Proc. Nat. Acad. Sci. USA
Vol. 72, No. 11, pp. 4650–4654, November 1975
Microbiology

Separation of sarcoma virus-specific and leukemia virus-specific genetic sequences of Moloney sarcoma virus

(mechanism of transformation)

EDWARD M. SCOLNICK*, RICHARD S. HOWK†, ANTHONY ANISOWICZ†, PAUL T. PEEBLES*, CHARLES D. SCHER‡, AND WADE P. PARKS*

* National Cancer Institute, Bethesda, Maryland; † Meloy Laboratories, Rockville, Maryland; and ‡ Harvard Medical School, Boston, Massachusetts

Communicated by Wallace P. Rowe, September 3, 1975

ABSTRACT **We have studied the nucleic acid sequences in nonproducer cells transformed by Moloney sarcoma virus or Abelson leukemia virus (two types of replication-defective, RNA-containing, viruses isolated by passage of Moloney leukemia virus in BALB/c mice). DNA probes from the Moloney leukemia virus detect RNA in both Abelson virus-transformed nonproducer cells and Moloney sarcoma virus-transformed nonproducer cells. A sarcoma-specific cDNA, prepared from the Moloney sarcoma virus, has extensive homology to RNA found in heterologous nonproducer cells transformed by Moloney sarcoma virus, has little homology to RNA in cells producing Moloney leukemia virus, and no detectable homology to RNA in nonproducer cells transformed by the Abelson virus. By analogy to earlier data on avian and mammalian sarcoma viruses, these results suggest that the Moloney sarcoma virus arose by recombination between a portion of the Moloney leukemia virus genome and additional sarcoma-specific information, and indicate that the expression of this information is not essential for Abelson virus-mediated fibroblast transformation.**

RNA-containing mammalian type-C viruses, which are defective for replication and which produce morphological transformation of fibroblasts in cell culture have been isolated from murine, feline, and primate hosts (1–7). Two isolates of such defective RNA tumor viruses, the Moloney sarcoma virus and Abelson leukemia virus, have been obtained by passage of Moloney murine leukemia virus (Mo-MuLV) in BALB/c mice; these viruses provide an excellent model system in which to investigate the question of the universality of the nucleic acid sequences which code for transformation of different types of cells.

From the Moloney sarcoma-leukemia complex (8), two forms of the Moloney sarcoma virus (Mo-SV) genome have been isolated free of leukemia virus in transformed "nonproducer" cells (9–11). One of these forms of the Mo-SV genome was isolated by injection of the sarcoma–leukemia complex into hamsters (9); this form of Mo-SV codes for no known structural proteins of Moloney leukemia virus (12, 13) and thus is referred to here as Mo-SV p^-. The other form of Mo-SV, isolated in cell culture from the sarcoma–leukemia complex, was called the S^+L^- strain of Mo-SV (11). This form of Mo-SV was later found to contain, as a part of its genome, information which codes for the p30 protein of Moloney leukemia virus (12, 14), and thus is referred to here as a p30 positive strain of Mo-SV (Mo-SV $p30^+$). In past experiments each of these forms has been shown to be genetically stable (12), and each of these forms of Mo-SV is defective by itself for replication. When the Mo-SV p^- form is rescued by replicating murine leukemia virus, the complex causes sarcomas when injected into animals (9).

The Abelson leukemia virus, isolated by passage of Mo-MuLV in prednisolone-treated BALB/c mice, (4), can also transform fibroblasts or other cells in cell culture (15–17) but produces B-cell (bone marrow derived cell) and null cell leukemias, not sarcomas, when injected into mice (15, 18). Like Moloney sarcoma virus, the Abelson genome has been isolated free of replicating helper virus, in transformed nonproducer cells (15, 19). Analysis of such nonproducer cells for structural proteins of Moloney murine leukemia virus has not to our knowledge been reported.

Since no molecular explanation of the biological difference between Moloney sarcoma virus and Abelson virus is available, we began nucleic acid hybridization experiments to compare the genomes of the two replication-defective viruses.

MATERIALS AND METHODS

Cells and Viruses. All cells were grown in Dulbecco's modification of Eagle's medium containing either 10% calf serum or 10% fetal calf serum obtained from the Colorado Serum Co. The N-tropic and xenotropic murine type-C viruses isolated from BALB/c cells were the gift of Dr. Raoul Benveniste, National Cancer Institute. The xenotropic virus was grown in the rabbit cell, SIRC, as previously described (22). The N-tropic virus was grown in NIH 3T3 cells. The Moloney leukemia virus was subjected to two cycles of passage at limiting dilution in NIH 3T3 cells, and the virus was then grown in NIH 3T3 cells as the source of virus for synthesis of cDNA probe homologous to Mo-MuLV (see below). The endogenous feline virus, RD-114, was obtained from Pfizer Laboratories, Maywood, N.J. through the Office of Program Resources and Logistics, Virus Cancer Program, NCI. The RD-114 virus was propagated in a human rhabdomyosarcoma cell, the RD cell (24), or in the Cf_2th dog cell (25). A population of $p30^+$ Moloney sarcoma virus and RD-114 virus grown in a canine kidney cell was derived by superinfection of a nonproducer canine cell transformed by Moloney sarcoma virus with RD-114 virus derived from the Cf_2th cell. This virus mixture was used to synthesize the sarcoma specific cDNA. The cells used are listed in Table 1.

Synthesis of Virus Specific [^{3}H]DNA. The endogenous reverse transcriptase reaction from viruses banded by sucrose density gradient was used to synthesize [^{3}H]deoxycytidine-labeled DNA; the conditions have been fully detailed in an earlier publication (13).

Isolation of Viral and Total Cellular RNA. Total cell RNA was isolated by the cesium chloride centrifugation method described by Glisin *et al.* (26), and purified RNA solutions were stored at −20°.

Abbreviation: C_rt, initial concentration of total RNA (moles of nucleotide/liter) × time (seconds); t_m, temperature at which 50% of the RNA-DNA hybrid unwinds. Definitions of abbreviations for cells are found in Table 1.

Table 1. Cells used in hybridization experiments

Cell	Description	Ref.
Transformed nonproducer cells		
Mo-NRK p30$^+$	Normal rat kidney cells (NRK) transformed by a p30 antigen positive form of Moloney sarcoma virus (Mo-SV p30$^+$).	11, 13, 30
Mo-dog p30$^+$	Canine kidney cell transformed by Mo-SV p30$^+$.	23
Mo-human p30$^+$	Human fibroblasts transformed by Mo-SV p30$^+$.	14
Mo-NRK p$^-$	NRK cells transformed by form of Moloney sarcoma virus, which produces no known MuLV structural proteins (Mo-SV p$^-$).	13
ANN-1	NIH 3T3 mouse cells transformed by Abelson virus.	15
Ab-NRK	NRK cells transformed by Abelson virus.	19
Ha-NRK	NRK cells transformed by Harvey sarcoma virus.	21
Other		
SR-V-NRK	Schmidt-Ruppin virus transformed normal rat kidney cell (NRK) spontaneously releasing endogenous rat type-C viruses.	19, 31
SIRC	Rabbit cornea cells.	22
NIH 3T3	NIH Swiss mouse cell.	32
Cf_2th	Canine thymus cell.	25
MDCK	Canine kidney cell.	23

Hybridization. For analytical hybridizations, cellular RNA and [^{3}H]DNA (2×10^7 cpm/μg) were incubated for 24–48 hr at 65° in 0.05 ml reaction mixtures containing: 0.01 M Tris·HCl at pH 7.2; 10^{-5} M EDTA; 0.60 M NaCl; 0.05% vol/vol sodium dodecyl sulfate; 60 μg/ml of yeast RNA; and 60 μg/ml of calf thymus DNA. Hybridizations were analyzed by S_1 nuclease digestion (27). C_rt [initial concentration of total RNA (moles of nucleotide/liter) × time (seconds)] values (29) were corrected to 0.18 M monovalent cation concentration [Britten *et al.* (28)].

Recycling of [^{3}H]DNA. DNA transcripts were recycled against the appropriate RNAs from various infected cells as follows. Hybridization reactions (2.5–5.0 ml) were carried out at a C_rt of about 10^4 moles·sec·liters^{-1} under conditions used for analytical reactions except carrier DNA was omitted. Reactions were diluted 4-fold with 0.025 M sodium phosphate at pH 6.8 and loaded onto a water-jacketed hydroxylapatite column (Bio Rad HTP) equilibrated in this buffer at room temperature. The column contained 1.0 g of hydroxylapatite per mg of RNA in the hybridization reaction. The column was washed with starting buffer. The temperature of the column was then raised to 65° with a Haake pump. The single-stranded [^{3}H]DNA was eluted at 65° with 0.14 M sodium phosphate, pH 6.8. Aliquots of each fraction were counted in Redisolve V. The hybridized [^{3}H]DNA was then eluted at 65° in a buffer containing 0.46 M sodium phosphate at pH 6.8. The nonhybridized and hybridized fractions were dialyzed against distilled water and treated for 5 hr with 0.5 M NaOH at 37°. After thorough dialyses against distilled water, the cDNAs were lyophilized, resuspended in 0.01 M Tris·HCl at pH 7.2 and stored at −20°. Recovery of the sarcoma-specific [^{3}H]DNA from the Mo-SV/RD-114 probe was about 25% of the starting cDNA. Recycling of [^{3}H]DNA from Mo-MuLV against RNA from Mo-SV p30$^+$ transformed NRK cells resulted in a recovery of 40% of the radioactivity in the nonhybridized, and 60% in the hybridized fractions, respectively.

RESULTS

A cDNA prepared from Mo-MuLV was hybridized with excess total cellular RNA from a variety of nonproducer rat cells transformed by various strains of mammalian sarcoma viruses, rat cells transformed by an avian sarcoma virus, or cells producing either Mo-MuLV or a mouse xenotropic virus. The results, shown in Table 2, indicate the final extent of hybridization at saturating C_rt values (up to 10^4 moles·sec·liters^{-1}) obtained with each of the indicated RNAs. Hybridization is greatest with the RNA from either NIH 3T3 cells or NRK cells producing Moloney leukemia virus. As previously noted (13, 21), heterologous nonproducer cells transformed by either a p30$^+$ or p$^-$ strain of Moloney sarcoma virus have RNA with significant homology to part of the cDNA probe prepared from Mo-MuLV. The final extent of hybridization achieved with RNA from these cells is significantly less than that achieved with RNA of the same NRK cell producing the Moloney leukemia virus. In studies to be fully detailed elsewhere, we also examined the DNA of the Mo-SV transformed nonproducer rat cells. Consistent with earlier studies on the woolly monkey sarcoma virus (33), the DNA from Mo-NRK p30$^+$ or Mo-NRK p$^-$ also lacked the full complement of Mo-MuLV sequences found in the DNA of Mo-MuLV-producing NRK cells. The results suggest that the failure to detect RNA complementary to a portion of the Mo-MuLV cDNA in the Mo-SV-transformed nonproducer cells was not due to a lack of expression of these sequences.

As indicated in Table 2, the RNA from Abelson virus-transformed NRK cells also contained sequences homologous to part, but not all, of the sequences in the cDNA from Moloney leukemia virus. The final extent of hybridization was approximately the same with RNA from Mo-NRK p$^-$ cells or Abelson-transformed NRK cells. Of the various RNAs tested, the RNA of Ha-SV-transformed NRK cells showed the least homology to cDNA from the Moloney leukemia virus. As previously reported, the RNA from rabbit cells infected with a mouse xenotropic virus from BALB/c cells also yields only about a 30–40% hybridization with the Moloney leukemia virus cDNA (22).

In Exp. 2 in Table 2, we mixed the RNAs from NRK cells transformed by the Abelson virus and the p$^-$ strain of Moloney sarcoma virus to determine whether or not the RNAs were identical, or whether they represented different portions of the Moloney leukemia virus genome. The results in Table 2 indicate that the RNAs in Ab-NRK and Mo-NRK p$^-$

Table 2. Hybridization with cDNA from Moloney leukemia virus

RNA source	Final extent of hybridization: Percent	Final extent of hybridization: cpm hybridized
Exp. 1		
Producer cells		
NIH 3T3/Mo-MuLV	100	1491
NRK/Mo-MuLV	100	1503
SIRC/xenotropic virus	33	588
Nonproducer cells		
Mo-NRK p30$^+$	48	738
Mo-NRK p$^-$	33	566
Ab-NRK	33	560
Ha-NRK	21	312
Other		
SR-V-NRK	6	127
NIH 3T3	3	60
Exp. 2		
Nonproducer cells		
Ab-NRK (500 μg)	33	562
Mo-NRK p$^-$ (500 μg)	33	565
Ab-Nrk (250 μg) + Mo-NRK p$^-$ (250 μg)	42	690
Ab-NRK (250 μg) + SR-V-NRK (250 μg)	32	543
Mo-NRK p$^-$ (250 μg) + SR-V-NRK (250 μg)	33	550

Hybridization conditions are in *Materials and Methods*. Each reaction contained 2000 cpm of [^{3}H]DNA. Background in the absence of RNA was 43 cpm and has been subtracted from all values; 100% hybridization is defined as the value obtained with the NIH cells or NRK cells producing Mo-MuLV.

cells homologous to Mo-MuLV cDNA are largely similar. However, a small increment in hybridization to the Mo-MuLV cDNA was observed with a mixture of the Ab-NRK and Mo-NRK p$^-$ RNA. The results indicate that the Abelson virus that transforms fibroblasts contains as part of its genome, RNA homologous to the Moloney leukemia virus, and that the Mo-MuLV portion of this RNA is largely overlapping but somewhat different from the Mo-MuLV portion of the p$^-$ strain of Moloney sarcoma virus.

Fractionation of Mo-MuLV cDNA by absorption with RNA of Mo-NRK p30$^+$ cells

To further examine the portions of the Moloney leukemia genome contained in the RNA of nonproducer cells transformed by the p$^-$ strain of Moloney sarcoma virus or Abelson virus, we prepared probes (as detailed in *Materials and Methods*) which represented the portion of the Moloney leukemia virus genome which did hybridize to Mo-NRK p30$^+$ RNA, and the portion which did not hybridize to Mo-NRK p30$^+$ RNA. The results of hybridization with the two fractions of the Mo-MuLV cDNA are shown in Table 3. The Mo-MuLV cycled probe, not homologous to the Mo-NRK p30$^+$ RNA, hybridized well to the RNA of NIH 3T3 cells producing Moloney leukemia virus, but essentially no hybridization was detected with uninfected NIH 3T3 cell RNA, SR-V-NRK cellular RNA, Mo-NRK p30$^+$ RNA, Mo-Human p30$^+$ RNA, Ha-SV transformed NRK RNA, or Ab-NRK RNA. Importantly, the RNA from NRK cells transformed by the p$^-$ strain of Moloney sarcoma virus hybridized appreciably (18%) to the absorbed probe. Thus, these results indicate that the RNA homologous to Mo-MuLV in cells transformed by the p$^-$ strain of Mo-SV is not identical to the RNA homologous to Mo-MuLV in the cells transformed by the p30$^+$ form of Mo-SV. In other studies, no hybridization was detected with the absorbed (not homologous) cDNA and the DNA from Mo-NRK p30$^+$ cells, whereas the absorbed cDNA could detect MuLV sequences in the DNA of Mo-NRK p$^-$ cells.

In contrast to the results with the defective transforming viruses, the RNA from the rabbit cell infected with a xenotropic virus hybridized well to the absorbed probe. In fact, the same proportion of the absorbed cDNA hybridized to the RNA of cells producing xenotropic virus as the proportion of the unabsorbed cDNA that hybridized to the same RNA.

Finally, when the absorbed [^{3}H]DNA was hybridized in 10-fold molar excess to limiting ^{32}P-labeled 60–70S RNA of Mo-MuLV, only 25–30% of the genome was protected, whereas the starting uncycled cDNA protected 70–80% of the ^{32}P-labeled RNA of Mo-MuLV.

The portion of the Moloney leukemia virus probe which had hybridized to the Mo-NRK p30$^+$ RNA ("homologous" cDNA) was also tested for its hybridization properties with the same series of RNAs (Table 3). In contrast to the portion of the genome not found in p30$^+$ Mo-NRK RNA, the portion of the Moloney leukemia virus genome that was present in the p30$^+$ Mo-NRK RNA hybridized well to all of the RNAs from the rat cells transformed by each of the murine viruses. All of the hybridizations were substantially above that seen with RNA from NRK cells transformed by avian sarcoma virus which were producing endogenous rate type-C viruses. Importantly, the RNA from rabbit cells infected with a xenotropic virus hybridized again to approximately the same

Table 3. Hybridization with Moloney leukemia virus cDNAs homologous and not homologous to RNA from Mo-SV p30$^+$ transformed NRK cells

RNA source	Final extent of hybridization: cDNA not homologous: Percent	cDNA not homologous: cpm	cDNA homologous: Percent	cDNA homologous: cpm
Producer cells				
NIH 3T3/Mo-MuLV	100	1430	100	1710
SIRC/xenotropic virus	36	522	35	598
Nonproducer cells				
Mo-NRK p30$^+$	<3	<50	75	1189
Mo-human p30$^+$	<3	<50	79	1213
Mo-NRK p$^-$	18	257	38	662
Ab-NRK	<3	<50	42	718
Ha-NRK	<3	<50	28	488
Other				
SR-V-NRK	<3	<50	6	104
NIH 3T3	<3	<50	3	51

Each hybridization contained approximately 2000 cpm (trichloroacetic acid-precipitable radioactivity) of either cDNA homologous to RNA in Mo-NRK p30+ cellular RNA or cDNA not homologous to the same RNA. Background in absence of RNA was 24 cpm and has been subtracted from all values.

extent as that observed with Mo-MuLV RNA. At a 10-fold excess, this part of the Mo-MuLV [^{3}H]cDNA protected 50–60% of the ^{32}P-labeled 60–70S RNA of Mo-MuLV. The results thus indicate that the two different portions of the Moloney leukemia virus genome tested, each of which represents a substantial part of the genome, are both partially homologous to the BALB/c xenotropic virus, but that they are differently represented in the various strains of replication-defective fibroblast transforming viruses derived from the Moloney leukemia virus. Thus, the incomplete homologies observed with each RNA to the uncycled cDNA from Mo-MuLV in one set of cases represents a missing portion of the Mo-MuLV genome, and in the case of the xenotropic virus represents partial homology with each portion of the genome. These results with the xenotropic virus are consistent with t_m data obtained earlier (22).

Mo-SV specific probe

To examine the potential sarcoma-virus-specific region of Moloney sarcoma virus, we used the p30$^+$ strain of Mo-SV to prepare a cDNA from a virus mixture containing this strain of Moloney sarcoma virus that had been rescued with a heterologous helper virus, the endogenous feline virus, RD-114. This virus complex was grown in a canine kidney cell. We prepared a probe from this virus mixture, absorbed the cDNA with a mixture of RNAs from RD-114 infected dog cells and Mo-MuLV infected NIH 3T3 cells in order to remove sequences homologous to Mo-MuLV and RD-114. In the absorption, each RNA was hybridized at a C_rt of 10^4 moles·sec·liter^{-1}. The results of hybridization with a probe absorbed a single time in this manner are shown in Table 4.

In contrast to any of the Mo-MuLV probes shown in Tables 2 and 3, the cycled probe was highly enriched for sequences present in RNA found in the p30$^+$ form of Mo-SV or in RNA from cells transformed by either form of Mo-SV. An identical final extent of hybridization was achieved with the viral RNA of a mixture of Mo-SV p30$^+$ and RD-114 viruses, or the cellular RNA of Mo-SV p30$^+$ transformed nonproducer rat cells or human cells. Similarly, an identical final extent was achieved with RNA from NRK cells transformed by the p$^-$ strain of Mo-SV. Importantly, each of these hybridization values was well in excess of the final extent of hybridization achieved with viral RNA from Moloney leukemia virus or RD-114 virus, or cellular RNA from cells producing Moloney leukemia virus or RD-114 virus. Interestingly, the RNA from the Abelson transformed nonproducer NRK or NIH cells failed to give detectable (<10%) hybridization to this Mo-SV specific probe. Also, the RNA from NRK cells transformed by Harvey sarcoma virus hybridized only slightly to this Moloney sarcoma virus specific probe. The Mo-SV specific sequences were not contained in RNA from cells producing either an N-tropic strain of murine leukemia virus, or a xenotropic strain of endogenous BALB/c virus. As other controls, the Mo-SV specific sequences were not detected in RNA from uninfected dog thymus cells, or in Schmidt-Ruppin transformed NRK cells.

Table 4. Hybridization with sarcoma virus specific cDNA

RNA source	Final extent of hybridization: Percent	Final extent of hybridization: cpm hybridized
Cellular RNA		
Producer cells		
Mo-MuLV in NIH 3T3 cells	18	105
Xenotropic virus in SIRC cells	<3	<20
N-tropic virus in NIH 3T3 cells	6	36
RD-114 virus in Cf_2th cells	<3	<20
Nonproducer cells		
Mo-NRK p30$^+$	100	563
Mo-human p30$^+$	100	575
Mo-NRK p$^-$	100	580
Ab-NRK	<3	<20
Ab-NIH (ANN-1)	6	43
Ha-NRK	11	65
Other		
SR-V-NRK	<3	<20
Dog kidney or thymus	<3	<20
Viral RNA		
Mo-MuLV (2 μg)	16	95
RD-114 (2 μg)	<3	<20
RD-114/Mo-SV p30$^+$ (3 μg)	100	596

Each hybridization reaction contained 1100 cpm (trichloroacetic acid-precipitable radioactivity) of cDNA absorbed as described in *Materials and Methods*. Background with no RNA was 18 cpm and is subtracted from all values.

DISCUSSION

In these studies we have investigated the genetic composition of Moloney sarcoma virus and Abelson leukemia virus (two types of RNA-containing oncogenic viruses, each of which is replication-defective but capable of transforming fibroblasts in cell culture, and each of which was isolated from BALB/c mice). To study potential molecular differences between the two viruses, we prepared cDNA probes representing different portions of the Moloney leukemia genome and a cDNA probe specific for the Moloney sarcoma virus genome. One cDNA from Mo-MuLV represented those sequences not contained in the RNA from nonproducer cells transformed by a p30 antigen positive class of Moloney sarcoma virus; the other cDNA of Mo-MuLV represented the Mo-MuLV sequences that *are* contained in the RNA of these same cells. Hybridization results with these two portions of the Moloney leukemia virus genome indicated that each represented a substantial portion of the Mo-MuLV genome, and that differences could be detected in the RNA of cells transformed by a p$^-$ strain of Mo-SV and a p30$^+$ strain of Mo-SV that were not detectable with uncycled cDNA from Mo-MuLV. Surprisingly, the RNA of cells transformed by the p$^-$ form of Mo-SV, which codes for no known MuLV structural proteins, contains some MuLV sequences not found in the RNA of cells transformed by a p30$^+$ strain of Mo-SV. These results are thus not consistent with the origin of the p$^-$ class of Mo-SV as simply a further deletion of the p30$^+$ Mo-SV genome. A more plausible explanation is that the p30$^+$ class and p$^-$ class of Moloney sarcoma virus arose as independent deletions of a larger genome of Moloney sarcoma virus.

Since the p$^-$ form of Mo-SV and the Abelson virus had been compared biologically (15), we also compared the two viruses biochemically. When the RNA in nonproducer cells transformed by the Abelson genome was examined with the same cDNA probes, RNA was detected which was homologous to Moloney leukemia virus. To examine the sarcoma-specific portion of the Moloney sarcoma virus genome, we prepared a cDNA from a mixture of Mo-SV and RD-114

virus grown in a dog cell. This cDNA probe was then absorbed with RNA from cells infected with the Moloney leukemia virus and RNA from cells infected with the heterologous helper virus, RD-114. This absorbed cDNA probe contained sequences found in the viral RNA of the $p30^+$ form of Mo-SV, and the cellular RNA of cells transformed by a p^- strain of Moloney sarcoma virus or the cellular RNA of cells transformed by a $p30^+$ strain of Moloney sarcoma virus. These sequences were not detected in viral RNA from Mo-MuLV or RD-114, or in cellular RNA from mouse cells or rat cells producing Moloney leukemia virus, or in dog cells producing RD-114 virus. In addition, this RNA was not detected in mouse cells producing a N-tropic strain of murine leukemia virus, in rabbit cells producing a mouse xenotropic virus, in rat cells transformed by the Schmidt-Ruppin strain of avian sarcoma virus, or in rat cells transformed by the Harvey strain of sarcoma virus. These results suggest that the sarcoma virus specific sequences detected are part of the Mo-SV genome and not a consequence of transformation. This RNA found in the Moloney sarcoma virus was not detected in cells transformed by the Abelson genome, even though RNA is found in Abelson-transformed nonproducer cells with homology to the Moloney leukemia virus. Thus, if the Mo-SV specific sequences measured contain information homologous to RNA which is necessary for the maintenance of transformation, the results would suggest that the sequences in Moloney sarcoma virus responsible for the maintenance of transformation are not identical to the sequences in Abelson leukemia virus or Harvey sarcoma virus which are responsible for their ability to transform fibroblasts in cell culture.

The current results are consistent with studies on avian and mammalian sarcoma viruses which have indicated that they arose by recombination between a replicating type-C helper virus and additional sequences. For the Kirsten and Harvey sarcoma viruses, these additional sequences originated in rats (19–21) and for Rous sarcoma virus the extra sequences (34) are present in normal chicken DNA[§]. Presumably by analogy to both systems, the additional sequences of Moloney sarcoma virus originated in BALB/c mice. However, since our Moloney sarcoma virus specific probe has not yet been sufficiently purified of Moloney leukemia virus sequences, we have not yet investigated further the origin of the Moloney sarcoma virus. However, after further purification of the probe it should be possible to examine the origin of the sequences and their relationship to other strains of oncogenic murine viruses (35, 36) and naturally occurring tumors in mice. In addition, application of similar technology to sarcoma virus isolates from feline and primate origin should help to determine in mammalian systems the number of classes of information and the types of proteins that cause transformation in cell culture and in naturally occurring tumors of rodents and primate species.

This work was supported by the Virus Cancer Program of the National Cancer Institute. The authors would like to thank H. Varmus and J. M. Bishop for helpful discussions about similar experiments on Rous sarcoma virus[§].

[§] D. Stehlin, J. M. Bishop, and H. Varmus, personal communication.

1. Harvey, J. J. (1964) *Nature* **204**, 1104–1106.
2. Moloney, J. B. (1966) *Nat. Cancer Inst. Monogr.* **22**, 139–142.
3. Kirsten, W. H. & Mayer, L. A. (1967) *J. Nat. Cancer Inst.* **39**, 311–319.
4. Abelson, H. T. & Rabstein, L. D. (1970) *Cancer Res.* **30**, 2113–2222.
5. Snyder, S. P. & Theilen, G. H. (1969) *Nature* **221**, 1074–1075.
6. Gardner, M. B., Rongey, R. W., Arnstein, P., Estes, J. O., Sarma, P., Huebner, R. J. & Rickard, C. G. (1970) *Nature* **226**, 807–809.
7. Wolfe, L. G., Deinhardt, G., Theilen, G. H., Rabin, H., Kawakami, T. & Bustad, L. E. (1971) *J. Nat. Cancer Inst.* **47**, 1115–1120.
8. Hartley, J. W. & Rowe, W. P. (1966) *Proc. Nat. Acad. Sci USA* **55**, 780–786.
9. Huebner, R. J., Hartley, J. W., Rowe, W. P., Lane, W. T. & Capps, W. I. (1966) *Proc. Nat. Acad. Sci USA* **56**, 1164–1169.
10. Aaronson, S. A. & Rowe, W. P. (1970) *Virology* **42**, 9–19.
11. Bassin, R. H., Tuttle, N. & Fischinger, P. J. (1970) *Int. J. Cancer* **6**, 95–107.
12. Aaronson, S. A., Bassin, R. H. & Weaver, C. (1972) *J. Virol.* **9**, 701–704.
13. Benveniste, R. & Scolnick, E. M. (1973) *Virology* **51**, 370–381.
14. Peebles, P. T., Fischinger, P. J., Bassin, R. H. & Papageorge, A. G. (1973) *Nature New Biol.* **242**, 98–117.
15. Scher, C. D. & Siegler, R. (1975) *Nature* **253**, 729–731.
16. Sklar, M. D., White, B. J. & Rowe, W. P. (1974) *Proc. Nat. Acad. Sci. USA* **71**, 4077–4081.
17. Rosenberg, N., Baltimore, D. & Scher, C. D. (1975) *Proc. Nat. Acad. Sci. USA* **72**, 1932–1936.
18. Potter, M., Sklar, M. D. & Rowe, W. P. (1973) *Science* **132**, 592–594.
19. Scolnick, E. M., Goldberg, R. J. & Parks, W. P. (1975) *Cold Spring Harbor Symp. Quant. Biol.* **39**, 885–895.
20. Scolnick, E. M., Rands, E., Williams, D. & Parks, W. P. (1973) *J. Virol.* **12**, 458–463.
21. Scolnick, E. M. & Parks, W. P. (1974) *J. Virol.* **13**, 1211–1219.
22. Benveniste, R. E., Lieber, M. M. & Todaro, G. J. (1974) *Proc. Nat. Acad. Sci USA* **71**, 602–606.
23. Peebles, P. T., Gerwin, B. I., Papageorge, A. G. & Smith, S. G. (1975) *Virology*, in press.
24. Giard, D. J., Aaronson, S. A., Todaro, G. J., Arnstein, P., Kersey, J. H., Dosik, H. & Parks, W. P. (1973) *J. Nat. Cancer Inst.* **51**, 1417–1424.
25. Benveniste, R. E., Lieber, M. M., Livingston, D. M., Sherr, C. J., Todaro, G. J. & Kalter, S. S. (1974) *Nature* **248**, 17–20.
26. Glisin, V., Crkvenjakov, R. & Byers, C. (1974) *Biochemistry* **13**, 2633–2637.
27. Leong, J. A., Garapin, A. C., Jackson, H., Fanshier, L., Levinson, W. & Bishop, J. M. (1972) *J. Virol.* **9**, 891–903.
28. Britten, R. J., Graham, D. E. & Neufeld, B. R. (1974) in *Methods in Enzymology*, eds. Moldave, K. & Grossman, L. (Academic Press, New York), Vol. 29, pp. 363–418.
29. Birnstiel, M. L., Sells, B. H. & Purdom, I. F. (1972) *J. Mol. Biol.* **63**, 21–29.
30. Bassin, R. H., Phillips, L. A., Kramer, M. J., Haapala, D. K., Peebles, P. T., Nomura, S. & Fischinger, P. J. (1971) *Proc. Nat. Acad. Sci. USA* **68**, 1520–1524.
31. Benveniste, R. & Todaro, G. J. (1973) *Proc. Nat. Acad. Sci. USA* **70**, 3316–3320.
32. Jainchill, J. F., Aaronson, S. & Todaro, G. J. (1969) *J. Virol.* **4**, 459–553.
33. Scolnick, E. M., Parks, W., Kawakami, T., Kohne, D., Okabe, H., Gilden, R. & Hatanaka, M. (1974) *J. Virol.* **13**, 363–369.
34. Duesberg, P. H. & Vogt, P. K. (1973) *Virology* **54**, 207–219.
35. Finkel, M. P., Biskis, B. O. & Jinkins, P. B. (1966) *Science* **151**, 698–700.
36. Bittner, J. J. (1942) *Cancer Res.* **2**, 710–721.