subunit II was about $3.2 \times 10^4$. The release of 4S, 5S, 5.5S, and 8S RNAs from 70S RNA at various temperatures was studied by composite polyacrylamide gel electrophoresis. It was found that the nature of hydrogen bonding to the 70S RNA was different for each LMW RNA species. A possible relationship of the association between the subunits and each 70S-associated LMW RNA, based on their $T_m$ values, is discussed.

The 70S RNA genome of murine oncornavirus can be converted by heat or any other conditions that disrupt hydrogen bonds to subunits and low-molecular-weight (LMW) associated RNAs (1, 4). The aim of this study was to investigate the genome structure by following the conversion at various temperatures of the 70S RNA to smaller components by polyacrylamide gel electrophoresis. We present evidence that the 70S RNA of murine leukemia-sarcoma virus [M-MSV (MLV)] is a molecule of great complexity. It was found that at low temperature one portion of the 70S RNA was dissociated into an intermediate RNA population (Im.P) of about $6.6 \times 10^4$ daltons. Both aggregates, the 70S and the Im.P RNA, were successively converted at higher temperature into two RNA populations, one with a molecular weight of about $5 \times 10^4$ (I RNA) and the other with a molecular weight of about $3.2 \times 10^4$ (II RNA), plus LMW associated RNAs. The temperature at which the LMW RNAs had melted off the 70S RNA indicates that the nature of hydrogen bonding is different for each LMW RNA species. Our results concerning the 4S and 5S RNAs are very close to those previously reported for avian oncornavirus RNAs (8). In addition, we show that the 8S RNA has stronger bonds to the 70S complex and is liberated in a second step of 70S thermal dissociation. The significance of this observation is discussed.

MATERIALS AND METHODS

Mouse sarcoma virus (Moloney strain) was prepared from growth fluids of the chronically infected cell line 78 A₁ (2). Cells were grown as monolayers in Eagle minimum essential medium supplemented with 10% calf serum. The cells were labeled with $^{32}$P as previously described (11). The supernatant was usually harvested after 18 h of labeling. No difference in results was noticed when virus was harvested after a labeling period of 8 h.

The procedures of virus purification have been described previously (7, 11).

Nucleic acids were extracted from the purified virus by the cold phenol-sodium dodecyl sulfate procedure as already described (9). Total nucleic acid was precipitated overnight with 2 volumes of cold ethanol in the presence of 0.1 M NaCl at $-20$ C. Nonradioactive RNA extracted from rat cells was added as carrier. All operations were performed in the presence of potassium polyvinyl-sulfate (20 µg/ml). The high-molecular-weight RNA was separated from the LMW RNAs by velocity centrifugation through a 5 to 20% sucrose gradient.

After precipitation, the purified 70S RNA was dissolved in NTE buffer (0.01 M Tris-hydrochloride, pH 7.4, 0.1 M NaCl, 0.001 M EDTA), heated at various temperatures for 3 min, and then rapidly chilled.

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Components obtained by thermal treatment were analyzed by electrophoresis through a composite polyacrylamide-agarose gel (1.9 to 0.5%) or a 10% polyacrylamide gel overlaid with a 2.5% polyacrylamide-agarose according to the experimental purpose.

RESULTS

Thermal stepwise dissociation of M-MSV (MLV) 70S RNA into subunits. As already stated, the experiments were performed to elucidate the means whereby the RNA species of the viral genome are held together. For this purpose, 70S RNA was heated at different temperatures and analyzed in composite polyacrylamide-agarose gels (1.9 to 0.5%). The polyacrylamide gel migration profiles of the treated 70S RNA is presented for each temperature (Fig. 1). The assumption was made that migration rates of the rRNA and tRNA markers could be used to calculate the approximate molecular weight of the different viral RNAs (3) (inserts in Fig. 1). On this basis, the molecular weight of the native viral genome was 1.2 × 10^7 (Fig. 1A). A change in the electrophoretic mobility of a portion of the 70S RNA complex mobility was already observed after treatment at 40°C (Fig. 1B). At this temperature, about one-third of the viral native RNA radioactivity was found in a peak representing a population with a molecular weight of approximately 6.6 × 10^6 (Im.P.).

After further heating, this intermediate RNA component generated I and II RNA subunits and light 70S-associated RNAs. The peak corresponding to the stable 70S RNA at 40°C, after a shift to an RNA population of about 10^7 daltons at 50°C and one of about 8.5 × 10^4 daltons at 55°C disappeared completely at 60°C and the radioactivity was transferred mainly to the I-II RNA region and to light associated RNAs as well (Fig. 1C–E).

A complete dissociation of the 70S RNA molecule was observed after treatment at 80°C. No difference in the electrophoretic pattern was noticed at higher temperatures. The completely denatured viral RNA (Fig. 1F) consisted of two major components: one with an electrophoretic mobility corresponding to a molecular weight of about 5 × 10^4 (I RNA) and the other to a molecular weight of about 3.2 × 10^4 (II RNA).

Other RNA dissociation products with faster electrophoretic mobility also appeared giving a heterogeneous region in the gel. It is not possible to further classify this heterogeneous population into subunit components. Their amount is probably related to the fact that in this study the virus was harvested at intervals of several hours.

The light associated RNAs released by the total denaturation of the viral genome exhibited 8% of the native 70S RNA radioactivity (Fig. 2).

Thermal transition of 70S-associated LMW RNAs. Since these RNAs are detected during the dissociation of the high-molecular-weight RNA, a further characterization was performed. The associative nature of each light RNA released with the 70S RNA complex was explored by heating at various temperatures. For this purpose we used 10% polyacrylamide gels overlaid with 2.5 to 0.5% composite polyacrylamide-agarose gels. To obtain a good separation of the subunits described above, we overlaid long (7 cm) 2.5% polyacrylamide gels on 10 cm of 10% polyacrylamide gels. All 12 to 38S species as the II RNA subunits accumulate at the interface of the two polyacrylamide concentration gels, whereas the I RNA subunits do not reach the interface because of their slower rate of migration. To obtain a more precise localization of the electrophoretic migration of the I and II RNAs, an additional marker was used in these experiments: the 45S RNA extracted from L 5178 Y cells (14).

The light RNAs associated with the M-MSV (MLV) genome are the 8S, 5.5S, 5S, and 4S RNAs, as we have described earlier (12). The thermal transition of these small RNAs was followed by their appearance in the 10% polyacrylamide gel. In Fig. 3 is shown the electrophoretic distribution of 70S RNA compounds after incubation at various temperatures. Among the light 70S-associated RNAs, the 4S RNA species appeared at rather low temperatures in contrast to the 8S RNA. The 5 and 5.5S RNAs appeared when the 70S RNA disappeared.

It is of interest to note that the 70S-associated 8S RNA was composed of two distinct species, A and B, when the genome was heated at low temperatures. The B molecules were converted into the A component at higher temperatures. These data corroborate our previous results on the secondary structure of free viral and cellular 8S RNA (6, 10, 13).

For a more extensive characterization of this phenomenon, we calculated the Tm values of each RNA species released from the 70S RNA complex as described by Faras et al. (8). These values represent the temperature at which half of a specific RNA component is dissociated from the viral genome.

The 4S RNA had a Tm value (47°C) lower than that of the other 70S-associated RNAs: 52°C for the 5S RNA, 54°C for the 5.5S RNA, and 56°C for the 8S RNA (Fig. 4). The Tm value of 70S RNA disappearance and that of II RNA subunit appearance were identical to that of 5S
Fig. 1. Electrophoresis of heat-treated 70S RNA in 1.9% polyacrylamide-0.5% agarose gels. Migration was performed at 5 mA per gel for 3.5 h. Slices were cut 1.5 mm thick, hydrolyzed for 1 h at 80 C in 1 ml of water, and counted with Bray solution in a Packard scintillation counter. Inserts show logarithm molecular weight electrophoretic mobility relations using cellular ribosomal 18S and 28S RNAs and yeast 4S RNA as markers. Thermal treatment: A = 20 C; B = 40 C; C = 50 C; D = 55 C; E = 60 C; and F = 80 C.

RNA (52 C), and that of the I RNA subunit appearance was identical to that of 8S RNA (56 C) (not shown). These values represent the average of three experiments done in identical conditions of gel electrophoresis as reported in Fig. 1 and 3. These results point out that not all of the light associated RNAs' release is concomitant with the dissociation of the 70S RNA into subunits.

DISCUSSION

The experiments described in this paper show that the genome of the mouse sarcoma-leukemia (Moloney strain) viral complex has an aggregated structure. It is composed of two RNA subunits, I and II, and 4S, 5S, 5.5S, and 8S associated RNAs.

The molecular weights of the subunits corre-
spond to approximately $3.2 \times 10^6$ (II) and $5 \times 10^6$ (I). At least one portion is derived from the dissociation of an intermediate RNA population whose molecular weight is $6.6 \times 10^6$. A similar intermediate RNA component was obtained by the action of formamide on the avian myeloblastosis virus genome by Travnicek and Riman (15). The viral preparation we studied is a complex of murine sarcoma and leukemia viruses. Moreover, the molecular weights of the genome subunits are very similar to those of the "a" and "b" Rous sarcoma virus RNA subunits described by Duesberg and Vogt (5), and also to those recently obtained by Lo and Ball (12) for a murine leukemia-sarcoma virus, MoMuSV. These similarities suggest precise biological functions for the M-MSV (MLV) I and II RNA.

**FIG. 2.** Thermal transition of the release of 70S-associated total LMW RNAs. The percentage of total LMW RNAs was computed from the data of Fig. 1 by summing the radioactivity present under the area of 8S to 4S RNA (155 to 200 mm).

**DISTANCE MOVED (mm)**

**FIG. 3.** Electrophoresis of heat-treated 70S RNA in composite gels of 2.5 and 10% polyacrylamide. The composite gels consisted of 10 cm of 10% polyacrylamide overlaid with 7 cm of 2.5% polyacrylamide. Migration was performed overnight at 3 mA/gel. Symbols: Two separate scales are present on the ordinate. The scale represented by the broken line corresponds to 70S and its subunits and the scale represented by the solid line corresponds to 70S-associated 8S, 5.5S, 5S, and 4S RNAs. Arrows indicate the positions of cellular 45S, 8S, 5S, and 4S markers.
subunits. However, the interpretation is made difficult because the viral preparation studied is a complex of viruses propagated on cell lines from heterologous species and also because it is a mixed MLV-MSV-producing culture. In a recent publication, Tsuchida and Green (16) reported that only one class of subunits (3 x 10^4 daltons) was detected after conversion by denaturation of 70S RNA isolated from M-MSV (MLV) virions. The discrepancy between our data and those of Tsuchida and Green may result from differences in the proportion of MLV-MSV by the producing culture or by differences in the experimental conditions (nature of the electrophoresis gels, distance of subunits migration, etc.).

The stepwise release of light RNAs upon thermal treatment indicates that the nature of their association with the different RNA species of the genome is different for each light RNA. The $T_m$ values suggest that the 5S RNA is released from the 70S RNA complex at the temperature required to dissociate 70S RNA into the II RNA subunits. At least one portion of the 4S RNA species is weakly hydrogen bonded and is probably released when the Im.P appears (see Fig. 1). The phenomenon is more complex as far as 8S RNA is concerned. Its high $T_m$ value indicates that its release is effectuated in a second step of the 70S RNA denaturation process. The $T_m$ value of the appearance of the I RNA subunit is identical to that of 8S RNA. These data suggest that both RNA populations are simultaneously formed with a certain lag phase on 70S RNA dissociation. Since a relationship exists between the liberation of 8S and I RNAs and the dissociation of the Im.P, it is tempting to hypothesize that the Im.P is composed of the association of I RNA subunits with 8S RNA. It can be concluded that the function of the majority of the 70S-associated LMW RNAs is to maintain the overall aggregate structure of the viral genome, the subunits of which are held together by uneven hydrogen bonds. Further characterization of the subunits and 70S-associated LMW RNAs in different leukemia or sarcoma virus systems is needed to specify their biological significance in virus replication and cell transformation. Such studies are in progress.

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LITERATURE CITED


