JOURNAL OF VIROLOGY, Jan. 1979, p. 328-335
0022-538X/79/01-0328/08$02.00/0

Vol. 29, No. 1

Selective Packaging of Host tRNA's by Murine Leukemia Virus Particles Does Not Require Genomic RNA

JUDITH G. LEVIN* AND J. G. SEIDMAN

Laboratory of Molecular Genetics, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland 20014

Received for publication 8 August 1978

The 4S RNA contained in RNA tumor virus particles consists of a selected population of host tRNA's. However, the mechanism by which virions select host tRNA's has not been elucidated. We have considered a model which specifies that 35S genomic RNA determines which tRNA's are to be encapsidated as well as the relative amounts of these tRNA's within the virion. The model was tested by comparing the free 4S RNA composition of normal murine leukemia virus (MuLV) particles and noninfectious virions from actinomycin D (ActD)-treated cells, which are deficient in genomic RNA (ActD virions). Viral 4S RNA was analyzed by two-dimensional polyacrylamide gel electrophoresis. Surprisingly, the patterns obtained for control and ActD 4S RNA were identical to each other and were clearly distinct from the cell 4S RNA pattern. The viral patterns had three prominent areas of radioactivity. One of the spots was identified on the basis of its oligonucleotide fingerprint as $tRNA^{Pro}$, the primer for MuLV RNA-directed DNA synthesis. These results were obtained with two different MuLV strains, AKR and Moloney, each grown in SC-1 cells. The demonstration that ActD virions contain primer tRNA and in general exhibit the characteristic MuLV tRNA pattern rather than the complete representation of cell 4S RNA leads to the conclusion that genomic RNA is not the major determinant in selective packaging of host tRNA's. A possible role for one or more viral proteins, including reverse transcriptase, is suggested.

RNA tumor viruses contain significant amounts of 4S RNA (5, 7, 18). This 4S RNA exhibits amino acid acceptor activity (7, 9, 16, 22, 51, 53, 54) and consists of approximately 15 different tRNA species (40, 44). Interestingly, all of the virion-associated tRNA's are derived from the host (1, 5, 7, 18, 57). However, evidence from many laboratories indicates that the viral 4S RNA population does not reflect the distribution of tRNA's in the cell but instead represents a selected class of host tRNA molecules (5, 9, 16, 22, 40, 41, 51, 53, 54). Although these observations were initially reported about 10 years ago, the mechanism by which virions selectively package host tRNA's is still not understood. To design an experimental approach which can be used to investigate this aspect of virus assembly, it is important to first consider some of the more recent information on viral 4S RNA.

Most of the viral tRNA's are isolated from particles as unassociated molecules, which are referred to as "free" 4S RNA, but a significant fraction is also found in association with 35S genomic RNA (17) as a high-molecular-weight 70S RNA complex. The 70S-associated 4S RNA is presumably bound to 35S RNA in the 70S RNA complex because dissociation of this 4S RNA occurs only under denaturing conditions which disrupt hydrogen bonds (8, 11, 17, 19, 54). A broad melting profile is obtained during thermal denaturation of 70S-associated 4S RNA, and it has been suggested that this is due to differences in the extent of binding between individual tRNA's and 35S genomic RNA in the 70S RNA complex (19). A prominent tRNA species which is the most tightly bound to 35S RNA (8, 11, 40, 49, 54) and which is also a major constituent of free 4S RNA (40, 44, 49, 54) serves as the primer for RNA-directed DNA synthesis by the reverse transcriptase enzyme (8, 11, 20, 49, 52). The interaction between primer tRNA and 35S RNA is highly specific and involves hybridization of 16 nucleotide base pairs (10, 14) at a site close to the 5′ end of 35S RNA (7a, 48, 50). In the case of the avian viruses, the primer has been identified as $tRNA^{Trp}$ (21, 25, 56) and for the murine viruses as $tRNA^{Pro}$ (40). The evidence indicating that tRNA's are bound to genomic RNA as well as the nature of the binding reaction between 35S RNA and the primer suggests that these interactions may be important in virus assembly. Moreover, it raises the

possibility that 35S genomic RNA is a major determinant in the selective encapsidation of cell tRNA's.

The availability of the actinomycin D (ActD) system, which has previously been used for studying virion assembly in the absence of genomic RNA (23, 30, 32, 33), provides a unique opportunity for evaluating this model. In this system, after several hours of treatment with ActD, murine leukemia virus (MuLV)-infected cells produce noninfectious particles (ActD virions) (32, 39) which lack genomic RNA and contain predominantly 4S RNA (32). Despite this deficiency in genomic RNA, ActD virions contain the normal complement of MuLV structural proteins (30, 33), and a reverse transcriptase which is functionally indistinguishable from the standard MuLV enzyme (23). In addition, these defective particles are identical to normal virions with respect to morphology (32) and sedimentation behavior in sucrose gradients (32).

In the present study, we have investigated the role of genomic RNA in tRNA selection by comparing the composition of free 4S RNA in normal and ActD virions. If genomic RNA is a major element controlling the selection process, virions lacking 35S RNA might be expected to contain a more typical representation of host tRNA's instead of the characteristic viral pattern. In testing this hypothesis, we were especially interested in determining whether the $tRNA^{Pro}$ primer is present in the defective particles and if so whether it occurs in the same proportion as in normal MuLV. By using two-dimensional (2-D) polyacrylamide gel electrophoresis (29) to analyze 4S RNA, the results showed that the patterns for control and ActD virion 4S RNA were identical to each other, but were distinct from the cell 4S RNA pattern. Both viral patterns had a prominent spot which was identified on the basis of its oligonucleotide fingerprint as $tRNA^{Pro}$.

MATERIALS AND METHODS

Materials. ActD was a gift from Merck, Sharp, & Dohme. $^{32}PO_4^{3-}$ (carrier-free) was purchased from New England Nuclear Corp.

Cells and viruses. SC-1 cells (26) and the AKR-L1 and Moloney strains of MuLV were graciously provided by Marilyn Lander. The cells were infected and maintained as described previously (33). To prepare ^{32}P-labeled viral RNA, the cells were grown in 100-mm plastic petri dishes in Eagle medium containing 10% fetal calf serum. One day before reaching confluence, each culture received Eagle medium with 1/10 the normal phosphate concentration, 3% fetal calf serum, and 1 mCi of $^{32}PO_4^{3-}$. After incubation overnight (16 to 17 h), Eagle medium containing 10% fetal calf serum and 0.1 μg of ActD per ml (where specified) was added (zero time). Four hours later, fresh medium with or without ActD was added, and virions were harvested after 2 more h of incubation (4- to 6-h harvest). With 0.1 μg of ActD per ml, viral RNA synthesis is inhibited by at least 90% (13, 39; unpublished data). The procedures for virus purification have been described (33) except that sedimentation at 96,000 × *g* was for 100 min. In each experiment, infectivity was determined by the XC plaque assay (42) on a parallel set of unlabeled cultures. The XC tests were expertly performed by Elizabeth von Kaenel, Microbiological Associates.

Isolation and analysis of viral RNA. RNA was extracted from purified virions as previously described (32). The low-molecular-weight RNAs were fractionated by the 2-D polyacrylamide gel electrophoresis procedure of Ikemura and Dahlberg (29) with a few modifications. Briefly, viral RNA was applied to a 1.5-mm-thick 10% polyacrylamide slab gel (10 by 35 cm) and was subjected to electrophoresis overnight at 4°C at a constant current of 6.5 mA. The run was terminated when the bromophenol blue dye marker reached 25 cm. The 4S RNA band was located by autoradiography, and a strip (2 by 6 cm) was cut out from the 10% gel and laid across the top of an already polymerized 20% polyacrylamide slab gel (13 by 18 cm). A 10% acrylamide gel mixture was then allowed to polymerize around the 10% gel strip. Electrophoresis in the second dimension was carried out at room temperature at a constant current of 6 mA overnight and 12 mA on the next day. Buffer was circulated through the upper and lower chambers of the apparatus. The run was terminated when the bromophenol blue had migrated 30 to 35 cm. The gel was then subjected to autoradiography. The 2-D gel patterns obtained by this procedure were very reproducible. Presumably, differences in experimental conditions account for the somewhat different pattern described by Peters et al. (40).

Oligonucleotide fingerprint analysis. The tRNA species to be analyzed were cut out and eluted from the 20% polyacrylamide gel in the presence of 50 μg of carrier *Escherichia coli* tRNA and were then concentrated on a small DEAE-cellulose column (46). The RNA was digested with RNase T1 and was then subjected to 2-D fingerprint analysis as described by Sanger et al. (43).

RESULTS

Analysis of RNA in purified supernatant fluids of uninfected and infected cells. To assess the possible role of the viral RNA genome in the selection of host tRNA's, it was necessary to compare the 4S RNA composition of control and ActD virions. During our initial attempts to analyze the tRNA of ActD virions, we found that sequential exposure of cells to high levels of $^{32}PO_4^{3-}$ and ActD (1 μg/ml) caused severe cytotoxic effects and contamination of virions with cellular debris. As a result, abnormally high amounts of rRNA were present in preparations of ActD virion RNA, and it seemed likely that the tRNA in such preparations would be largely

contaminating host cell 4S RNA. Several approaches were employed in an effort to minimize these effects, but the simplest was to lower the dose of ActD. Control experiments without label indicated that for a 4- to 6-h harvest, varying the ActD concentration 10-fold, from 0.1 to 1 μg/ml, gave only a small variation in the inhibition of viral infectivity (93 to 97%); however, when viewed by phase-contrast microscopy, cells treated with 0.1 μg of the drug per ml appeared undamaged and were essentially indistinguishable from untreated controls. Thus, the inhibitory effect of ActD was preserved under conditions of minimal toxicity to cells.

In addition, the previous observation that ActD virions are deficient in 70S RNA (32) was confirmed when 0.1 μg of ActD per ml was used (Fig. 1). Viral RNA was analyzed in composite 1.8% polyacrylamide–0.5% agarose gels under conditions which resolve 70S and 4S RNA (32). For comparison, similar amounts of ^{32}P-labeled RNA from control and ActD virions were used. As may be seen from Fig. 1, a large peak of 70S RNA and a much smaller peak of 4S RNA were observed in the control. In contrast, the ActD sample contained predominantly 4S RNA and a small peak of radioactivity which comigrated with 70S RNA in the ^{3}H-viral RNA marker. This material presumably corresponds to 70S RNA in infectious particles, which are always present in small amounts in ActD virion preparations (32, 33). The ratio of 70S to 4S RNA in the two virus preparations was calculated from the total amounts of radioactivity in each of the major peaks and was found to be 3.2 for the control and 0.39 for the ActD sample. Thus, the 70S/4S RNA ratio of the control was eightfold greater than that of the ActD sample, in accord with the lowered infectivity of the ActD virion preparation.

Because control and ActD virions differ in their RNA composition, it was of interest to determine whether they also differ in the total amount of particle-associated RNA. By using virion-associated reverse transcriptase activity (23) as a measure of total virus particles (33) and then comparing total RNA with total enzyme activity for each preparation, it can be seen from Table 1 that ActD and normal virions contain roughly the same amounts of RNA per virion. Moreover, the data of Fig. 1 as well as earlier observations (32) suggest that the 70S RNA deficiency in the defective particles is at least partially compensated by a 1.5- to 2-fold increase in 4S RNA content.

The background level of cell 4S RNA in virion preparations was determined by comparing the 4S RNA content of ^{32}P-labeled RNA from purified fluids of uninfected and infected cells, incubated in either the presence or absence of 0.1 μg of ActD per ml. All four RNA samples were subjected to electrophoresis in a 10% polyacrylamide slab gel, which resolves low-molecular-weight RNAs. As shown in Fig. 2, most of the

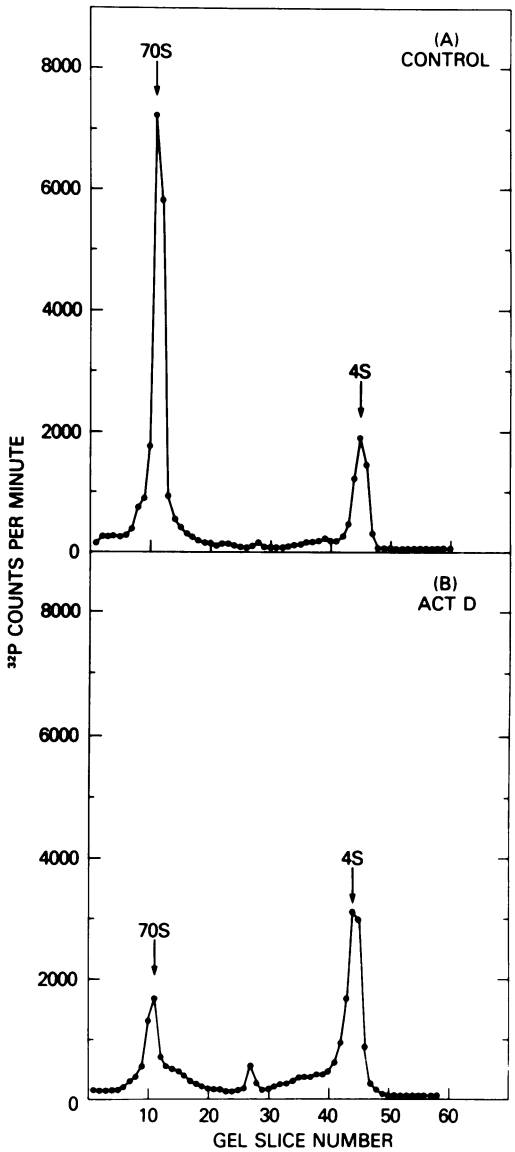

FIG. 1. *Polyacrylamide gel electrophoresis of ^{32}P-labeled RNA from control and ActD virions. ^{32}P-labeled viral RNA was subjected to co-electrophoresis with ^{3}H-labeled marker RNA from control virions in 1.8% polyacrylamide–0.5% agarose gels, as described previously (32). The arrows denote the positions to which ^{3}H-labeled 70S and 4S RNA migrated. A parallel gel with ^{32}P-labeled cell RNA was also run. 28S and 18S RNA peaks were at gel slice numbers 23 and 29, respectively.*

TABLE 1. *Amounts of RNA and reverse transcriptase activity associated with control and ActD virions*

Virus	Total RNA[a] (cpm)	Total reverse transcriptase[b] (pmol of [^{3}H]dTMP incorporated)	RNA/reverse transcriptase
Control	176,298	4,044	43.6
ActD	132,684	3,000	44.2

[a] Total RNA extracted from virion preparations used for analysis in Fig. 1.

[b] The reverse transcriptase activity in the peak fractions of the discontinuous sucrose gradient employed in virus purification (see text) was summed.

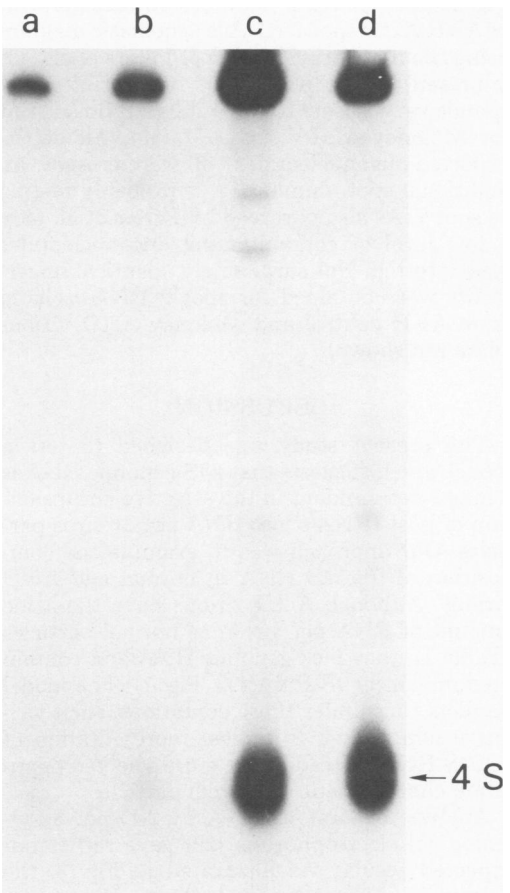

FIG. 2. *Analysis of ^{32}P-labeled RNA from culture fluids of uninfected and AKR virus-infected SC-1 cells incubated in the presence or absence of ActD. Four parallel sets of cultures (five plates per set) were each labeled with $^{32}PO_4^{3-}$ and were then incubated with or without 0.1 μg of ActD per ml, as described in the text. ^{32}P-labeled RNA was extracted from the purified supernatant fluids and was then subjected to electrophoresis in a 10% polyacrylamide slab gel. (a) Uninfected, no ActD; (b) uninfected, plus ActD; (c) infected, no ActD; (d) infected, plus ActD.*

radioactivity in the uninfected samples (a and b) remained at the origin, with only a small portion migrating to the 4S region of the gel. In contrast, a relatively large proportion of the radioactivity present in the infected samples (Fig. 2c and d) was 4S RNA. These observations were verified by cutting out and counting the origin and 4S regions of the gel. The results showed that despite differences in the amount of radioactivity at the origin, ActD treatment had essentially no effect on the respective amounts of 4S RNA in fluids from uninfected and infected cells. Furthermore, the infected samples contained approximately 30-fold more 4S RNA than the uninfected samples (5,800 versus 200 cpm). These data demonstrate that under the conditions used, virtually all of the 4S RNA in preparations of control and ActD virions is contained within virus particles; only insignificant amounts of contaminating cell 4S RNA are present. Similar results were obtained by Bishop et al. (5) in experiments comparing the 4S RNA content of fluids from Rous sarcoma virus-infected and uninfected chicken cells. It should be pointed out that in addition to providing information on 4S RNA, Fig. 2 shows that ActD virions contain other small RNAs (6, 15, 19) which also occur in normal virus particles (compare c and d, in area between origin and 4S region of the gel).

2-D polyacrylamide gel analysis of viral RNA. A more detailed analysis of viral 4S RNA was obtained by fractionation in a 2-D gel system (Fig. 3). For comparison, each set of control and ActD samples was run as a pair in the same 10 and 20% gels. Fig. 3a and b illustrates the patterns observed for AKR virus control and ActD 4S RNA, respectively. As may be seen, these patterns were essentially identical to each other while clearly different from the cell pattern (see below, Fig. 3e). In both virus patterns there were three prominent radioactive spots which are designated by the numbers 1, 2, and 3 in the schematic diagram in Fig. 3f. Occasionally spot 1 was smaller than shown here and another spot was resolved, immediately to its left. Other radioactive areas consistently appeared in the autoradiograms, although it is not known whether these correspond to individual tRNA species. Fingerprint analysis of spots 1 and 2 is discussed below.

Inspection of the autoradiograms at different exposure times indicated that the relative intensities of corresponding radioactive spots were similar for the control and ActD samples. Individual areas of the 20% gel were also cut out and counted, and the results supported the visual observations. Some variation was observed from one experiment to another but on the average, differences in the relative amounts of radioactivity in corresponding spots of control and ActD

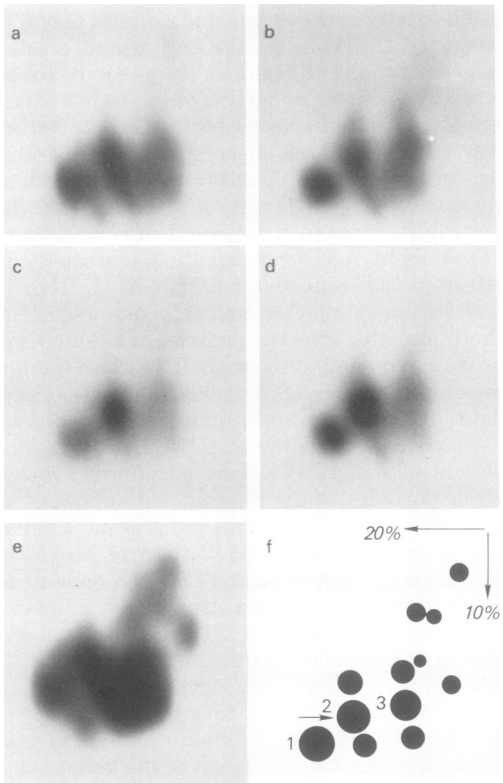

FIG. 3. *2-D gel electrophoresis patterns of ^{32}P-labeled 4S RNAs from MuLV control and ActD virions and MuLV-infected SC-1 cells. Electrophoresis in a 10% polyacrylamide gel was followed by electrophoresis in a 20% gel, as described in the text. The direction of each dimension is indicated in (f). (a) AKR control virus; (b) AKR ActD virus; (c) Moloney control virus; (d) Moloney ActD virus; (e) AKR virus-infected SC-1 cells; (f) schematic diagram of the AKR ActD viral 4S RNA pattern shown in (b). The three major areas of radioactivity are numbered. The arrow denotes the tRNA^{Pro} primer present in spot 2 of each of the virus samples (see text). Each set of control and ActD RNA samples (a and b; c and d) were run on the same 10 and 20% gels.*

4S RNA were not greater than a factor of two. Thus, the data indicate that control and ActD virions contain the same population of tRNA's and in similar proportions. This suggests that the tRNA composition of MuLV particles is not determined by the viral RNA genome.

To further verify this conclusion, a different strain of MuLV, Moloney virus, was grown in the SC-1 cell line (26) used to propagate AKR virus. Comparison of Fig. 3c and d with Fig. 3a and b shows that the Moloney pattern was indistinguishable from the AKR pattern. Furthermore, as expected from the AKR results, patterns for Moloney control and ActD 4S RNA were identical.

Fig. 3e presents the 2-D gel pattern of 4S RNA from AKR-infected cells. As may be seen, the cell pattern was more complex than the viral pattern. This observation confirms earlier findings of Peters et al. (40) and lends further support to the notion that viral tRNA's represent a subset of cell 4S RNA species rather than a collection of the entire cell tRNA population (5, 9, 16, 22, 40, 41, 51, 53, 54).

Fingerprint analysis. Two of the spots seen in the viral 2-D gel pattern of Fig. 3 were analyzed further by oligonucleotide fingerprinting techniques (43). Spot 1 appeared to be the same as spot 5 of Peters et al. (40). However, it was spot 2 that turned out to be of greatest interest. Fig. 4a illustrates the oligonucleotide fingerprint of AKR ActD spot 2 RNA; a schematic diagram using the numbering system of Peters et al. (40) is presented in Fig. 4b. This fingerprint corresponds very closely to the published fingerprint for Moloney MuLV tRNA^{Pro} (40). All of the expected oligonucleotides (40) were present. An additional spot, number 15, is probably related to spot 7. As also observed by Peters et al. (40), a low level of contaminating oligonucleotides was detected. Not surprisingly, identical fingerprints were obtained for spot 2 RNA isolated from AKR control and Moloney ActD virions (data not shown).

DISCUSSION

The present study was designed to test a model which suggests that 35S genomic RNA is a major determinant in the selective encapsidation of host tRNA's into RNA tumor virus particles. Our approach was to examine the composition of free 4S RNA in normal and ActD virions. Although ActD virions have the same amount of RNA per virion as normal particles (Table 1), they lack genomic RNA and contain predominantly 4S RNA (32; Fig. 1). The model predicts that under these conditions, such virions would contain a typical representation of cell 4S RNA instead of the more selected population characteristic of normal particles.

Analysis of viral 4S RNA by 2-D polyacrylamide gel electrophoresis (29) gave rather unexpected results. As illustrated in Fig. 3, the patterns for control and ActD 4S RNA were identical, and in both cases these patterns were simpler than the pattern for cell 4S RNA. In addition, the relative intensities of corresponding spots in control and ActD samples were similar (i.e., within a factor of two or three), suggesting that the tRNA's are present in similar proportions in total control and ActD viral 4S RNA. These observations were made with two different strains of MuLV, AKR and Moloney, each grown in SC-1 (26) mouse embryo cells.

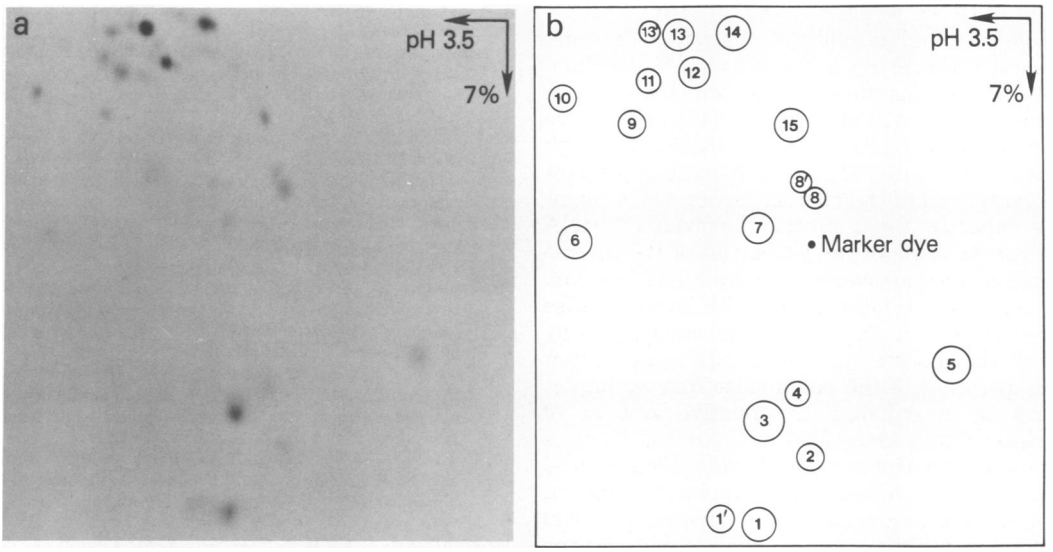

FIG. 4. *Oligonucleotide fingerprint of spot 2 RNA purified from AKR MuLV ActD virions. The RNA from spot 2 was eluted from the 20% gel, digested with RNase T1, and fingerprinted as described in the text. The autoradiogram was exposed at −70°C with the aid of a Kodak Lightening-Plus intensifying screen. (a) Fingerprint; (b) schematic diagram. For simplicity, only authentic tRNA^{Pro} oligonucleotides (40) are shown. The numbering system for the oligonucleotide spots is the same as that used by Peters et al. (40). The position of the xylene cyanol dye is shown in the diagram.*

Thus, ActD virions contain a selected population of host tRNA's which appears to be the same as that observed for normal virus particles. The data presented in Fig. 3 therefore lead to the general conclusion that genomic RNA is not required for the selective packaging of cell tRNA's.

In view of the highly specific binding of primer tRNA to genomic RNA (7a, 10, 14, 48, 50) and the fact that the primer is bound to 35S RNA more tightly than any other viral tRNA (8, 11, 40, 49, 54), it was of particular interest to find that the tRNA^{Pro} primer (40) is present in ActD virions (Fig. 4). Under our labeling conditions, we estimate that normal virions contain approximately three copies of free tRNA^{Pro} per 35S RNA subunit, in general agreement with the value of three to four copies calculated by Peters et al. (40). Thus, because virions are thought to contain one copy (3, 7, 19, 44) of the 70S RNA complex consisting of two 35S RNA subunits (3, 4, 31, 34), there should be a total of six to eight copies of free tRNA^{Pro} per virion of normal MuLV. Moreover, based on the similar intensity of tRNA^{Pro} spots (spot 2) in 20% gels of control and ActD 4S RNA (Fig. 3), we would expect the presence of a comparable number of tRNA^{Pro} copies in ActD virions.

In the past, two possibilities were suggested to account for differences between host and viral 4S RNA. In 1971, Randerath et al. (41) proposed that possible differences between membrane-associated tRNA and tRNA in the cytosol would allow virions to acquire a select class of host tRNA's during budding from the host plasma membrane. Subsequently, a study showing different amino acid acceptor patterns for a few avian sarcoma and leukosis viruses grown in the same cells led to the proposal that the viral RNA genome determines which tRNA's are to be encapsidated (53). The first hypothesis is difficult to evaluate because no data are available on the tRNA composition of cell membranes, and the second is not consistent with our present findings. A third possibility which has not been considered previously is that one or more viral proteins are required for the selection of host tRNA's.

Because the genome itself does not appear to be a major determinant in the selection process, the requirement for a particular viral gene product(s) would provide a rationale for the rather significant differences in the tRNA composition of viruses from different species (40, 55). The involvement of viral proteins in the selection process would also be consistent with our previous conclusion that virus assembly depends upon protein interactions (23) and is not directed by the viral RNA genome (23, 33). In this context, ActD virions can be regarded like normal particles, because they contain all of the MuLV structural proteins (30, 33) as well as a normal reverse transcriptase (23).

Presumably, a protein involved in encapsida-

tion of tRNA would have an affinity for tRNA molecules, and its ability to select tRNA's would be expected to be a species-specific function. MuLV contains three proteins which are known to bind RNA: p10, (12, 45), p12, (47), and reverse transcriptase (2, 24, 27, 28, 37, 38, 58). Although none of the three MuLV RNA binding proteins possesses all of the characteristics which might be expected for a protein involved in tRNA selection, some of the properties of the reverse transcriptase are consistent with such a function. Primer tRNA interactions with avian reverse transcriptase (2, 7a, 24, 27, 28, 38) and potentially with the murine enzyme (27, 37) suggest that the presence of the polymerase may be important in determining the relative amount of primer tRNA assembled into virions. There is some very recent evidence that this may indeed be the case in the avian system (G. Peters, personal communication; H. Hanafusa, personal communication). It is also conceivable that the selection process is more complex than previously expected and that to achieve maximum specificity and efficiency in this process interactions between several viral proteins are required. Furthermore, interactions involving viral protein precursors which bind nucleic acid (35, 36) may occur. Thus, the surprising result of this study that the viral RNA genome is not involved in the selection of host tRNA's points to the importance of experiments designed to assess a possible role for reverse transcriptase and other viral proteins in this aspect of virus assembly.

ACKNOWLEDGMENTS

We are grateful to Marilyn Lander for cells and virus and to Wallace Rowe for providing XC assays. We also thank James Dahlberg, Gordon Peters, and Barbara Cordell for helpful discussions and Brenda Gerwin, Philip Leder, and Alan Rein for critical readings of the manuscript. Cora Harvey rendered expert assistance in the preparation of the manuscript.

LITERATURE CITED

1. **Baluda, M. A., and D. P. Nayak.** 1970. DNA complementary to viral RNA in leukemic cells induced by avian myeloblastosis virus. Proc. Natl. Acad. Sci. U.S.A. **66**:329–336.
2. **Baroudy, B. M., M. Fournier, J. Labouesse, T. S. Papas, and J. G. Chirikjian.** 1977. $tRNA^{Trp}$ (bovine) binding to the reverse transcriptase of avian myeloblastosis virus and function as a heterologous primer. Proc. Natl. Acad. Sci. U.S.A. **74**:1889–1893.
3. **Bellamy, A. R., S. C. Gillies, and J. D. Harvey.** 1974. Molecular weight of two oncornavirus genomes: derivation from particle molecular weights and RNA content. J. Virol. **14**:1388–1393.
4. **Bender, W., and N. Davidson.** 1976. Mapping of poly(A) sequences in the electron microscope reveals unusual structure of type C oncornavirus RNA molecules. Cell **7**:595–607.
5. **Bishop, J. M., W. E. Levinson, N. Quintrell, D. Sullivan, L. Fanshier, and J. Jackson.** 1970. The low molecular weight RNAs of Rous sarcoma virus. I. The 4S RNA. Virology **42**:182–195.
6. **Bishop, J. M., W. E. Levinson, D. Sullivan, L. Fanshier, N. Quintrell, and J. Jackson.** 1970. The low molecular weight RNAs of Rous sarcoma virus. II. The 7S RNA. Virology **42**:927–937.
7. **Bonar, R. A., L. Sverak, D. P. Bolognesi, A. J. Langlois, D. Beard, and J. W. Beard.** 1967. Ribonucleic acid components of BAI strain A (myeloblastosis) avian tumor virus. Cancer Res. **27**:1138–1157.

7a. **Brown, R. D., and R. W. Armentrout.** 1977. Primer recognition by avian myeloblastosis virus RNA-directed DNA polymerase. J. Virol. **21**:1236–1239.

8. **Canaani, E., and P. Duesberg.** 1972. Role of subunits of 60 to 70*S* avian tumor virus ribonucleic acid in its template activity for the viral deoxyribonucleic acid polymerase. J. Virol. **10**:23–31.
9. **Carnegie, J. W., A. O'C. Deeney, K. C. Olson, and G. S. Beaudreau.** 1969. An RNA fraction from myeloblastosis virus having properties similar to transfer RNA. Biochim. Biophys. Acta **190**:274–284.
10. **Cordell, B., E. Stavnezer, R. Friedrich, J. M. Bishop, and H. M. Goodman.** 1976. Nucleotide sequence that binds primer for DNA synthesis to the avian sarcoma virus genome. J. Virol. **19**:548–558.
11. **Dahlberg, J. E., R. C. Sawyer, J. M. Taylor, A. J. Faras, W. E. Levinson, H. M. Goodman, and J. M. Bishop.** 1974. Transcription of DNA from the 70*S* RNA of Rous sarcoma virus. I. Identification of a specific 4*S* RNA which serves as primer. J. Virol. **13**:1126–1133.
12. **Davis, J., M. Scherer, W. P. Tsai, and C. Long.** 1976. Low-molecular-weight Rauscher leukemia virus protein with preferential binding for single-stranded RNA and DNA. J. Virol. **18**:709–718.
13. **Duesberg, P. H., and W. S. Robinson.** 1967. Inhibition of mouse leukemia virus (MLV) replication by actinomycin D. Virology **31**:742–746.
14. **Eiden, J. J., K. Quade, and J. L. Nichols.** 1976. Interaction of tryptophan transfer RNA with Rous sarcoma virus 35S RNA. Nature (London) **259**:245–247.
15. **Emanoil-Ravicovitch, R., C. J. Larsen, M. Bazilier, J. Robin, J. Périès, and M. Boiron.** 1973. Low-molecular-weight RNAs of murine sarcoma virus: comparative studies of free and 70*S* RNA-associated components. J. Virol. **12**:1625–1627.
16. **Erikson, E., and R. L. Erikson.** 1970. Isolation of amino acid acceptor RNA from purified avian myeloblastosis virus. J. Mol. Biol. **52**:387–390.
17. **Erikson, E., and R. L. Erikson.** 1971. Association of 4S ribonucleic acid with oncornavirus ribonucleic acids. J. Virol. **8**:254–256.
18. **Erikson, R. L.** 1969. Studies on the RNA from avian myeloblastosis virus. Virology **37**:124–131.
19. **Faras, A. J., A. C. Garapin, W. E. Levinson, J. M. Bishop, and H. M. Goodman.** 1973. Characterization of the low-molecular-weight RNAs associated with the 70S RNA of Rous sarcoma virus. J. Virol. **12**:334–342.
20. **Faras, A. J., J. M. Taylor, W. E. Levinson, H. M. Goodman, and J. M. Bishop.** 1973. RNA-directed DNA polymerase of Rous sarcoma virus: initiation of synthesis with 70S viral RNA as template. J. Mol. Biol. **79**:163–183.
21. **Folk, W. R., and A. J. Faras.** 1976. Initiation of DNA synthesis by the avian oncornavirus RNA-directed DNA polymerase: tryptophan tRNA as the major species of primer RNA. J. Virol. **17**:1049–1051.
22. **Gallagher, R. E., and R. C. Gallo.** 1973. Chromatographic analyses of isoaccepting tRNAs from avian myeloblastosis virus. J. Virol. **12**:449–457.
23. **Gerwin, B. I., and J. G. Levin.** 1977. Interactions of murine leukemia virus core components: characterization of reverse transcriptase packaged in the absence of

70S genomic RNA. J. Virol. **24**:478-488.
24. **Grandgenett, D. P., A. C. Vora, and A. J. Faras.** 1976. Different states of avian myeloblastosis virus DNA polymerase and their binding capacity to primer $tRNA^{Trp}$. Virology **75**:26-32.
25. **Harada, F., R. C. Sawyer, and J. E. Dahlberg.** 1975. A primer ribonucleic acid for initiation of *in vitro* Rous sarcoma virus deoxyribonucleic acid synthesis: nucleotide sequence and amino acid acceptor activity. J. Biol. Chem. **250**:3487-3497.
26. **Hartley, J. W., and W. P. Rowe.** 1975. Clonal cell lines from a feral mouse embryo which lack host-range restrictions for murine leukemia viruses. Virology **65**: 128-134.
27. **Haseltine, W. A., A. Panet, D. Smoler, D. Baltimore, G. Peters, F. Harada, and J. E. Dahlberg.** 1977. Interaction of tryptophan tRNA and avian myeloblastosis virus reverse transcriptase: further characterization of the binding reaction. Biochemistry **16**: 3625-3632.
28. **Hizi, A., J. P. Leis, and W. K. Joklik.** 1977. The RNA-dependent DNA polymerase of avian sarcoma virus B77: binding of viral and nonviral ribonucleic acids to the α, β_2, and $\alpha\beta$ forms of the enzyme. J. Biol. Chem. **252**:6878-6884.
29. **Ikemura, T., and J. E. Dahlberg.** 1973. Small ribonucleic acids of *Escherichia coli*. I. Characterization by polyacrylamide gel electrophoresis and fingerprint analysis. J. Biol. Chem. **248**:5024-5032.
30. **Jamjoom, G. A., R. B. Naso, and R. B. Arlinghaus.** 1976. Selective decrease in the rate of cleavage of an intracellular precursor to Rauscher leukemia virus p30 by treatment of infected cells with actinomycin D. J. Virol. **19**:1054-1072.
31. **King, A. M. Q.** 1976. High molecular weight RNAs from Rous sarcoma virus and Moloney murine leukemia virus contain two subunits. J. Biol. Chem. **251**:141-149.
32. **Levin, J. G., P. M. Grimley, J. M. Ramseur, and I. K. Berezesky.** 1974. Deficiency of 60 to 70S RNA in murine leukemia virus particles assembled in cells treated with actinomycin D. J. Virol. **14**:152-161.
33. **Levin, J. G., and M. J. Rosenak.** 1976. Synthesis of murine leukemia virus proteins associated with virions assembled in actinomycin D-treated cells: evidence for persistence of viral messenger RNA. Proc. Natl. Acad. Sci. U.S.A. **73**:1154-1158.
34. **Mangel, W. F., H. Delius, and P. H. Duesberg.** 1974. Structure and molecular weight of the 60-70S RNA and the 30-40S RNA of the Rous sarcoma virus. Proc. Natl. Acad. Sci. U.S.A. **71**:4541-4545.
35. **Oroszlan, S., C. W. Long, and R. V. Gilden.** 1976. Isolation of a murine type-C virus p30 precursor protein by DNA-cellulose chromatography. Virology **72**: 523-526.
36. **Oskarsson, M. K., C. W. Long, W. G. Robey, M. A. Scherer, and G. F. Vande Woude.** 1977. Phosphorylation and nucleic acid binding properties of m1 Moloney murine sarcoma virus-specific $pP60^{src}$. J. Virol. **23**:196-204.
37. **Panet, A., and H. Berliner.** 1978. Binding of tRNA to reverse transcriptase of RNA tumor viruses. J. Virol. **26**:214-220.
38. **Panet, A., W. A. Haseltine, D. Baltimore, G. Peters, F. Harada, and J. E. Dahlberg.** 1975. Specific binding of tryptophan transfer RNA to avian myeloblastosis virus RNA-dependent DNA polymerase (reverse transcriptase). Proc. Natl. Acad. Sci. U.S.A. **72**:2535-2539.
39. **Paskind, M. P., R. A. Weinberg, and D. Baltimore.** 1975. Dependence of Moloney murine leukemia virus production on cell growth. Virology **67**:242-248.
40. **Peters, G., F. Harada, J. E. Dahlberg, A. Panet, W. A. Haseltine, and D. Baltimore.** 1977. Low-molecular-weight RNAs of Moloney murine leukemia virus: identification of the primer for RNA-directed DNA synthesis. J. Virol. **21**:1031-1041.
41. **Randerath, K., L. J. Rosenthal, and P. C. Zamecnik.** 1971. Base composition differences between avian myeloblastosis virus transfer RNA and transfer RNA isolated from host cells. Proc. Natl. Acad. Sci. U.S.A. **68**: 3233-3237.
42. **Rowe, W. P., W. E. Pugh, and J. W. Hartley.** 1970. Plaque assay techniques for murine leukemia viruses. Virology **42**:1136-1139.
43. **Sanger, F., G. G. Brownlee, and B. G. Barrell.** 1965. A two-dimensional fractionation procedure for radioactive nucleotides. J. Mol. Biol. **13**:373-398.
44. **Sawyer, R. C., and J. E. Dahlberg.** 1973. Small RNAs of Rous sarcoma virus: characterization by two-dimensional polyacrylamide gel electrophoresis and fingerprint analysis. J. Virol. **12**:1226-1237.
45. **Schulein, M., W. N. Burnette, and J. T. August.** 1978. Stoichiometry and specificity of binding of Rauscher oncovirus 10,000-dalton (p10) structural protein to nucleic acids. J. Virol. **26**:54-60.
46. **Seidman, J. G., B. G. Barrell, and W. H. McClain.** 1975. Five steps in the conversion of a large precursor RNA into bacteriophage proline and serine transfer RNAs. J. Mol. Biol. **99**:733-760.
47. **Sen, A., C. J. Sherr, and G. J. Todaro.** 1976. Specific binding of the type C viral core protein p12 with purified viral RNA. Cell **7**:21-32.
48. **Staskus, K. A., M. S. Collett, and A. J. Faras.** 1976. Initiation of DNA synthesis by the avian oncornavirus RNA-directed DNA polymerase: structural and functional localization of the major species of primer RNA on the oncornavirus genome. Virology **71**:162-168.
49. **Taylor, J. M., B. Cordell-Stewart, W. Rohde, H. M. Goodman, and J. M. Bishop.** 1975. Reassociation of 4S and 5S RNA's with the genome of avian sarcoma virus. Virology **65**:248-259.
50. **Taylor, J. M., and R. Illmensee.** 1975. Site on the RNA of an avian sarcoma virus at which primer is bound. J. Virol. **16**:553-558.
51. **Travnicek, M.** 1969. Some properties of amino acid-acceptor RNA isolated from avian tumor virus BAI strain A (avian myeloblastosis). Biochim. Biophys. Acta **182**: 427-439.
52. **Verma, I. M., N. L. Meuth, E. Bromfeld, K. F. Manly, and D. Baltimore.** 1971. Covalently linked RNA-DNA molecule as initial product of RNA tumour virus DNA polymerase. Nature (London) New Biol. **233**:131-134.
53. **Wang, S., R. M. Kothari, M. Taylor, and P. Hung.** 1973. Transfer RNA activities of Rous sarcoma and Rous associated viruses. Nature (London) New Biol. **242**:133-135.
54. **Waters, L. C.** 1975. Transfer RNAs associated with the 70S RNA of AKR murine leukemia virus. Biochem. Biophys. Res. Commun. **65**:1130-1136.
55. **Waters, L. C., and B. C. Mullin.** 1977. Transfer RNA in RNA tumor viruses. Prog. Nucleic Acid Res. Mol. Biol. **20**:131-160.
56. **Waters, L. C., B. C. Mullin, T. Ho, and W. K. Yang.** 1975. Ability of tryptophan tRNA to hybridize with 35S RNA of avian myeloblastosis virus and prime reverse transcription *in vitro*. Proc. Natl. Acad. Sci. U.S.A. **72**: 2155-2159.
57. **Wollmann, R. L., and W. H. Kirsten.** 1968. Cellular origin of a mouse leukemia viral ribonucleic acid. J. Virol. **2**:1241-1248.
58. **Yamaura, I., and L. F. Cavalieri.** 1978. Inhibition of reverse transcription of 70S and 35S avian myeloblastosis RNAs by nonprimer tRNA's. J. Virol. **27**:300-306.