0022-538X/78/0025-0238$02.00/0
JOURNAL OF VIROLOGY, Jan. 1978, p. 238-252

Vol. 25, No. 1
Printed in U.S.A.

# Physical Map of the Kirsten Sarcoma Virus Genome as Determined by Fingerprinting RNase T1-Resistant Oligonucleotides

THOMAS Y. SHIH,[1]* HOWARD A. YOUNG,[1] JOHN M. COFFIN,[2] AND EDWARD M. SCOLNICK[1]

*Laboratory of Tumor Virus Genetics, National Cancer Institute, Bethesda, Maryland 20014,[1] and Department of Molecular Biology and Microbiology and Cancer Research Center, Tufts University School of Medicine, Boston, Massachusetts 02111[2]*

Received for publication 29 June 1977

From analysis of the large RNase T1-resistant oligonucleotides of Kirsten sarcoma virus (Ki-SV), a physical map of the virus genome was deduced. Kirsten murine leukemia virus (Ki-MuLV) sequences were detected in T1 oligonucleotides closest to the 3′ end of the viral RNA and extended approximately 1,000 nucleotides into the genome. The rat genetic sequences started at this point and extended all the way to the very 5′ end of the RNA molecules, where a small stretch of Ki-MuLV sequence was detected. By comparison of the fingerprints of Ki-SV RNA and the RNA of the endogenous rat *src* genetic sequences, it was found that more than 50% of the T1 oligonucleotides were similar between Ki-SV and the endogenous rat *src* RNA, suggesting an identical primary nucleotide sequence in over 50% of the viral genomes. The results indicate that Ki-SV arose by recombination between the 5′ and 3′ ends of Ki-MuLV and a large portion of the homologous sequences of the endogenous rat *src* RNA.

Kirsten and Harvey sarcoma viruses (Ki-SV and Ha-SV, respectively) were isolated by inoculation of murine leukemia viuses (MuLV's) into various strains of rats (11, 13). Although the original isolates were mixtures of leukemia and sarcoma viruses, subsequent studies clearly showed that the viruses that induce solid tumors in animals and transform fibroblasts in tissue culture ($FT^+$) are the replication-defective components of the leukemia-sarcoma complexes with smaller genomes (1, 15, 16, 22). Studies in this laboratory (4, 22, 23) and recently by other investigators (2) have demonstrated that the genomes of these two sarcoma viruses contain sequences homologous to the leukemia viruses from which the sarcoma viruses were derived and, in addition, sequences derived from rat genetic information. Recent studies have revealed that this rat genetic information, which is expressed in a variety of rat cells as RNA, has all of the characteristics of an endogenous type C virus (19, 24, 27): (i) the genetic information exists as multiple copies (20 to 40) per haploid rat cell genome; (ii) it is inducible with halogenated pyrimidines to high levels of expression; (iii) it is specifically packaged by type C helper viruses; (iv) the subunit size is approximately 30S, and it forms dimer structures of approximately 60S; and (v) it is transmissible to some host cells (Scolnick, unpublished data). Since the sarcomagenic potential of Ki-SV and Ha-SV seems to have been associated with the acquisition of this class of endogenous type C virus sequences and for simplicity, we shall hereafter in this paper refer to this virus RNA as the endogenous rat "sarcoma" virus RNA, although the sarcomagenicity of this endogenous virus has not yet been conclusively demonstrated.

In the present paper, we attempt by fingerprinting the viral RNA in a two-dimensional gel electrophoresis system, to: (i) characterize the endogenous sarcoma virus RNA, (ii) compare the homologous sequences between Ki-SV and the endogenous sarcoma virus and between Ki-SV and Ki-MuLV, and (iii) map the rat and the Ki-MuLV sequences in the Ki-SV genome. A possible mechanism for the generation of Ki-SV and a functional implication of the organization of the viral genome will be discussed.

## MATERIALS AND METHODS

**Cells and viruses.** All cells were grown in Dulbecco-Vogt-modified Eagle minimal essential medium supplemented with 10% calf serum or 10% fetal calf serum (Grand Island Biological Co., Grand Island, N.Y.). The virus producer cells used in the present study were as follows. A mink fibroblast transformed by Ki-SV and superinfected with feline leukemia virus (FeLV) subgroup C [Ki-SV(FeLV)] was described ear-

lier (21). The HTP cells containing high levels of rat endogenous sarcoma virus RNA were originally derived from a uterine carcinoma induced by 7,12-dimethylbenzanthracene in a Sprague-Dawley rat and were obtained from S. Sekiya (Chiba, Japan) (25). The endogenous virus RNA was rescued by superinfection with woolly leukemia virus [HTP(WoLV)] (24). A clone, LTP, of the rat cells derived from the same uterine tumor expressing very low levels of the endogenous sarcoma virus RNA was superinfected in the same manner with WoLV [LTP(WoLV)] and was used as a control for the helper virus. The SR V-NRK cells (V-NRK transformed by the Schmidt-Ruppin strain of Rous sarcoma virus) spontaneously releasing a typical rat endogenous leukemia virus and the endogenous sarcoma virus was also used (21). Ki-MuLV was produced by a wild mouse SC-1 cell line infected with this virus. Harvey sarcoma virus (Ha-SV) grown in dog or mouse cells was described previously (22, 24). All cells were monitored for mycoplasma by both aerobic and anaerobic techniques and were found to be negative.

**Labeling and preparing viral RNA.** The cells, grown to confluence in 150-cm$^2$ Falcon flasks, were labeled with 12 ml of phosphate-free medium supplemented with 1 to 1.5 mCi of carrier-free [$^{32}$P]orthophosphate (NEX-054, New England Nuclear, Boston, Mass.) per ml and 10% dialyzed fetal calf serum. The $^{32}$P labeling was for 20 to 24 h at 37°C after incubating the cells with phosphate-free medium for about 2 h. After clarifying the culture medium at low-speed centrifugation, the virus was pelleted through 10 ml of 20% (wt/vol) sucrose in TNE buffer (0.01 M Tris [pH 7.4]–0.1 M NaCl–1 mM EDTA) in 30-ml polycarbonate centrifuge tubes with a Spinco 30 rotor at 29,000 rpm for 3 h (4°C). The virus pellets were lysed at room temperature in 1 ml of TNE buffer containing, in addition, 1% (wt/vol) sodium dodecyl sulfate (SDS) and 0.2% (vol/vol) diethylpyrocarbonate to inactivate RNase. The lysate was layered on 11 ml of a 15 to 30% sucrose gradient in TNE buffer and centrifuged at 39,000 rpm in an SW41 rotor at 20°C for 2.5 h. The [$^{32}$P]viral RNA at the 50 to 70S peak region was collected and precipitated with 100 μg of carrier yeast RNA and 2.5 volumes of absolute ethanol. After standing at −20°C overnight, the RNA was pelleted and dissolved in a small volume of water for fingerprinting.

**Fingerprinting [$^{32}$P]viral RNA.** The two-dimensional gel electrophoresis method for fingerprinting RNA was performed by the procedure of Billeter et al. (6) and Coffin and Billeter (7) as modified from the original method described by DeWachter and Fiers (9). The [$^{32}$P]RNA sample, containing 100 μg of yeast RNA in a small volume of water, was lyophilized and digested with 10 μl of RNase T1 (22 U) (Calbiochem, San Diego, Calif.) in 20 mM Tris-hydrochloride (pH 7.5)–1 mM EDTA–3.5 mM NaCl at 35°C for 30 min. The digested sample was mixed with xylene cyanol FF and bromophenol blue dye markers and applied to the first-dimension gel (160 by 380 by 2 mm) of 10% acrylamide–6 M urea–0.025 M citric acid (pH 3.5). Electrophoresis was terminated when xylene cyanol had migrated 20 cm from the origin. The second-dimension gel (250 by 250 by 2 mm) was of 21.8% acrylamide–0.04 M Tris-citrate (pH 8.0), and the experiment was terminated when bromophenol blue reached the 16-cm mark. Autoradiography was performed with Kodak No-Screen X-ray film at 4°C for 1 to 4 days. Oligonucleotides were identified by comparing their positions in the autoradiographs with standard fingerprints. Only fingerprints with good overlapping were chosen for direct comparison. The minimum criterion of superimposition of at least 10 adjacent spots was applied in most studies, except in a few fingerprints with a small number of large T1 oligonucleotides.

**Preparation of RNA fragments with poly(A) tags.** For the preparation of RNA fragments with polyadenylic acid [poly(A)] tags, the 50 to 70S [$^{32}$P]-RNA was precipitated with ethanol together with 150 μg of yeast RNA, and the RNA precipitate was dissolved in 300 μl of 0.16 M Tris (pH 7.2)–1% SDS. It was extracted with an equal volume of phenol-chloroform (1:1) at room temperature. The RNA was again precipitated with ethanol, dissolved in 200 μl of 0.01 M Tris (pH 7.2)–0.1 mM EDTA, and heat denatured at 95°C for 30 s. To obtain smaller fragments of RNA, the [$^{32}$P]RNA was first degraded with 0.05 M $Na_2CO_3$ at 50°C for 6 min (7). After neutralization with 1 M Tris-hydrochloride (pH 7.2) to a final concentration of 0.1 M, it was phenol-chloroform extracted as above. The RNA fragments were sedimented as described in the legend of Fig. 8. The RNA fragments with different size ranges were pooled and precipitated with 50 μg of yeast RNA. The RNA precipitate was dissolved in 1 ml of 0.01 M Tris–1 mM EDTA. After it was briefly heated (95°C, 10 s), LiCl was added to 0.5 M and SDS was added to 0.05%. The [$^{32}$P]RNA was then applied at room temperature to a small oligodeoxythymidylic acid$_{12\text{-}18}$ [oligo(dT)$_{12\text{-}18}$]-cellulose column containing 0.25 g of dry cellulose powder (Collaborative Research, Inc., Waltham, Mass.) equilibrated with the binding buffer (0.5 M LiCl–0.01 M Tris [pH 7.2]–0.05% SDS). The [$^{32}$P]RNA not absorbed by cellulose was washed off the column with the binding buffer, and the bound poly(A)$^+$ RNA was eluted with 0.01 M Tris–0.05% SDS (4 ml). After the LiCl concentration was adjusted to 0.5 M, it was bound to a new oligo(dT)-cellulose column for a second-cycle experiment. The twice-bound poly(A)$^+$ RNA was fingerprinted (28).

**Protection of T1 oligonucleotides against digestion with RNase A by hybridization to cDNA.** The complementary DNA (cDNA) protection experiments to identify homologous T1 oligonucleotides, as shown in Fig. 1, were performed as follows. [$^{32}$P]RNA (2 × 10$^6$ to 6 × 10$^6$ cpm with a specific activity of about 4 × 10$^6$ cpm/μg) was annealed to cDNA in 50 μl of 0.5 M NaCl–20 mM Tris (pH 7.2). After annealing at 65°C for 20 h, 5 μl of RNase A (5 μg) and RNase T1 (4.4 U) was added. It was further incubated at 37°C for 30 min. At the end of incubation, 100 μl of 0.2% (vol/vol) diethylpyrocarbonate–0.5 M NaCl–0.01 M Tris–0.2% SDS was added to the mixture. It was passed through a small Sephadex G-75 column equilibrated with the same buffer but omitting diethylpyrocarbonate. The RNA-cDNA hybrid in the excluded volume was pooled, twice phenol-chloroform extracted, and precipitated with ethanol. The RNA-cDNA hybrid dissolved in 150 μl of water was disso-

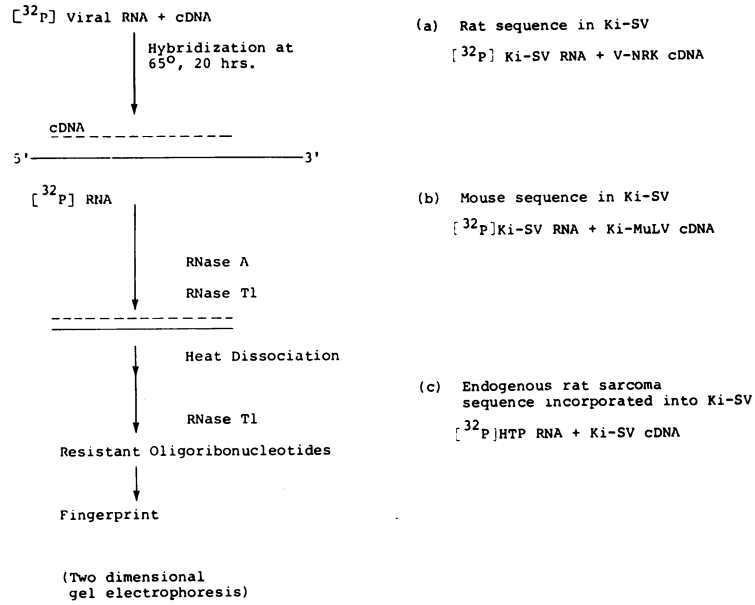


FIG. 1. *Scheme employed to study the sequence relationship in the cDNA protection experiments.*

ciated by heating at 95°C for 30 s and lyophilized. The RNA was further digested with 15 μl of T1 solution (33 U) and fingerprinted as above. The procedure was that of Coffin and Haseltine (8).

**Preparation of cDNA and RNA-DNA hybridization.** The cDNA used in [$^{32}$P]RNA protection experiments was prepared as follows. The cDNA of viruses produced by V-NRK and Ki-SV(FeLV) was prepared by the endogenous reverse transcriptase reactions primed by the calf thymus DNA fragments (21, 26). The Ki-MuLV cDNA was prepared by transcription of the heat-denatured viral 70S RNA primed by calf thymus DNA fragments with purified avian myeloblastosis virus reverse transcriptase (26). All of these cDNA preparations used herein protected more than 70% of the input homologous viral 70S [$^{32}$P]RNA under stringent hybridization conditions employing an S1 nuclease digestion assay (14). The preparation of [$^{3}$H]cDNA probes and conditions for molecular hybridization were described elsewhere (21).

## RESULTS

**Virus preparations and the scheme employed to study the sequence relationship.** The sequence homology among viruses studied herein as detected by molecular hybridization is summarized in Table 1. Ki-SV is a replication-defective virus, and any virus preparation therefore contains in addition a helper type C virus component. For a detailed study on the organization of the Ki-SV genome by fingerprinting viral RNA, it is mandatory to obtain Ki-SV RNA in a fairly pure form. We have employed a culture of Ki-SV with a feline type C virus (FeLV) pseudotype growing in a mink cell. This virus preparation has approximately a 20-fold excess of Ki-SV RNA as compared with FeLV RNA, as determined by molecular hybridization experiments with [$^{3}$H]cDNA probes used to detect either the rat sequences of Ki-SV or the cat sequences of the FeLV helper (Table 1). Therefore, this $^{32}$P-labeled virus preparation could be used directly without further purification of the subunit of the defective Ki-SV for fingerprint analysis. For the endogenous rat sarcoma virus RNA, no virus preparation with such a good ratio of sarcoma to helper as Ki-SV(FeLV) is yet available. However, the virus preparation from the HTP cells infected with WoLV contains an approximately 1:1 ratio of the sarcoma virus and WoLV components, and Ki-SV has no appreciable homology with the helper component in this virus preparation (24) (Table 1). A difference in the subunit and dimer size of these two viral RNA components permits partial separation upon sedimentation of the $^{32}$P-labeled RNA in a sucrose gradient (see below). In addition, the HTP culture appears to be more readily labeled with [$^{32}$P]orthophosphate than does the V-NRK culture and has been chosen for this purpose. As a control for the WoLV grown in rat cells, the LTP cell line, derived from the same tumor as the HTP cell line, was used, which does not express any appreciable level of either endogenous rat leukemia or endogenous rat sarcoma virus RNA (Table 1).

To identify the T1 oligonucleotides of the Ki-

TABLE 1. *Sequence homology of sarcoma viruses and helper leukemia viruses*[a]

| Producer cells | Ratio of viruses | Sequence homology | | | | |
|---|---|---|---|---|---|---|
| | | Ki-SV | Endogenous rat "sarcoma" virus | Endogenous rat leukemia virus | WoLV | Ki-MuLV |
| Ki-SV(FeLV) | 20:1 | + | + | − | − | + |
| V-NRK | 1:1 | + | + | + | − | − |
| "Sarcoma" component | | + | + | − | − | − |
| Leukemia component | | − | − | + | − | − |
| HTP(WoLV) | 1:1 | + | + | − | + | NT[b] |
| "Sarcoma" component | | + | + | − | − | NT |
| Leukemia component | | − | − | − | + | NT |
| LTP(WoLV) | 1:50 | − | − | − | + | NT |
| Ki-MuLV | | + | − | − | NT | + |

[a] The sequence homology was examined by cross-hybridization of excess cDNA prepared from each virus to [$^3$H]viral RNA under conditions previously described with S1 nuclease (21). Hybridization levels above the 5% S1 nuclease background were considered to be positive. The virus ratios were determined by hybridization of [$^3$H]cDNA probe ($10^7$ cpm/$\mu$g) specific for each virus to varying concentrations of 50 to 70S viral RNA. Ratios of virus RNA were determined by a comparison of their $C_rt_{1/2}$ values. Data are as previously published (19, 22, 24).

[b] NT, Not tested.

SV RNA homologous to Ki-MuLV and the endogenous rat sarcoma virus RNA, cDNA protection experiments were performed by the scheme shown in Fig. 1. To detect Ki-MuLV homologous sequences in the Ki-SV genome, Ki-SV [$^{32}$P]RNA was protected with cDNA prepared from Ki-MuLV. To insure that all of the homologous sequences were detected, the following titration experiments were performed to determine the appropriate ratios of cDNA to [$^{32}$P]RNA for adequate protection. Ki-SV [$^{32}$P]RNA (1,000 cpm) was hybridized to increasing amounts of Ki-MuLV cDNA (2 ng to 1 $\mu$g) in a stringent hybridization and nuclease S1 digestion assay described above. At the plateau (cDNA/RNA = 20 to 40), 11% of Ki-SV [$^{32}$P]RNA was hybridized with Ki-MuLV cDNA, whereas 70% of input homologous Ki-MuLV [$^{32}$P]RNA was hybridized under the same condition. Thus, by molecular hybridization, 15% (11/70 × 100) of the Ki-SV genome appears to be homologous to the Ki-MuLV genome.

To detect rat sequences in Ki-SV, Ki-SV [$^{32}$P]RNA was protected with cDNA prepared from virus released by V-NRK cells (Fig. 1). The absolute amount of virus produced from the V-NRK culture was somewhat greater than that from HTP(WoLV), and, therefore, it was a more convenient source of virus for synthesizing cDNA. Table 1 shows that the ratio of sarcoma and leukemia components is approximately 1:1, and the rat leukemia component apparently possesses little homology with Ki-SV (24). To identify endogenous rat sarcoma sequences incorporated into Ki-SV, HTP [$^{32}$P]RNA was protected with Ki-SV(FeLV) cDNA. In similar titration experiments described above, this cDNA preparation hybridized to 75% of the input Ki-SV [$^{32}$P]RNA, whereas no detectable hybridization was observed with [$^{32}$P]RNA from the control LTP(WoLV). However, appreciable hybridization (about 20%) was detected in [$^{32}$P]RNA of HTP(WoLV) containing sarcoma RNA. Thus, for above-mentioned experimental reasons, two cultures, HTP and V-NRK, releasing the endogenous rat sarcoma virus RNA were employed to study the sequence relationship. It is worthwhile to mention that they are derived from different strains of rats, HTP from Sprague-Dawley rats and V-NRK from Osborne-Mendel rats, and this may account for small differences observed in fingerprint analysis to be described in the next sections.

**Characterization of the sarcoma virus RNA released from HTP cells.** The endogenous rat sarcoma virus DNA sequences are present in multiple copies in rat cell genomes. One question that we asked is whether the sarcoma virus RNA that is expressed in a given rat cell line is a single and unique viral RNA species. To determine this, we analyzed the viral RNA produced by the HTP cells superinfected with WoLV and as a control analyzed the RNA produced by the LTP cells similarly infected with WoLV. As shown in Fig. 2, the 50 to 70S viral RNA peak from HTP cells (Fig. 2A) sediments more slowly than the viral RNA released from the control LTP cells (Fig. 2B). The fingerprint analysis of the leading edge and the slow edge of the broad 50 to 70S viral RNA peak as compared with that of viral RNA released from the LTP(WoLV) culture is shown in Fig. 3.

Although the two viral RNA components are not clearly separated in the sucrose gradient, a

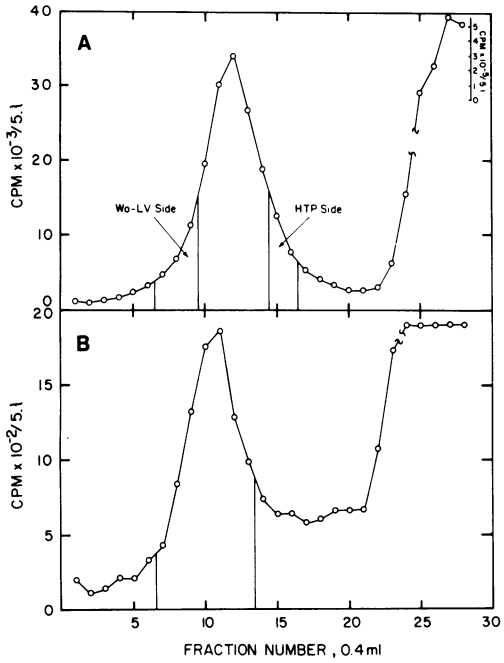


FIG. 2. *Sedimentation profiles of 50 to 70S viral RNA of (A) HTP(WoLV) and (B) LTP(WoLV). The viruses labeled with $^{32}P$ medium were lysed in SDS buffer; [$^{32}P$]RNA was sedimented in a 15 to 30% sucrose gradient in TNE buffer in a Spinco SW41 rotor at 39,000 rpm for 2.5 h at 20°C. (A) (total RNA in the peak region, 16 × $10^6$ cpm) is the profile of the pseudotype of HTP(WoLV) with a ratio of HTP to WoLV of approximately 1:1, and (B) (total RNA, 0.7 × $10^6$ cpm) is the profile of WoLV released from the LTP(WoLV) cells. Both [$^{32}P$]RNA preparations were sedimented in the same rotor, and 0.4-ml fractions were collected from the bottom of the centrifuge tubes.*

fingerprint of the fast side (Fig. 3A) is almost identical to that of WoLV RNA produced by the control LTP(WoLV) (Fig. 3C). The slower side of the HTP(WoLV) culture contains a distinct set of T1 oligonucleotide spots characteristic of the sarcoma virus RNA. By superimposition of Fig. 3B and C, most of the WoLV spots in Fig. 3B that appear to be lighter in intensity as compared with the darker distinctive set of spots can be discerned (Fig. 4). The remaining darker sarcoma virus-specific oligonucleotides are indicated by numbers from 101 to 137, whereas, the WoLV spots are numbered from 1 to 35. Some WoLV spots, such as 103(4), 105(5), 111(4), and 115(19), apparently possess very similar electrophoretic mobilities to those of the sarcoma virus RNA. A consistently darker appearance in the fingerprints of the HTP side and their protection by Ki-SV cDNA suggest that they are HTP specific. The characteristic pattern of HTP-specific oligonucleotides indicates that it is a unique RNA species. The number of large RNase T1-resistant oligonucleotides below the bromophenol blue dye marker (chain length larger than 15 nucleotides) attributable to HTP *src* RNA is 35, which is comparable to 38 spots for Ki-SV. This number is, however, slightly smaller than that of the helper virus with a larger subunit size, i.e., Moloney MuLV (Mo-MuLV), 42 spots; WoLV, 43 spots. If one assumes the random distribution of the guanine residues along the viral genomes, the number of large T1 spots is indicative of the complexity of the viral genome. By comparison with the Mo-MuLV genome complexity of 11,000 nucleotides (3), the complexity of sarcoma viral RNA in HTP cells is estimated to be about 8,000 to 9,000 nucleotides.

**Comparison of the homologous sequences among genomes of Ki-SV, Ki-MuLV, and HTP.** Figure 5 shows the fingerprint of the Ki-SV RNA (Fig. 5A). A faintly visible set of spots, probably due to the helper FeLV RNA or the endogenous mink viral RNA, is visible in addition to the major spots that we attribute to the Ki-SV genome. Figure 5 also shows Ki-SV RNA protected by V-NRK cDNA (Fig. 5B) and that protected by Ki-MuLV cDNA (Fig. 5C). The corresponding T1 oligonucleotides are identified by superimposition of these fingerprints and are summarized in Fig. 6. More than 10 adjacent spots of each oligonucleotide shown in Fig. 5B can be found to overlap well with corresponding spots in total Ki-SV (Fig. 5A). Although the number of spots in Ki-MuLV cDNA-protected oligonucleotides (Fig. 5C) is small, a complete superimposition of spots 4, 22, 25, 31, 37, and an additional three spots above the bromophenol blue dye marker was observed. These results are summarized in Fig. 6. A total of 23 out of 45 (51%) numbered T1 oligonucleotides of Ki-SV RNA were positively identified as homologous to the endogenous rat sarcoma viral RNA, and 5 out of 45 (11%) were derived from Ki-MuLV RNA. The remaining 17 spots were not protected by either cDNA. The percentage of homologous Ki-SV oligonucleotides protected by the corresponding cDNA's is in good agreement with that observed by molecular hybridization, i.e., 51% observed by fingerprinting versus 45% observed by hybridization for the V-NRK sequences and 11% found in fingerprints versus 15% found by hybridization for the Ki-MuLV sequences.

One question that we asked is whether the homologous sequences observed by molecular hybridization between Ki-SV and endogenous sarcoma virus are of identical primary nucleotide sequence. RNase T1 cleaves the phosphodiester linkage at the 3′ side of every guanine residue,

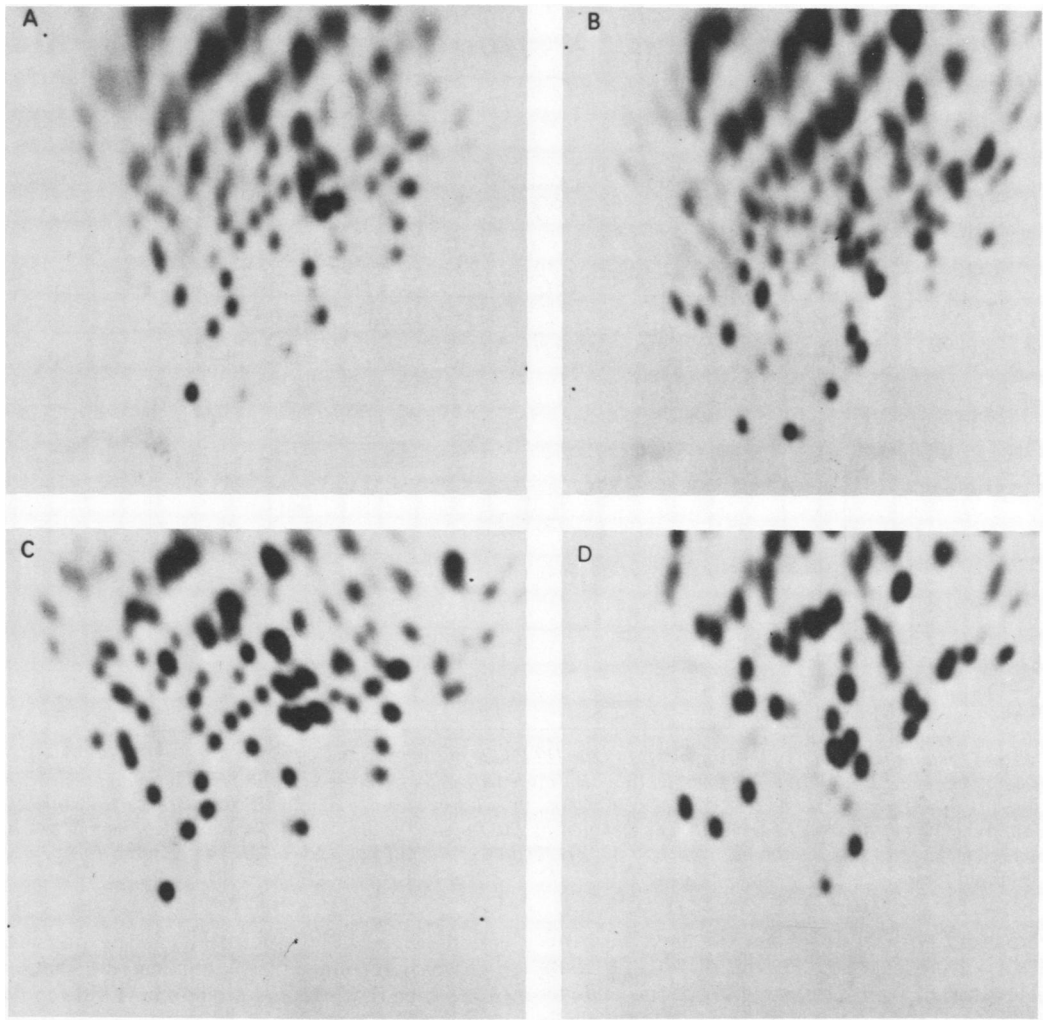


FIG. 3. *Comparison of fingerprints of HTP and helper WoLV. [$^{32}$P]RNA fractions pooled from sucrose gradients shown in Fig. 2 were fingerprinted. (A) WoLV side of the 50 to 70S RNA (1.9 × 10$^6$ cpm) from HTP(WoLV); (B) HTP side of the 50 to 70S RNA (1.5 × 10$^6$ cpm) from HTP(WoLV); (C) 50 to 70S RNA (1.2 × 10$^6$ cpm) from LTP(WoLV), which is essentially WoLV; (D) HTP side of the 50 to 70S RNA from HTP(WoLV) protected by Ki-SV cDNA as described in the text. Ki-SV cDNA (60 μg) was hybridized to the HTP half of the 50 to 70S [$^{32}$P]RNA (6 × 10$^6$ cpm) with a ratio of cDNA to RNA of approximately 40. After RNase digestion and purification, 20% of the [$^{32}$P]RNA (1.2 × 10$^6$ cpm) was protected and recovered for fingerprinting. Autoradiography was for 1 to 3 days.*

thus producing a unique set of large oligonucleotides in fingerprints. If the primary sequences are indeed identical, the fingerprints would be expected to be the same. Figure 7 shows the striking resemblance of the homologous sequences between Ki-SV and endogenous sarcoma virus genomes. In fact, most of the T1 oligonucleotides are superimposable, and the common oligonucleotides are shown in the hatched spots and indicated with the corresponding identification number in Fig. 7. This observation strongly suggests that the primary nucleotide sequence shared by Ki-SV and the endogenous sarcoma virus RNA produced by HTP is almost identical. The small number of spots not common in these two fingerprints may be due to small differences in the Ki-SV homologous sequences of these two strains of sarcoma virus RNA (HTP and V-NRK) or equally possible maybe due to an incomplete representation of viral sequences in the cDNA transcripts.

In summary, these fingerprint studies suggest that: (i) at least 50% of the Ki-SV genome is conserved in a primary nucleotide sequence as

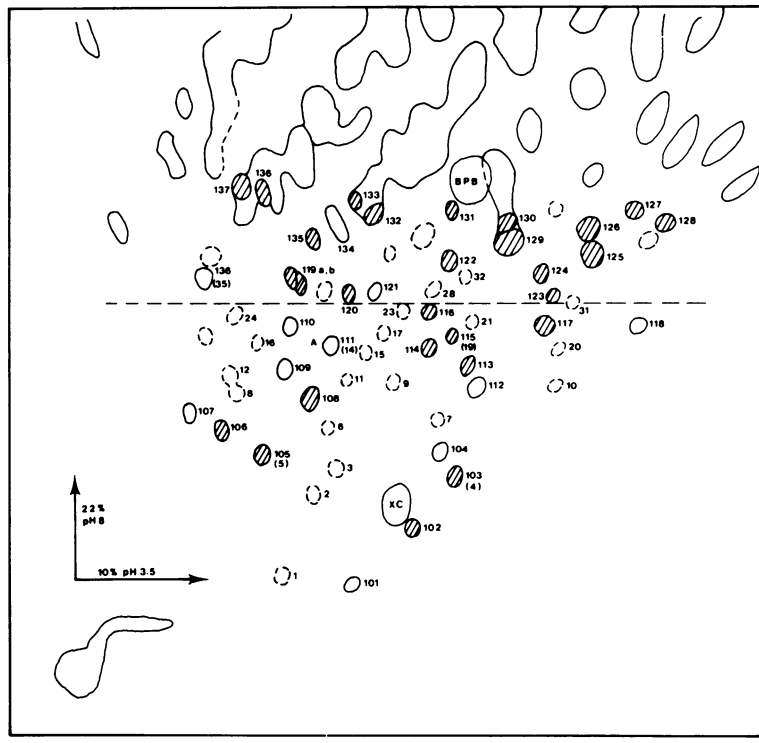


FIG. 4. *Summary sketch of the fingerprint of the HTP side of the 50 to 70S viral RNA from the HTP(WoLV) pseudotype. HTP and WoLV oligonucleotides were identified as described in the text by a comparison of fingerprints shown in Fig. 3. The HTP-specific spots in solid circles are numbered from 101 to 137, and the spots further protected by Ki-SV cDNA are shown by the hatched spots. WoLV-contaminating spots are in broken circles and are numbered from 1 to 35. The origin of all but one spot below the dashed line can be accounted for.*

compared with endogenous rat sarcoma virus RNA; (ii) at least 56% (10 out of 18 HTP spots below the dashed line in Fig. 4) of the endogenous rat sarcoma virus genome in HTP cells is incorporated into Ki-SV; and (iii) about 11% of the Ki-SV genome is homologous to the Ki-MuLV genome.

**Map location of rat endogenous sequences and Ki-MuLV sequences in the Ki-SV genome.** The mapping of large T1 oligonucleotides within the Ki-SV genome was performed by procedures developed by Wang et al. (28) and by Coffin and Billeter (7). It is based on determining the relative distance of each oligonucleotide to the 3′ poly(A) termini of the RNA molecules. Ki-SV [$^{32}$P]RNA naturally or further chemically degraded by mild $Na_2CO_3$ treatment was sedimented in a sucrose gradient (Fig. 8). Pools were made encompassing molecules of ±2 to 3S in size. The poly(A)-containing RNA fragments within each size range were selected by two cycles of oligo(dT)-cellulose chromatography. The poly(A)$^+$ RNA was then fingerprinted after RNase T1 digestion. The location of each oligonucleotide in the physical map was determined by the smallest size fraction from which that particular oligonucleotide could be fully recovered.

The results are shown in Fig. 9. The largest 26S fraction (Fig. 9A) contains all of the oligonucleotides of the unfractionated Ki-SV RNA (Fig. 5A), and it is used as the reference fingerprint for full recovery of each oligonucleotide. It is evident in the 21S fraction (Fig. 9B) that spots 5, 6, 20, 26, 29, 36, and probably 1 are already decreased in relative intensity as compared with the 26S fingerprint (Fig. 9A) and are completely absent in the 16S (Fig. 9C) and smaller fractions. These oligonucleotides seem to be located at the far end of the 5′ termini. On the other hand, the smallest 4S fraction (Fig. 9E) contains only 12 darker spots, presumably located at the very 3′ end close to the poly(A) termini. The relative order of these oligonucleotides can be determined from the relative molar yield of these spots in this two-dimensional gel as shown in Fig. 10. The closer the oligonucleotide is to the poly(A) terminus, the higher the

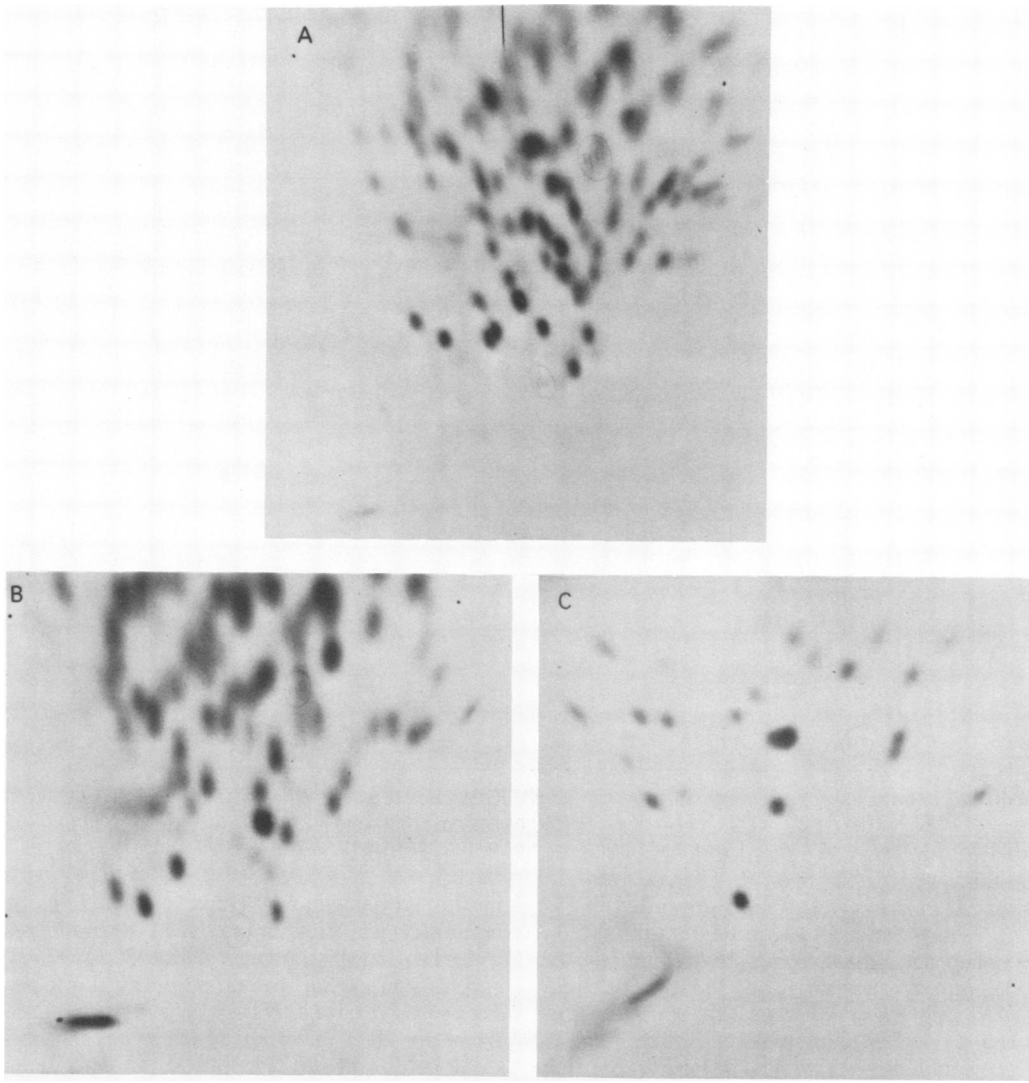


FIG. 5. *Fingerprints of Ki-SV RNA and oligonucleotides protected by hybridization to V-NRK cDNA and Ki-MuLV cDNA. (A) Fingerprint of Ki-SV RNA. Ki-SV [$^{32}$P]RNA (2 × 10$^6$ cpm) was fingerprinted and autoradiographed for 25 h. (B) Fingerprint of Ki-SV RNA protected by V-NRK cDNA. Ki-SV [$^{32}$P]RNA (2 × 10$^6$ cpm with 10 μg of yeast RNA carrier) was hybridized with 62 μg of V-NRK cDNA (ratio of cDNA to RNA of 120). After RNase digestion and purification, 39% of the Ki-SV [$^{32}$P]RNA (0.8 × 10$^6$ cpm) was protected and recovered for fingerprinting. Autoradiography was for 4 days. (C) Fingerprint of Ki-SV RNA protected by Ki-MuLV cDNA. Ki-SV [$^{32}$P]RNA (3 × 10$^6$ cpm) was hybridized to 40 μg of Ki-MuLV cDNA (ratio of cDNA to RNA of 53). After RNase digestion and purification, 10% of the Ki-SV [$^{32}$P]RNA (0.3 × 10$^6$ cpm) was protected and recovered for fingerprinting. Autoradiography was for 2 days.*

yield expected in this size fraction. The relative location of all of the other oligonucleotides is ordered by visual estimation of the yield of each spot in Fig. 9 and another series of similar experiments. As shown in Fig. 11, except for the few spots closest to the poly(A) end that drastically decrease in their molar yield in the 4S fraction, no attempt was made to further place the relative order within each bracket. The unique relationship of oligonucleotides to the poly(A) end of the RNA molecule indicates that there is no large circular permutation of the viral genome, an observation similar to that documented in the avian sarcoma viruses (7, 28).

Oligonucleotides protected by either V-NRK cDNA (rat-specific spots) or Ki-MuLV cDNA (mouse-specific spots) seen in Fig. 5 and 6 are

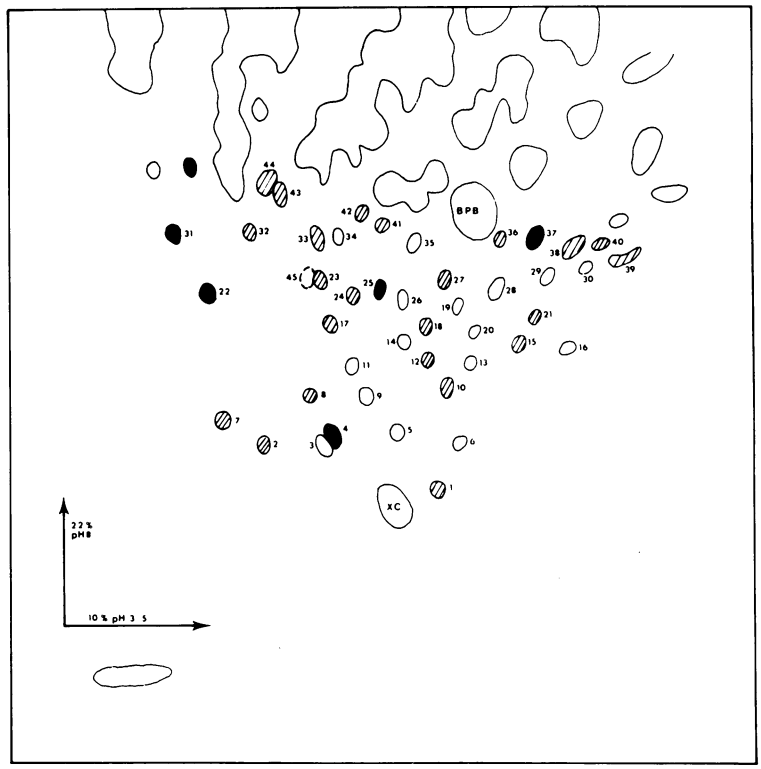


FIG. 6. *Summary sketch of the fingerprints of Ki-SV RNA. The oligonucleotides of Ki-SV RNA protected either by V-NRK cDNA (▨) or by Ki-MuLV cDNA (●) were identified as described in the text by a comparison of fingerprints shown in Fig. 5. Open spots are those not protected by any of these two cDNA's.*

indicated in the physical map presented in Fig. 11. The most interesting finding is that the oligonucleotides closest to the poly(A) end, i.e., 22, 4, 31, and 25, are all protected by Ki-MuLV cDNA. The one in between (no. 28) is not protected by either Ki-MuLV cDNA or V-NRK cDNA and may very well be of Ki-MuLV origin but differ slightly in its nucleotide sequence. Thus, out of 45 numbered oligonucleotides, these 5 oligonucleotides account for approximately 11% of the genome, consistent with the 15% hybridization of Ki-SV [$^{32}$P]RNA with excess Ki-MuLV cDNA. The closest rat sequences start from this junction at no. 25 and 33, and rat-specific spots extend intermittently all the way to the very 5′ end of the Ki-SV genome. The mouse-rat junction at the 3′ end appears to be located at approximately 11% of the genome from the poly(A) termini, i.e., close to 1,000 nucleotides.

**Differences between sarcoma-virus-specific sequences of Ki-SV, Ha-SV, V-NRK, and HTP.** To explore the relationship of the sarcoma virus specific sequences in Ki-SV, Ha-SV, V-NRK, and HTP and to explain the incomplete protection of all of the T1 oligonucleotides in the Ki-SV genome (Fig. 11), the sequence homology was further analyzed by molecular hybridization as shown in Table 2. An Ha-SV specific [$^{3}$H]cDNA was prepared by the following procedure. First, Ha-SV cDNA was prepared from an Ha-SV(FeLV) pseudotype grown in mink cells, and cat- and Mo-MuLV-specific sequences were removed by adsorption of the cDNA on hydroxyapatite after hybridization with Mo-MuLV RNA and FeLV RNA by the procedure previously described (20). The Ki-SV-specific [$^{3}$H]cDNA was prepared similarly from the Ki-SV(FeLV) culture, except that Ki-MuLV and FeLV RNA were used to remove the helper sequences. The [$^{3}$H]cDNA specific for Ha-SV or Ki-SV was then hybridized to RNA from cells transformed by either Ki-SV or Ha-SV or to RNA from HTP or V-NRK. As shown in Table 2, the [$^{3}$H]cDNA specific for Ha-SV hybridized completely to RNA from cells transformed by it, whereas this cDNA hybridized only incompletely to RNA of Ki-SV-transformed cells or HTP or V-NRK. The same was also true for cDNA specific for Ki-SV. A change in melting temperature ($\Delta T_m$) of 2 to 2.5°C was also observed for these incomplete hybrids.

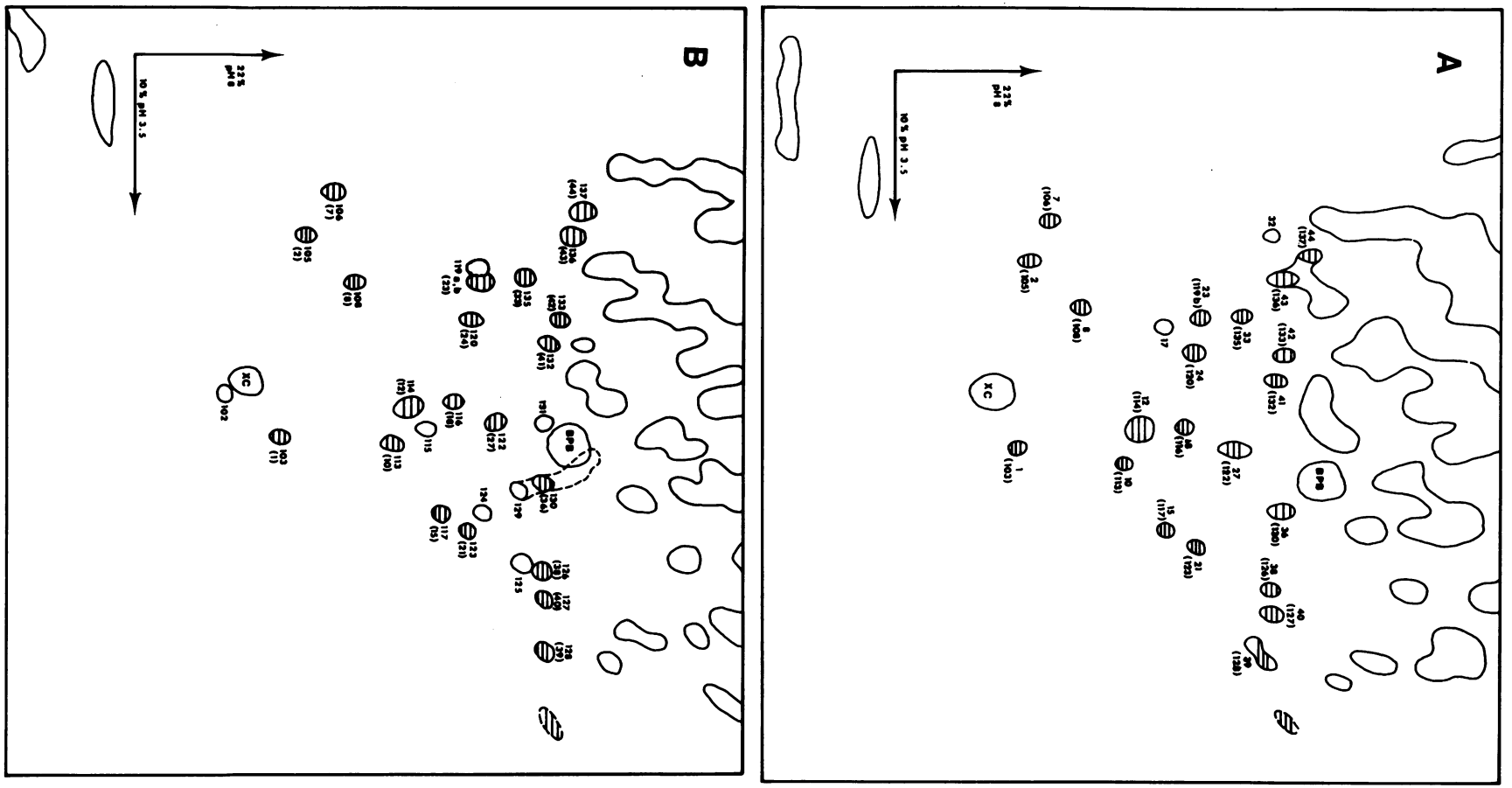


FIG. 7. *Comparison of the fingerprints of Ki-SV [$^{32}$P]RNA protected by V-NRK cDNA and HTP [$^{32}$P]RNA protected by Ki-SV cDNA. (A) Sketch of the fingerprint of Ki-SV [$^{32}$P]RNA protected by V-NRK cDNA shown in Fig. 5; (B) sketch of the fingerprint of HTP [$^{32}$P]RNA protected by Ki-SV cDNA shown in Fig. 3D. Oligonucleotide spots that appear to be common in these two fingerprints are shown in the hatched spots with the corresponding identification numbers.*

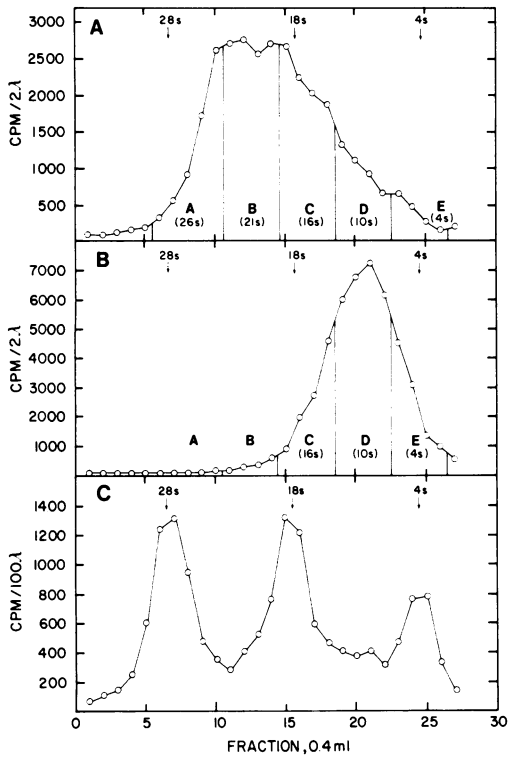


FIG. 8. *Sedimentation profiles of the Ki-SV [$^{32}$P]-RNA fragments from which poly(A)$^+$ RNA was selected. Three RNA samples were sedimented in 10 to 25% sucrose gradients (0.01 M Tris [pH 7.2]-0.01 M NaCl-1 mM EDTA-0.1% SDS) at 40,000 rpm in the same SW41 rotor (20°C) for 7 h. (A) Ki-SV [$^{32}$P]-RNA (14 × 10$^6$ cpm) naturally degraded. (B) Ki-SV [$^{32}$P]RNA (16 × 10$^6$ cpm) further degraded by $Na_2CO_3$ treatment (0.05 M at 50°C for 6 min). (C) [$^3$H]-rRNA marker prepared from mouse cells. RNA in each indicated size fraction (4 to 6s range) were pooled and purified by two cycles of oligo(dT)-cellulose chromatography as described in the text. The s value of the midpoint in each pool is indicated in this figure. The recovery of the poly(A)$^+$ RNA from oligo(dT)-cellulose columns is as follows: fraction A (26s), 73%; fraction B (21s), 60%; fraction C (16s), 45%; fraction D (10s), 29%; fraction E (4s), 22%.*

These results suggest that homologous sarcoma-specific sequences among these viruses are not completely the same and mismatched sequences are susceptible to S1 nuclease, i.e., 40 to 45% resistant versus 100% for homologous hybrids (Table 2). Additional evidence suggesting that these mismatched sequences occur diffusely is the failure to absorb out completely these incomplete hybrids by a hydroxyapatite procedure used to fractionate the [$^3$H]cDNA probes (20). For example, repeated attempts to cross-absorb the Ha-SV cDNA with Ki-SV-transformed cell RNA to prepare a probe from Ha-SV that would not hybridize to either Ki-SV, HTP, or V-NRK were unsuccessful. The same was also true for Ki-SV cDNA.

In conclusion, the region of Ki-SV genome homologous to Ha-SV or the endogenous rat sarcoma virus RNA may represent the entire region starting from the mouse-rat junction at 1,000 nucleotides from the 3′ poly(A) end and extending to the very 5′ end of the molecules (Fig. 12). In this homologous region, the mismatched sequences occur throughout the genome, and the 50% identical nucleotide sequences suggested by fingerprint analysis represent sequences of "diffuse homology" within the genomes.

## DISCUSSION

Many earlier studies conclude that recombinant genomes of Ki-SV and Ha-SV are composed of two distinctive sets of sequences; one derived from leukemia viruses used for sarcoma virus isolation and another set of sequences derived from a novel endogenous rat sarcoma virus (2, 19, 21, 24). The present fingerprint studies reveal that at least 50% of the Ki-SV genome appears to be conserved in primary nucleotide sequences as compared with the genome of the endogenous sarcoma virus. The close similarity of these viral genomes strongly indicates that Ki-SV arose by the direct recombination of Ki-MuLV RNA and an endogenous rat *src* RNA similar to that found in HTP cells.

The organization of rat and mouse sequences in Ki-SV is such that almost all of the Ki-MuLV homologous sequences are at the 3′ end immediately next to the poly(A) termini of RNA molecules. This part of the Ki-MuLV homologous sequences includes 4 (or 5) large oligonucleotides, representing a stretch of about 1,000 nucleotides from the poly(A) end. The sequences homologous to the rat endogenous sarcoma virus RNA, however, extend from this distance continuously all the way close to the 5′ termini of Ki-SV RNA molecules. Since the "strong stop" cDNA (about 135 nucleotides) copied from Ki-SV RNA, starting at the tRNA primer located close to the 5′ end and presumably terminating at the 5′ termini of the RNA molecules, contains both Ki-MuLV and endogenous rat sarcoma virus sequences (Young et al., manuscript in preparation), it is very likely that a small stretch of Ki-MuLV sequence terminally redundant with that at the 3′ end of the molecules is located at the 5′ terminus of the Ki-SV genome. In Rous sarcoma virus, the terminally redundant sequence is an identical sequence of 21 nucleotides at both ends (8, 12, 18). In Mo-MuLV, an analogous redundant sequence of about 50 nucleotides has been found (J. M. Coffin and W. A. Haseltine, submitted for publication). The or-

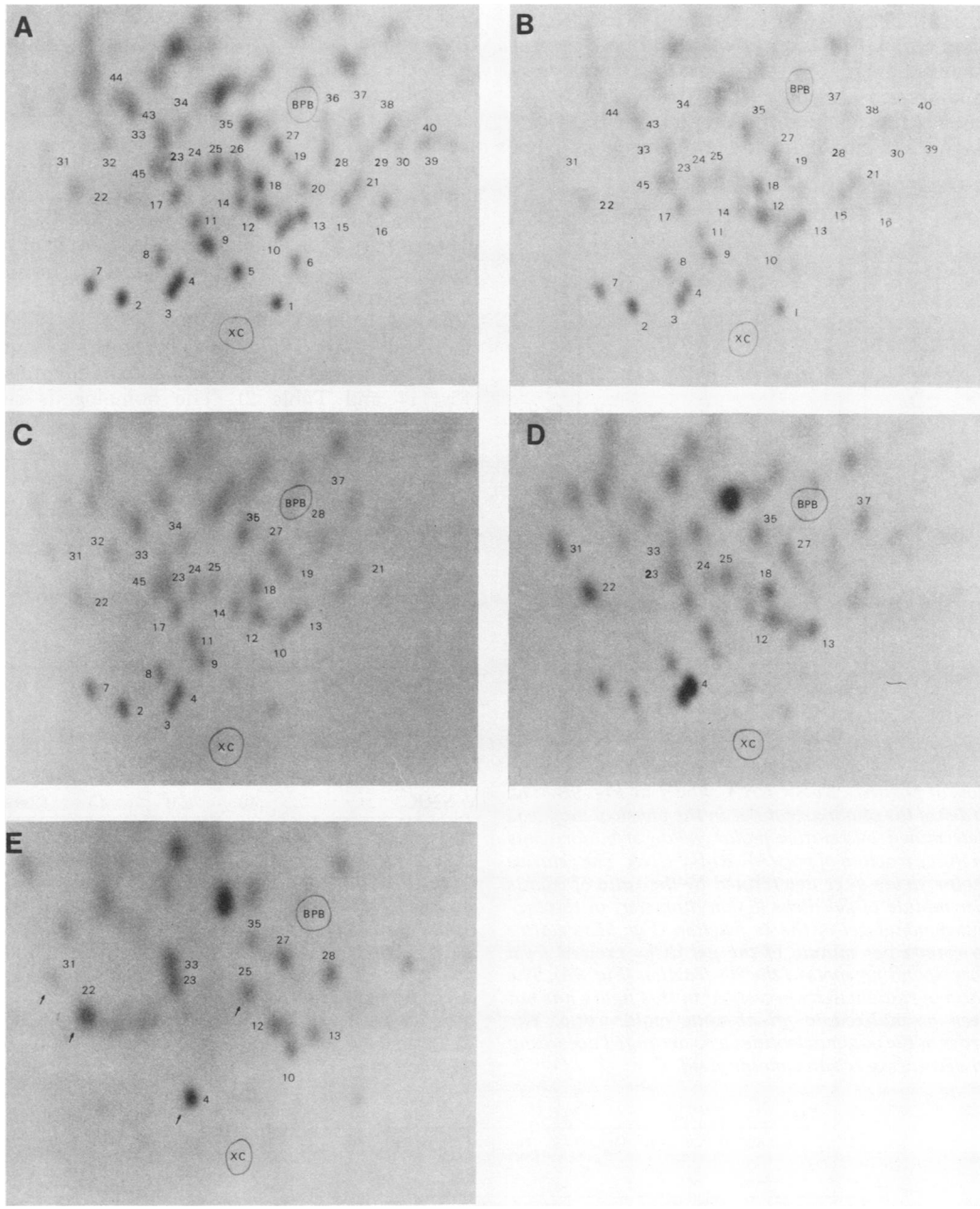


FIG. 9. *Fingerprints of poly(A)⁺ Ki-SV RNA of different sizes. poly(A)⁺ RNA twice purified by oligo(dT)-cellulose chromatography as shown in Fig. 8 was fingerprinted. (A) 26s fraction (530,000 cpm); (B) 21s fraction (900,000 cpm); (C) 16s fraction (800,000 cpm); (D) 10s fraction (650,000 cpm); (E) fraction (300,000 cpm). Autoradiography was for 24 to 48 h. Oligonucleotides that appear to be fully recovered in each fingerprint are numbered. Arrows in (E) indicate mouse-specific spots.*

ganization of the Ki-SV genome is depicted in Fig. 12.

One mechanism by which Ki-SV could have arisen is seen in Fig. 12. In a very rare event during the course of reverse transcription of Ki-MuLV RNA, the cDNA was copied from the 5′ end of Ki-MuLV RNA and, after jumping the gap, was copied from the 3′ end (8). At a point, approximately 1,000 nucleotides, reverse transcriptase switched to copy endogenous sarcoma

virus RNA instead of Ki-MuLV RNA. The finished circular proviral DNA would be a recombinant of most of the endogenous sarcoma virus genome and a small part of the Ki-MuLV genome. After opening the circular proviral DNA at the 5'-3' junction and integrating into the host cell chromosome, the transcribed viral RNA would be the progenitor of Ki-SV. However, the possibility that the recombination event occurred at the level of proviral DNA either in the free or in the integrated state cannot be excluded from the available evidence. Although the endogenous rat sarcoma virus is not readily transmissible to most host cells, acquisition of Ki-MuLV termini within the Ki-SV genome may thus enable a Ki-SV to be readily replicated in a variety of host cells with the aid of helper type C viruses.

Although Ki-SV shares 50% of its T1 oligonucleotides with HTP RNA, the entire homologous sequences are not completely identical (Fig. 11 and Table 2). The homologous sequences appear to represent portions of identical

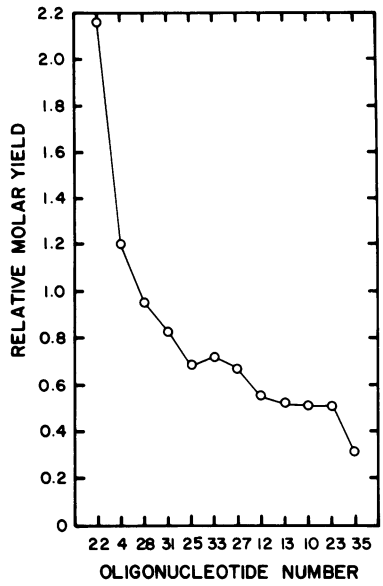


FIG. 10. *Order of oligonucleotides in the 4s fraction of poly(A)⁺ Ki-SV RNA shown in Fig. 9E. The order of the oligonucleotides in the physical map was determined by relative molar yields of major spots in the 4s fraction of poly(A)⁺ Ki-SV RNA. The relative molar yields were determined by the ratio of counts per minute of gel disks (8-mm diameter) in the two-dimensional gel of the 4s fraction (Fig. 9E) relative to counts per minute of the gel disks excised from corresponding spots of the 26s fraction (Fig. 9A). The ratio of radioactivity presented in this figure has not been normalized to an absolute molar ratio. The order of the oligonucleotides was arranged according to decreasing relative molar yield.*

TABLE 2. *Differences between sarcoma virus-specific sequences of Ki-SV and Ha-SV*[a]

| RNA Tested | Absorbed [³H]cDNA of: | | | |
|---|---|---|---|---|
| | Ha-SV | | Ki-SV | |
| | % Hybridization | $\Delta T_m$ | % Hybridization | $\Delta T_m$ |
| Ki-SV (mink) | 40 | 2.5 | 100 | |
| Ki-SV (mouse) | 40 | 2.5 | 100 | |
| Ha-SV (dog) | 100 | | 45 | 2.0 |
| Ha-SV (mouse) | 100 | | 45 | 2.0 |
| HTP | 45 | 2.0 | 45 | 2.0 |
| V-NRK | 45 | 2.0 | 45 | 2.0 |

[a] [³H]cDNA's were prepared from either Ha-SV(FeLV) subgroup C or Ki-SV(FeLV) subgroup C grown in mink cells. The Ki-SV(FeLV) probe was absorbed with Ki-MuLV and FeLV RNA, and the Ha-SV(FeLV) probe was absorbed with Mo-MuLV and FeLV RNA. The resulting cDNA's were hybridized to saturating levels of RNA from the indicated cells, and hybridization was determined with the use of S1 nuclease (21). The source of cells and the method of $T_m$ analysis have been described previously (5, 19, 24).

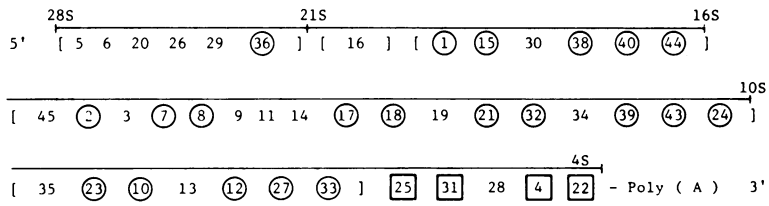


FIG. 11. *Physical map of large T1 oligonucleotides of the Ki-SV 70S genome. The physical map was deduced from fingerprints of poly(A)⁺ RNA fragments of various sizes as shown in Fig. 9 and another similar series of experiments. The location of each oligonucleotide in the physical map was determined by the smallest size fraction from which that particular oligonucleotide could be fully recovered; e.g., oligonucleotides present in the 26S fraction but not in the 21S fraction were placed between the 28S and 21S marks. The relative order of the last 12 oligonucleotides was determined by the increasing relative molar yield seen in Fig. 10. Squares indicate oligonucleotides protected by Ki-MuLV cDNA, and circles are those protected by V-NRK cDNA (see also Fig. 6).*

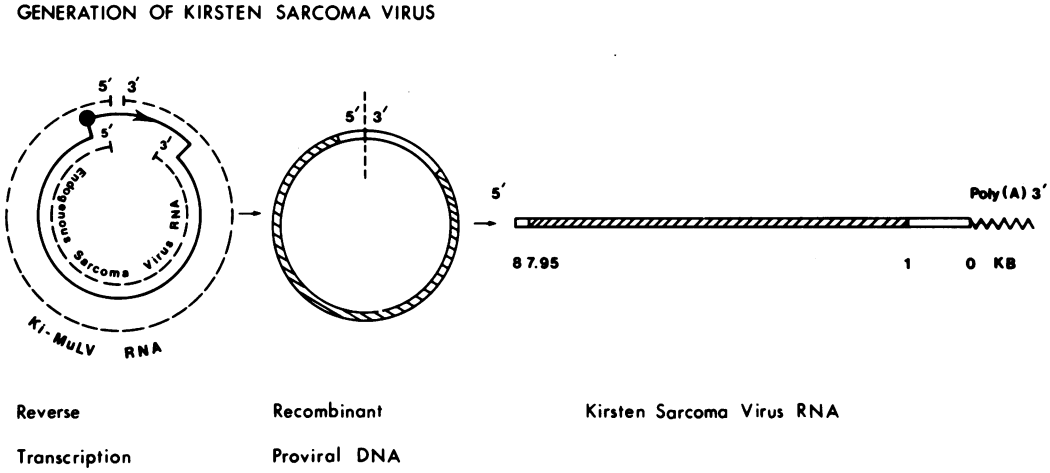


FIG. 12. *Physical map and a possible mechanism for the generation of the Ki-SV genome. For details, see text.*

sequences with many intervening mismatched sequences. The following three possibilities can be considered for explaining the observed sequence divergence. (i) These sarcoma virus sequences are derived independently from different strains of rats, i.e., Ki-SV from Wistar-Furth rats, Ha-SV from Chester-Beatty rats, HTP from Sprague-Dawley rats, and V-NRK from Osborne-Mendel rats. Strain divergence of gene sequences may account for the viral RNA sequence differences. (ii) These sarcoma virus sequences may represent transcripts of different copies of the family of closely related 20 to 40 copies found in rat cell genome in different strains of rat and different cell lines (19). (iii) Some sequence divergence may be the consequence of long serial passage of the transmissible sarcoma viruses in the laboratory. In Ki-SV genomes, the origin of sequences other than those derived from Ki-MuLV and endogenous rat sarcoma virus RNA has not been rigorously ruled out by the present data. However, the clustering of the unprotected spots close to the 5′ end may reflect the inadequate representation of cDNA as presently prepared from the endogenous reverse transcriptase reaction.

It is of interest to speculate whether sarcomagenicity of Ki-SV is a direct consequence of the endogenous rat sarcoma virus sequence in Ki-SV genome, or whether recombination of rat and Ki-MuLV sequences at the 3′ end of the RNA molecules creates the *src* gene. The AKR-MCF virus is an intragenic recombinant of AKR N-tropic and xenotropic viruses involving the *env* gene region (10). Ki-MuLV sequences at the 3′ end of the Ki-SV genome suggest the possibility of the formation of Ki-SV in a manner analogous to the formation of AKR-MCF virus, possibly involving a portion of the *env* gene region of Ki-MuLV and the corresponding genetic region of the endogenous rat sarcoma virus.

Finally, the present study also sheds light on the origin of polypeptide synthesized from Ki-SV RNA in in vitro translation experiments (17; Scolnick et al., manuscript in preparation). Since there are rat sequences at the 5′ end of the Ki-SV genome and no appreciable mouse sequences in this region, the results indicate that protein(s) translated in vitro must be translated from rat sequences of Ki-SV and that this approach can provide a protein marker(s) for rat sequences of Ki-SV.

## ACKNOWLEDGMENTS

We thank William Vass, David Williams, and Maureen Weeks for excellent technical assistance.

This work was supported by the Virus Cancer Program of the National Cancer Institute and Public Health Service grant CA 17659 from the National Cancer Institute to J.M.C.

## LITERATURE CITED

1. **Aaronson, S. A., and C. Weaver.** 1971. Characterization of murine sarcoma virus (Kirsten) transformation of mouse and human cells. J. Gen. Virol. **13**:245–252.
2. **Anderson, G. R., and K. C. Robbins.** 1976. Rat sequences of the Kirsten and Harvey murine sarcoma virus genomes: nature, origin, and expression in rat tumor RNA. J. Virol. **17**:335–351.
3. **Beemon, K. L., A. J. Faras, A. T. Haase, P. H. Duesberg, and J. E. Maisel.** 1976. Genomic complexities of murine leukemia and sarcoma, reticuloendotheliosis, and visna viruses. J. Virol. **17**:525–537.
4. **Benveniste, R. E., and E. M. Scolnick.** 1973. RNA in mammalian sarcoma virus transformed nonproducer cells homologous to murine leukemia virus RNA. Virology **51**:370–382.
5. **Benveniste, R. E., and G. J. Todaro.** 1974. Evolution of type-C viral genes. I. Nucleic acid from baboon type-

C virus as a measure of divergence among primate species. Proc. Natl. Acad. Sci. U.S.A. **71**:4513–4518.

6. **Billeter, M. A., J. T. Parsons, and J. M. Coffin.** 1974. The nucleotide sequence complexity of avian tumor virus RNA. Proc. Natl. Acad. Sci. U.S.A. **71**:3560–3564.
7. **Coffin, J. M., and M. A. Billeter.** 1976. A physical map of the Rous sarcoma virus genome. J. Mol. Biol. **100**:293–318.
8. **Coffin, J. M., and W. A. Haseltine.** 1977. Terminal redundancy and the origin of replication of Rous sarcoma virus RNA. Proc. Natl. Acad. Sci. U.S.A. **74**:1908–1912.
9. **DeWachter, R., and W. Fiers.** 1972. Preparative two-dimensional polyacrylamide gel electrophoresis of $^{32}P$ labeled RNA. Anal. Biochem. **49**:184–197.
10. **Hartley, J. W., N. K. Wolford, L. J. Old, and W. P. Rowe.** 1977. A new class of murine leukemia virus associated with development of spontaneous lymphomas. Proc. Natl. Acad. Sci. U.S.A. **74**:789–792.
11. **Harvey, J. J.** 1964. An unidentified virus which causes the rapid production of tumors in mice. Nature (London) **204**:1104–1105.
12. **Haseltine, W. A., A. M. Maxam, and W. Gilbert.** 1977. Rous sarcoma virus genome is terminally redundant: the 5′ sequence. Proc. Natl. Acad. Sci. U.S.A. **74**:989–993.
13. **Kirsten, W. H., and L. A. Mayer.** 1967. Morphologic responses to a murine erythroblastosis virus. J. Natl. Cancer Inst. **39**:311–335.
14. **Leong, J. A., A. C. Garapin, N. Jackson, L. Fanshier, W. Levinson, and J. M. Bishop.** 1972. Virus-specific RNA in cells producing Rous sarcoma virus: detection and characterization. J. Virol. **9**:891–903.
15. **Levy, J. A.** 1973. Xenotropic viruses: murine leukemia virus associated with NIH Swiss, NZB and other mouse strains. Science **182**:1151–1153.
16. **Maisel, J., M. Klement, M. C. Lai, W. Ostertag, and P. H. Duesberg.** 1973. RNA components of murine sarcoma and leukemia viruses. Proc. Natl. Acad. Sci. U.S.A. **70**:3536–3540.
17. **Parks, W. P., and E. M. Scolnick.** 1977. In vitro translation of Harvey murine sarcoma virus RNA. J. Virol. **22**:711–719.
18. **Schwartz, D. E., P. C. Zamecnik, and H. L. Weith.** 1977. Rous sarcoma virus genome is terminally redundant: the 3′ sequence. Proc. Natl. Acad. Sci. U.S.A. **74**:994–998.
19. **Scolnick, E. M., R. J. Goldberg, and D. Williams.** 1976. Characterization of rat genetic sequences of Kirsten sarcoma virus: distinct class of endogenous rat type C viral sequences. J. Virol. **18**:559–566.
20. **Scolnick, E. M., R. S. Howk, A. Anisowicz, P. T. Peebles, D. D. Scher, and W. P. Parks.** 1975. Separation of sarcoma virus-specific and leukemia virus-specific genetic sequences of Moloney sarcoma virus. Proc. Natl. Acad. Sci. U.S.A. **72**:4650–4654.
21. **Scolnick, E. M., J. M. Maryak, and W. P. Parks.** 1974. Levels of rat cellular RNA homologous to either Kirsten sarcoma virus or rat type-C virus in cell lines derived from Osborne-Mendel rats. J. Virol. **14**:1435–1444.
22. **Scolnick, E. M., and W. P. Parks.** 1974. Harvey sarcoma virus: a second murine type C sarcoma virus with rat genetic information. J. Virol. **13**:1211–1219.
23. **Scolnick, E. M., E. Rands, D. Williams, and W. P. Parks.** 1973. Studies on the nucleic acid sequences of Kirsten sarcoma virus: a model for formation of a mammalian RNA-containing sarcoma virus. J. Virol. **12**:458–463.
24. **Scolnick, E. M., D. Williams, J. Maryak, W. Vass, R. J. Goldberg, and W. P. Parks.** 1976. Type C particle-positive and type C particle-negative rat cell lines: characterization of the coding capacity of endogenous sarcoma virus-specific RNA. J. Virol. **20**:570–582.
25. **Sekiya, S., H. Takamizawa, F. Wang, T. Takane, and T. Kuwata.** 1972. In vivo and in vitro studies on uterine adenocarcinoma of the rat induced by 7,12-dimethylbenz[A] anthracene. Am. J. Obstet. Gynecol. **113**:691–695.
26. **Taylor, J. M., R. Illmensee, and J. Summers.** 1976. Efficient transcription of RNA into DNA by avian sarcoma virus polymerase. Biochim. Biophys. Acta **442**:324–330.
27. **Tsuchida, N., R. V. Gilden, and M. Hatanaka.** 1974. Sarcoma-virus related RNA sequences in normal rat cells. Proc. Natl. Acad. Sci. U.S.A. **71**:4503–4507.
28. **Wang, L. H., P. Duesberg, K. Beemon, and P. K. Vogt.** 1975. Mapping RNase $T_1$-resistant oligonucleotides of avian tumor virus RNAs: sarcoma-specific oligonucleotides are near the poly(A) end and oligonucleotides common to sarcoma and transformation-defective viruses are at the poly(A) end. J. Virol. **16**:1051–1070.