Ribonucleic Acid Components of Murine Sarcoma and Leukemia Viruses

(NRK rat cells/fingerprinting/gel electrophoresis)

J. MAISEL, V. KLEMENT*, M. M-C. LAI, W. OSTERTAG†, AND P. DUESBERG

Department of Molecular Biology and Virus Laboratory, University of California, Berkeley, Calif. 94720; * University of Southern California, School of Medicine, Los Angeles, Ca. 90032. † Max-Planck-Institut für experimentelle Medizin, Abt. Molekulare Biologie, 34 Göttingen, Germany

ABSTRACT Defective Kirsten murine sarcoma virus was present as leukemia virus pseudotype [Ki-MSV(MLV)] in a 10- to 100-fold excess over its helper, Kirsten murine leukemia virus (Ki-MLV), when the two viruses were propagated in an NRK rat cell line. The $s_{w,20}$ of the fast-sedimenting RNA complex of Ki-MLV and of Ki-MSV-(MLV) was 62 S and 55 S, respectively. Gel electrophoresis in buffered aqueous or formamide solution of the dissociated 62S RNA complex of Ki-MLV showed a single major peak of molecular weight about 2.5×10^6. Dissociated 55S RNA of Ki-MSV(MLV) was resolved into a major component with a higher electrophoretic mobility than that of Ki-MLV RNA and molecular weight about 2.3×10^6. Occasionally, a minor component with the same electrophoretic mobility as Ki-MLV RNA was observed in Ki-MSV(MLV) RNA; it is thought to be the RNA of Ki-MLV present as helper virus in our stocks of Ki-MSV(MLV). The RNA of an endogenous rat C-type RNA virus was electrophoretically different from both Ki-MLV RNA and Ki-MSV(MLV) RNAs. Oligonucleotide fingerprinting of the RNAs digested with RNase T1 indicated that the RNAs of Ki-MSV(MLV) and Ki-MLV are different. However, the extent of the difference between the two RNAs could not be estimated by this method.

The heat-dissociated 50-70S RNAs of two other defective murine sarcoma-leukemia viruses; Harvey-MSV(MLV) and Moloney-MSV(MLV) and of defective spleen-focus-forming Friend virus were resolved electrophoretically into two components. The larger components had the same electrophoretic mobility as the RNA of Ki-MLV or Moloney MLV. The smaller were not present in leukemia virus. It is suggested that the small RNA components of the two murine sarcoma viruses and of Friend virus represent specific genetic information of these replication-defective transforming viruses.

Possible relationships between the RNAs of murine leukemia viruses and replication-defective murine sarcoma and Friend viruses are discussed.

The known murine sarcoma viruses are replication-defective (1,2). They depend upon the presence of an associated helper MLV for their replication, antigenicity and host range (1,2), while MLV is independent of helper (1–3). The sarcoma virus produced in the presence of helper virus is a phenotypically mixed virion or pseudotype, which is designated MSV(MLV) (2). Infection by certain defective MSV(MLV) strains in the absence of helper virus leads to transformed nonproducer cells, which lack virus particles and viral antigens (1–5). The genetic relationship between the known replication-defective murine sarcoma viruses and their respective helper leukemia viruses is not clear (1, 2, 6, 7, 8, 37).

It is the purpose of this study to compare the RNAs of MSV and MLV. In the avian system, the RNAs of nondefective sarcoma viruses are larger than those of corresponding leukosis viruses (6, 26, 31, 35, 37). However, in the murine system, there has been no evidence of physical differences between sarcoma and leukemia virus RNA, except for our preliminary experiments which suggested that Ki-MSV RNA is smaller than Ki-MLV RNA (6). Experiments by others have suggested little homology between these two RNAs in one study (7) and over 50% homology in another study (8).

A comparison of the RNAs of a defective sarcoma virus and a helper leukemia virus such as Ki-MLV and Ki-MSV is complicated because the defective Ki-MSV is of necessity in a mixture with the leukemia helper virus. Therefore, its RNA must be separated from that of the helper virus to be identified. The ratio of defective murine sarcoma viruses to that of their respective helper leukemia viruses is greatly influenced by the host in which the viruses are propagated (9, 10). Therefore, it was to our advantage to use a NRK rat-kidney cell line, which was infected and transformed by Ki-MSV(MLV) and which continuously produces Ki-MLV and Ki-MSV-(MLV) (11) with Ki-MSV(MLV) in excess (Klement, unpublished; and see below). Consequently we thought that comparison of the RNA of pure Ki-MLV to that of a mixture of Ki-MLV and excess Ki-MSV(MLV) should reveal differences if the RNAs of the two viruses are in fact different. We found that the RNA of Ki-MSV(MLV) is smaller than that of Ki-MLV. Subsequently RNA components which are smaller than those of leukemia viruses were also found in Harvey and Moloney sarcoma viruses and in spleen-focus-forming Friend virus.

MATERIALS AND METHODS

Propagation of Ki-MSV(MLV) and Ki-MLV in NRK cells were described (11). Moloney (M)-MSV(MLV) and M-MLV were obtained from A. J. Hackett (Naval Biomedical Laboratory, U.C., Berkeley). M-MSV(MLV) was propagated in a transformed NRK cell line derived by J. Maisel (unpublished) and M-MLV in a Swiss mouse embryo cell line obtained from A. J. Hackett. A 3T3 mouse cell line transformed by Harvey (H)-MSV(MLV) was obtained from H. Temin

---

Abbreviations: Ki-MSV and Ki-MLV, Kirsten murine sarcoma virus and leukemia virus, respectively; PR RSV, Prague strain of Rous sarcoma virus. M-MLV and M-MSV(MLV), Moloney murine leukemia and sarcoma-leukemia virus; H-MSV(MLV). Harvey murine sarcoma-leukemia virus; SSFV, spleen-focus-forming Friend virus.

(McArdle Laboratory, University of Wisconsin, Madison). Spleen-focus-forming Friend virus (SFFV) was propagated in a spleen-derived transformed cell line FSD-1/F4 (36), which produces both spleen-focus-forming virus and helper leukemia virus (W. Ostertag, unpublished). [³H]Virus was made by addition of 100–200 μCi of [³H]uridine (New England Nuclear Corp., 20–30 Ci/mmol) to a confluent infected culture (10-cm petri dish) in 6 ml of medium. Eagle's Minimal Essential Medium supplemented with 5% fetal-calf serum, 1% dimethylsulfoxide omitted in propagation SFFV, 0.5 μg/ml of Fungizone, 100 units/ml of penicillin, and 50 μg/ml of streptomycin was used. To propagate H-MSV(MLV) the same medium was used but supplemented with 10% fetal calf serum and 10% tryptosephosphate broth. After a primary incubation of 5 hr, medium was collected for virus purification and subsequently collected three or four times more at intervals of 3 hr without additional radioactive precursor. [³²P]-Virus was made by the addition of 1 mCi of $H_3{}^{32}PO_4$ (carrier free, Schwarz BioResearch Inc.) to an infected culture in 6 ml of phosphate-free Dulbecco's modified Eagle's medium containing 2% dialyzed fetal-calf serum and otherwise supplemented as described above. The schedule of [³²P]virus collections was as described for [³H]-uridine-labeled virus, except that phosphate containing Minimal Essential Medium supplemented as above was used after the first 3-hr collection.

RESULTS

Stocks of Ki-MLV and Ki-MSV(MLV) Containing Excess of Ki-MSV(MLV). Both Ki-MLV and Ki-MSV(MLV) can be propagated in mouse, rat, and other cells (11–14). When Ki-MSV and Ki-MLV are propagated together in the NRK rat cell line no. 58967 (11), a virus mixture is obtained which consists of Ki-MSV(MLV) and Ki-MLV. The amount of sarcoma virus pseudotype is always higher than that of leukemia virus, though the ratio varies in different virus harvests. In Table 1 an assay on NIH Swiss mouse 3T3 cells (15) of sarcoma virus by focus formation (16) and leukemia virus by plaque formation (17) demonstrates an excess of sarcoma virus of about 50-fold. In similar assays the excess of Ki-MSV(MLV) over Ki-MLV ranged between 10- and 100-fold. Although the efficiency of the two assay techniques may not be the same for each of the two viruses, it is likely that the assays give a sufficiently accurate estimate of the concentrations of biologically active Ki-MSV(MLV) and Ki-MLV in our virus stocks, particularly since both viruses were derived

TABLE 1. *Focus and XC plaque assay on NIH Swiss mouse 3T3 cells of Ki-MSV(MLV) released from the NRK line* no. 58967*

Virus dilution	Foci/ml	Plaques/ml
10⁰	TMTC†	≥479
10⁻¹	TMTC	96
10⁻²	≥293	7
10⁻³	40	0
10⁻⁴	7	0
10⁻⁵	0	0
Average titer	10⁴·⁷ ·	10²·⁹

* 73rd passage after transformation; harvested 24 hr after fluid change.

† TMTC, too many to count.
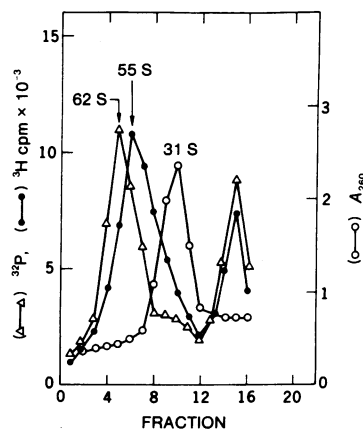

FIG. 1. Sedimentation analysis of the RNAs of Ki-MLV (△), Ki-MSV(MLV) (●), and tobacco mosaic virus (○). Purification of the viruses and extraction of the viral RNAs were described (19, 21, 22). Centrifugation was in a 10–25% sucrose gradient containing standard buffer [0.1 M NaCl–0.01 M Tris·HCl (pH 7.0)–1 mM EDTA] and 0.1% Na dodecyl sulfate for 45 min in a Spinco SW 65 rotor at 20°. The tobacco mosaic virus marker was located by its absorbance at 260 nm. Radioactivity was determined in a Tricarb spectrometer on appropriate aliquots dissolved in toluene-based scintillation fluid containing 10% NCS (Nuclear Chicago Corp.).

from the same NRK cells and were assayed on the same mouse indicator cell line. To exclude a possible host-cell influence, such as selection of virus variants or host modification, Ki-MLV propagated in the rat-kidney (NRK) cell line no. 5859 (Klement, unpublished) was used for comparison with Ki-MSV (MLV). This NRK line is chronically infected with murine erythroblastosis virus (12, 13) which has also been termed Ki-MLV (7). The line releases virus at a concentration of around 10³ plaque-forming units/ml when assayed on NIH Swiss mouse 3T3 cells (17).

Physical Properties of RNA of Ki-MSV(MLV) and of Ki-MLV. Sedimentation analysis of the RNAs of Ki-MSV(MLV) and Ki-MLV together with a standard of 31S tobacco mosaic virus RNA (18) is shown in Fig. 1. The RNAs of both RNA tumor viruses were resolved into a fast sedimenting and slowly sedimenting species. The distribution was typical of MLV RNAs (19–23) and similar to avian tumor virus RNA (21). However, the sedimentation coefficient of the fast sedimenting RNA component of Ki-MSV(MLV) was 55 S. This is lower than that of the fast sedimenting RNA species of Ki-MLV, which was 62 S based on the 31S tobacco mosaic virus RNA standard.

The 60–70S RNAs of all known RNA tumor viruses have a complex subunit structure, consisting of a major class of 30–40S RNAs and minor heterogeneous RNAs (21). Therefore, the difference between the RNAs of Ki-MSV(MLV) and Ki-MLV could be due to differences in the superstructure of their 50–70S RNA complex or to differences in the primary structure of the major 30–40S class of RNA subunits. To decide between these possibilities, we dissociated the 50–70S complexes of the two RNAs into subunits and analyzed them by electrophoresis in polyacrylamide gels. The 50–70S RNAs of both viruses consist of a major 30–40S class and faster migrating heterogeneous RNAs (Fig. 2A), compatible with earlier analyses of MLV RNA (19–23, 38). However, the major 30–
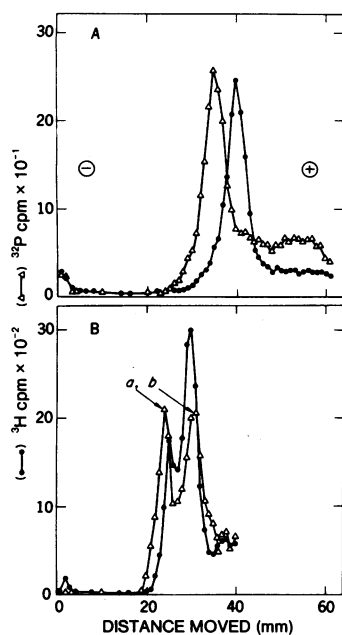
FIG. 4. Gel electrophoresis of heat-dissociated 50–70S Ki-MSV(MLV) RNA (△) and endogenous rat C-type virus RNA (●). Conditions were as described for Fig. 2. *Insert* shows the sedimentation distribution of rat C-type virus RNA (●) and a 31S tobacco mosaic virus RNA marker (○). Fractions 4–11 were pooled for electrophoretic analysis.

FIG. 2. Gel electrophoreses of heat-dissociated 50–70S RNA of Ki-MLV (*A*,△), Ki-MSV(MLV) (*A* and *B*,●), and a marker of Prague Rous sarcoma virus containing class *a* and class *b* 30–40S RNA species (*B*,△) (24, 35). Heat dissociation of viral RNAs and electrophoresis in 2% bisacrylamide crosslinked-polyacrylamide gels were described (24, 35).

40S subunit class of Ki-MLV RNA has a lower electrophoretic mobility than that of Ki-MSV(MLV) RNA.

The peak of Ki-MSV(MLV) RNA shown in Fig. 2*A* has a slower migrating shoulder, perhaps due to a minor (<10%) RNA component. In the preparation analyzed in Fig. 2*B*, a minor component consisting of about 25% of the total RNA of Ki-MSV(MLV) was resolved. The ratio of this minor component to the major RNA component of Ki-MSV(MLV) RNA varied between the extremes, <10% and 25%, shown in Figs. 2*A* and *B* in different virus harvests (see Figs. 2–4). This minor RNA component of Ki-MSV(MLV) is thought to represent the RNA of helper Ki-MLV. Assuming that this is
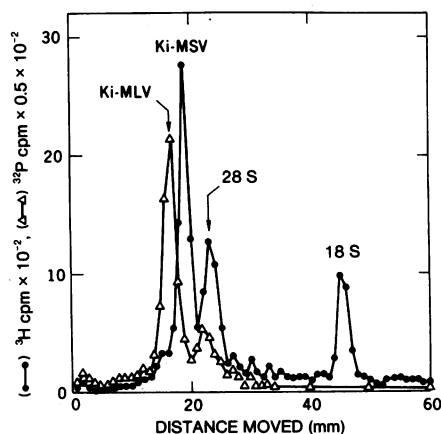
correct, the ratios of Ki-MSV(MLV) and Ki-MLV in our stocks of Ki-MSV(MLV) determined physically by analysis of their RNA ranged between >10:1 and 4:1 and those determined biologically ranged between 100:1 and 10:1 (see above and Table 1). The electrophoretic mobility of the 30–40S species of the presumed Ki-MLV RNA component of Ki-MSV(MLV) is slightly higher than that of the class *a* RNA of Prague (PR) Rous sarcoma virus (RSV) (24) and the mobility of the major component of Ki-MSV(MLV) RNA is slightly lower than that of class *b* RNA of PR RSV (24) (Fig. 2*B*).

The relative differences in electrophoretic mobility between the 30–40S species of Ki-MSV(MLV) RNA and Ki-MLV RNA were retained in formamide gels (25, 26) (Fig. 3). Based on the 28S (1.9 × 10⁶) and 18S (0.7 × 10⁶) (27) ribosomal RNA markers, the molecular weight of dissociated Ki-MLV RNA is 2.5 × 10⁶ and that of dissociated Ki-MSV(MLV) is 2.3 × 10⁶ from their electrophoretic mobilities in buffered formamide (25). However, these molecular weights must be considered as approximations only, because a linear logarithmic molecular weight-mobility calibration curve was not obtained for different RNA standards with molecular weights higher than 2 × 10⁶ in the formamide gels used (26). We conclude that the difference between the sedimentation coefficients of 50–70S Ki-MLV and Ki-MSV(MLV) RNA is due to a difference between the size of their major 30–40S class of subunits.

Recently an endogenous C-type RNA virus was isolated from NRK cells (28). Therefore, it could be argued that Ki-MSV(MLV) may induce such an endogenous virus in NRK cells while Ki-MLV does not, and that the RNA of Ki-MSV-(MLV) contains components of this endogenous virus. Thus the difference observed between Ki-MLV RNA and Ki-MSV-(MLV) RNA could be due to RNA components of endogenous rat virus in our preparations of Ki-MSV(MLV). To test this, we compared the 50–70S RNAs of Ki-MSV(MLV) and endogenous rat C-type virus (Fig. 4, *insert*) after heat dissociation. The 30–40S class of RNA of endogenous rat virus was resolved into two peaks, neither of which coincided with the major 30–40S species of Ki-MSV(MLV) (Fig. 4).

FIG. 3. Electrophoresis of 50–70S Ki-MLV RNA and Ki-MSV(MLV) RNA and of chick 28S and 18S ribosomal [³H]RNAs in a 3.5% polyacrylamide–formamide gel. Conditions of the electrophoresis were described (25, 26).
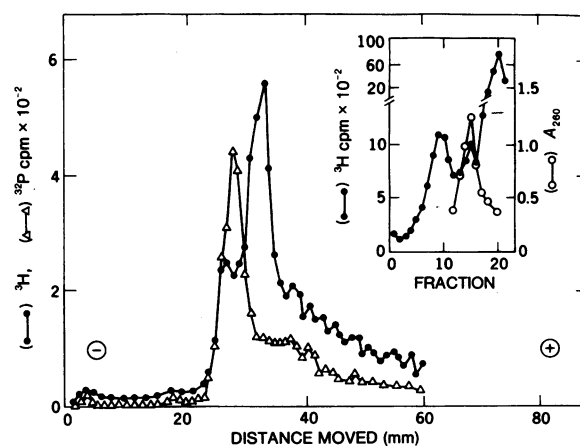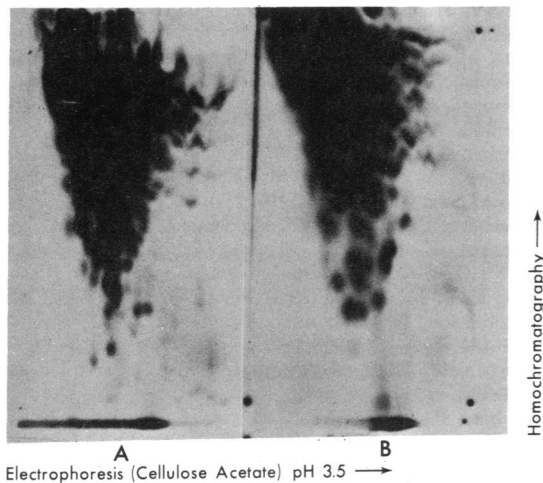
FIG. 5. Two-dimensional separation of RNase-T1 digests of 50–70S RNA of Ki-MSV(MLV) (*A*) and Ki-MLV (*B*). The method was a modification (31) of that described by Brownlee and Sanger (30). 1 to 3 × 10⁶ cpm of [³²P]RNA were digested with RNase T1 (1 unit of RNase per 20 μg of RNA) at 37° for 3–4 hr.

The RNAs of Harvey- and Moloney Murine Sarcoma Virus and of Friend Spleen-focus-forming Virus. The finding that the RNA of defective Ki-MSV is smaller than that of its helper virus Ki-MLV led us to investigate the RNA of other defective, transforming murine tumor viruses. The 50–70S RNAs of the Harvey- and Moloney sarcoma-leukemia virus complexes and of the Friend SFFV-leukemia virus complex contain two components (Fig. 6,A–C). One has an electrophoretic mobility identical to that of Moloney-MLV, while the other component is smaller than that of the leukemia virus. The RNA component of Harvey-, Moloney- and Friend-virus that co-migrates with that of leukemia virus is thought to be the RNA of the respective helper leukemia virus present in stocks of the three defective, transforming viruses (2). Differences in the electrophoretic mobilities of several murine leukemia viruses including Ki-MLV, M-MLV and the helper viruses of Friend virus and Harvey virus have not been detected so far (cf. Fig. 6 and unpublished). The RNAs of higher electrophoretic mobility, present in each of the three transforming virus preparations analyzed (Fig. 6, A–C), presumably represent genetic information of the replication-defective transforming components in each virus complex. Each of these three viruses contained more of the presumed helper leukemia virus RNA component than did
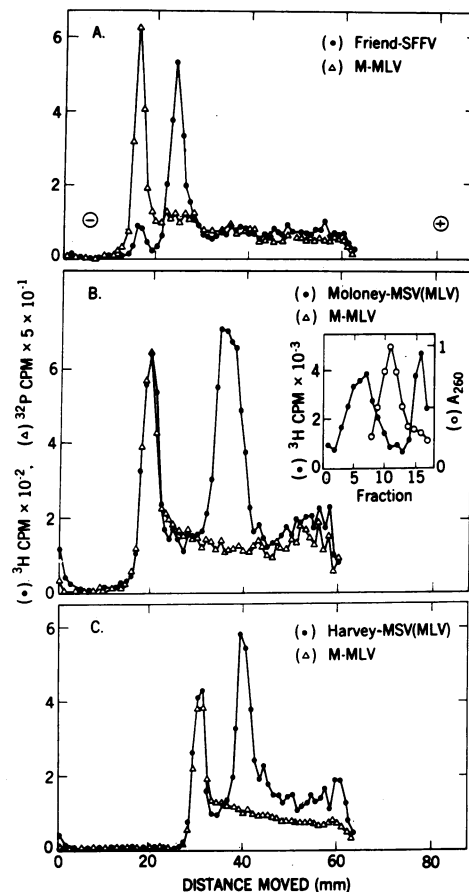
One component migrated ahead of the MSV peak, while the other coincided with the trailing half of the Ki-MSV(MLV) peak. This characterization of the RNA of endogenous rat virus is considered preliminary. However, we can conclude that the major 30–40S RNA of Ki-MSV(MLV) is probably the RNA of Ki-MSV(MLV) rather than the RNA of endogeneous NRK rat C-type virus.

This conclusion is further supported by serological studies, which indicate that Ki-MSV(MLV) propagated in the NRK line no. 58967 contains the *gs*-1 determinant of the group-specific antigen of mouse leukemia virus (29), whereas the endogenous NRK C-type virus contains a rat virus-specific *gs*-1 determinant (ref. 28, Gilden R. V. & Klement, V., unpublished).

Oligonucleotide Fingerprints of RNAs of Ki-MLV and Ki-MSV(MLV). To determine how the physically distinct RNAs of Ki-MLV and Ki-MSV(MLV) are related chemically, we prepared oligonucleotide fingerprints. Appropriate amounts of 50–70S [³²P]RNAs were subjected to exhaustive digestion with RNase *T1*, which hydrolyzes RNA at guanylic-acid residues and, subsequently, to two-dimensional electrophoretic-chromatographic analysis (30, 31). About 97–98% of the radioactivity appeared as a large black area in the upper portion of the autoradiograph. This area represents an unresolved mixture of monocleotides and very small oligonucleotides. About 2–3% of the radioactivity of each RNA was present in 20–25 large RNase *T1*-resistant oligonucleotides with molecular weights ranging from 6,000–12,000 (32) (Fig. 5).

It is evident from comparison of the large RNase *T1*-resistant oligonucleotides that the fingerprint patterns of Ki-MLV RNA and Ki-MSV(MLV) RNA are different. The extent of the difference between the two RNAs cannot be estimated by this method because only 2–3% of the total RNA is available for this comparison and because it has not been possible to obtain enough Ki-MLV [³²P]RNA for further analyses of distinct large oligonucleotides.

FIG. 6. Gel electrophoreses of heat-dissociated 50–70S RNAs of Moloney-MLV and (A) Friend spleen-focus-forming virus (SFFV) complex, (B) Moloney sarcoma-leukemia virus and (C) Harvey sarcoma-leukemia virus. Electrophoresis was as described for Fig. 2. The insert in Fig. 6B, shows the sedimentation distribution of the 50–70S RNA of M-MSV(MLV).

Ki-MSV(MLV). Based on a Ki- or M-leukemia virus RNA standard the presumed transformation-specific RNA component, appears to be smaller in Harvey, Moloney and Friend Virus than in Kirsten virus (cf. Fig. 2 and 6).

DISCUSSION

We have recently proposed that among certain avian RNA tumor viruses a correlation exists between the size of the viral RNA and defects in transformation and/or replication genes of corresponding viruses. The greater the extent of the defectiveness, the smaller is the size of the viral RNA (6, 37). We have found here that the RNA of Ki-MSV is smaller than the RNA of its helper leukemia virus and that the RNA components of high electrophoretic mobility present in the Harvey-, Moloney- and Friend virus complexes are also smaller than that of pure helper leukemia virus. This may indicate that replication-defective, transforming murine viruses lack genetic information present in helper leukemia virus which is reflected in the smaller size of their presumably transformation-specific RNA components. This hypothesis is consistent with the lack of all evidence of phenotypic virion expression by cells infected with certain strains of MSV (2), which suggests that defective MSV may even be unable to code for any detectable product in common with those of any competent helper leukemia virus.

We found that the RNAs of Ki-MSV and Ki-MLV appear to be chemically dissimilar on the basis of their different oligonucleotide patterns. This finding is compatible with an earlier DNA·RNA hybridization study of Stepheson and Aaronson (7), comparing the RNAs of Ki-MSV(MLV) and Ki-MLV. They concluded that Ki-MSV(MLV) RNA and Ki-MLV RNA may be only 20% or less homologous. Yet their result may have been influenced by their use of different host cells to propagate Ki-MLV (mouse) and Ki-MSV(MLV) (rat) in that different host cells may favor putatively different variants of helper virus. By contrast, a more recent DNA·RNA hybridization study by Benveniste and Scolnick suggests that Ki-MSV(MLV) and Ki-MLV RNA may be 50-60% homologous (8). Thus, further work is necessary to more accurately determine the extent of the chemical differences between the two RNAs.

Several hypothetical relationships may be suggested for the RNAs of replication-defective, transforming viruses and helper leukemia viruses: (i) For example, Ki-MSV and H-MSV which were isolated from rat cells infected with MLV (1, 2, 12, 13), may be recombinants between MLV and a rat RNA tumor virus. In this case the RNAs of the two viruses should be at least partially homologous. (ii) The RNAs of replication-defective, transforming murine viruses may be defective genomes of nondefective murine sarcoma viruses, analogous to nondefective avian sarcoma viruses (26, 31, 35, 37), entirely unrelated or perhaps partially related to their helper leukemia viruses. (iii) The RNAs of the replication-defective, transforming viruses may be cellular oncogenic information transducible by Ki-MLV and other RNA tumor viruses. This information may represent cellular genes (33, 34) evolving into viruses by a process of somatic evolution, as proposed by Temin's protovirus hypothesis (34).

1. Harvey, J. J. & East, J. (1971) *Int. Rev. Exp. Pathol.* **10**, 265–360.
2. "The Molecular Biology of Tumour Viruses" (1973) ed. J. Tooze, Cold Spring Harbor Laboratory.
3. Aaronson, S. A. & Weaver, C. A. (1971) *J. Gen. Virol.* **13**, 245–252.
4. Aaronson, S. A. & Rowe, W. P. (1970) *Virology* **42**, 9–19.
5. Klement, V., Nicolson, M. O. & Huebner, R. J. (1971) *Nature New Biol.* **234**, 12–14.
6. Duesberg, P. H., Vogt, P. K., Maisel, J., Lai, M. M.-C. & Canaani, E. (1973) in *Virus Research: Proceedings of the 1973 I.C.N.-U.C.L.A. Symposium on Molecular Biology,* ed. Fox, F. C. (Academic Press, Inc., New York,) pp. 327–338.
7. Stephenson, J. R. & Aaronson, S. A. (1971) *Virology* **46**, 480–484.
8. Benveniste, R. E. & Scolnick, E. M. (1973) *Virology* **51**, 370–382.
9. Peebles, P. T., Bassin, R. H., Haapala, D. K., Phillips, L. A., Nomura, S. & Fischinger, P. J. (1971) *J. Virol.* **8**, 690–694.
10. Long, C., Kelloff, G. & Gilden, R. V. (1972) *Int. J. Cancer* **10**, 310–319.
11. Klement, V., Freedman, M. M., McAllister, R. M., Nelson-Rees, W. H. & Huebner, R. J. (1971) *J. Nat. Cancer Inst.* **47**, 65–73.
12. Kirsten, D. H. & Mayer, L. A. (1967) *J. Nat. Cancer Inst.* **39**, 311–336.
13. Kirsten, D. H. & Mayer, L. A. (1969) *J. Nat. Cancer Inst.* **43**, 735–746.
14. Aaronson, S. A. & Todaro, G. J. (1970) *Nature* **225**, 458–459.
15. Jainchill, J. L., Aaronson, S. A. & Todaro, G. J. (1969) *J. Virol.* **4**, 549–553.
16. Hartley, J. W. & Rowe, W. P. (1966) *Proc. Nat. Acad. Sci. USA* **55**, 780–786.
17. Rowe, W. P., Pugh, W. E. & Hartley, J. W. (1970) *Virology* **42**, 1136–1138.
18. Spirin, A. S. (1963) *Prog. Nucl. Acid Res. Mol. Biol.* **1**, 301–345.
19. Duesberg, P. H. & Robinson, W. S. (1966) *Proc. Nat. Acad. Sci. USA* **55**, 219–227.
20. McClain, K. & Kirsten, W. M. (1972) *Cancer Res.* **32**, 1470–1475.
21. Duesberg, P. H. (1970) *Curr. Top. Microbiol. Immunol.* **51**, 79–104.
22. Blair, C. D. & Duesberg, P. H. (1968) *Nature* **220**, 396–399.
23. Watson, J. D. (1971) *Virology* **45**, 586–597.
24. Duesberg, P. H. & Vogt, P. K. (1970) *Proc. Nat. Acad. Sci. USA* **67**, 1673–1680.
25. Staynov, D. F., Pinder, I. C. & Gratzer, W. B. (1972) *Nature New Biol.* **235**, 108–110.
26. Duesberg, P. H. & Vogt, P. K. (1973) *J. Virol.*, **12**, 594–599.
27. McConkey, E. H. & Hopkins, I. W. (1969) *J. Mol. Biol.* **39**, 545–550.
28. Klement, V., Nicolson, M. O., Nelson-Rees W., Gilden, R. V., Oroszlan, S., Rougey, R. W. & Gardner, M. B. (1973) *Int. J. Cancer*, in press.
29. Gilden, R. V. (1972) in *RNA Viruses and Host Genome Inoncogenesis,* eds. Emmelot, P. & Bentvelzen, P. (North-Holland Publ. Co., Amsterdam), pp. 193–196.
30. Brownlee, G. C. & Sanger, F. (1969) *Eur. J. Biochem.* **11**, 395–399.
31. Lai, M. M. C., Duesberg, P. H., Horst, J. & Vogt, P. K. (1973) *Proc. Nat. Acad. Sci. USA* **70**, 2266–2270.
32. Horst, J., Content, J., Mandeles, S., Fraenkel-Conrat, H. & Duesberg, P. H. (1972) *J. Mol. Biol.* **69**, 209–215.
33. Huebner, R. J. & Todaro, G. H. (1969) *Proc. Nat. Acad. Sci. USA* **64**, 1087–1094.
34. Temin, H. M. (1971) *J. Nat. Cancer Inst.* **46**, III–VI.
35. Duesberg, P. H. & Vogt, P. K. (1973) *Virology,* **54**, 207–219.
36. Ostertag, W., Melderis, H., Steinheider, G., Kluge, N. & Dube, S. (1972) *Nature New Biol.* **239**, 231–234.
37. Duesberg, P. H., Lai, M. M-C., & Maisel, J. (1973, in press) in "Modern Trends in Human Leukemia—Biological, Biochemical and Virological Aspects," eds. Neth, R., Gallo, R. & Stohlman, F. (J. F. Lehmanns Verlag, Munich, Germany).
38. Bader, J. P. and Steck, T. L. (1969) *J. Virol.* **4**, 454–459.