JOURNAL OF VIROLOGY, May 1977, p. 361-372
Vol. 22, No. 2
Copyright © 1977 American Society for Microbiology
Printed in U.S.A.

Friend Strain of Spleen Focus-Forming Virus: a Recombinant Between Mouse Type C Ecotropic Viral Sequences and Sequences Related to Xenotropic Virus

DAVID H. TROXLER,* JANICE K. BOYARS, WADE P. PARKS, AND EDWARD M. SCOLNICK

Laboratory of Tumor Virus Genetics, National Cancer Institute, Bethesda, Maryland 20014

Received for publication 14 January 1977

The genome of the Friend strain of the spleen focus-forming virus (SFFV) has been analyzed by molecular hybridization. SFFV is composed of genetic sequences homologous to Friend type C helper virus (F-MuLV) and SFFV-specific sequences not present in F-MuLV. These SFFV-specific sequences are present in both the Friend and Rauscher strains of murine erythroleukemia virus. The SFFV-specific sequences are partially homologous to three separate strains of mouse xenotropic virus but not to several cloned mouse ecotropic viruses. Thus, the Friend strain of SFFV appears to be a recombinant between a portion of the F-MuLV genome and RNA sequences that are highly related to murine xenotropic viruses. The implications of the acquisition of the xenotropic virus-related sequences are discussed in relation to the leukemogenicity of SFFV, and a model for the pathogenicity of other murine leukemia-inducing viruses is proposed.

To study the mechanisms by which RNA tumor viruses produce malignancies in vertebrate species, extensive studies have been done on the nucleic acid sequences of fibroblast-transforming (FT^+) RNA tumor viruses isolated from both avian (35, 39, 40) and mammalian (7, 28-31) hosts. In the avian system, genetic (39) and molecular experiments (35, 40) have indicated that the Rous sarcoma virus contains a set of RNA sequences, designated "sarc," which plays an important role in the ability of the virus to transform cells. This FT^+ virus apparently arose by recombination between an avian type C leukosis virus and endogenous sarc sequences present in the DNA of all avian species (35). Similar analyses by nucleic acid hybridization have also been performed on several replication-defective FT^+ viruses isolated from rodent species (7, 28-32). These studies have indicated that these FT^+ viruses are also recombinants between portions of murine type C viral genomes and distinct sequences which are specific for sarcoma virus. For example, the Harvey and Kirsten strains of murine sarcoma virus (HaSV and KiSV, respectively), originally isolated by passage of murine leukemia virus (MuLV) through rats (13, 15), are recombinants between murine type C viruses and a special class of endogenous rat type C genetic sequences (32). Similarly, the Moloney sarcoma virus, derived by passage of Moloney MuLV through BALB/c mice (21), seems to be a recombinant between a portion of Moloney MuLV and a distinct set of sequences that are partially homologous to sequences present in normal mouse cellular DNA (7, 31).

To extend the concepts learned from such studies on murine FT^+ viruses to the study of the leukemia-inducing potential of MuLV's, we chose to study the genomes of the Friend (8) and Rauscher (24) strains of murine erythroleukemia virus by molecular hybridization. These viruses do not transform fibroblasts in cell culture, but when they are injected as pseudotypes into susceptible animals, each causes abundant splenic foci, rapid splenomegaly, and fatal erythroleukemia. Like the murine helper virus-sarcoma virus complexes, the Friend virus complex (which has been studied in greater detail than the Rauscher virus complex) contains two discrete viral components (33): (i) a replicating murine type C virus, designated F-MuLV, or LLV-F, which causes a lymphatic leukemia after latent intervals of several months after inoculation into weanling mice and provides helper function for defective spleen focus-forming virus (SFFV; 34); and (ii) SFFV (2), which is defective for replication and responsible for the rapid malignant transformation of hematopoietic precursor cells in vivo.

Recently we reported for the first time the clonal isolation by cell culture techniques of the Friend strain of SFFV free of replicating MuLV in nontransformed, nonproducer BALB/c 3T3 cells (38). After the isolation of this SFFV-containing nonproducer cell, the current studies

were undertaken to characterize RNA sequences present in the genome of the Friend strain of SFFV. Based on prior studies (30, 31) characterizing the genomes of KiSV and HaSV, we considered it mandatory to isolate the SFFV genome in nonproducer cells of a heterologous species rather than in cells of murine origin, so that we would be able to distinguish sequences contained in SFFV from potentially similar endogenous type C viral sequences present in mouse cells. Thus, we have isolated several rat cell clones containing SFFV in the absence of replicating MuLV. Using these SFFV-containing rat cells, we have demonstrated that the Friend strain of SFFV is a recombinant virus containing at least two sets of ribonucleotide sequences. One portion of the SFFV genome is homologous to a part of replicating F-MuLV; a second portion of the SFFV genome is not contained in replicating F-MuLV and has a high degree of homology to mouse xenotropic viruses. The implications of these results are discussed with regard to: (i) the analogy between the genomes of KiSV, HaSV, and SFFV and (ii) a model for the leukemia-inducing potential of SFFV as well as for other strains of leukemia-inducing viruses derived from mice.

MATERIALS AND METHODS

Cells and viruses. All cells were grown in Dulbecco-modified Eagle medium containing either 10% calf serum or 10% fetal calf serum. In general, all mouse and rat cells were grown in medium containing 10% calf serum, and all other cells were grown in medium containing 10% fetal calf serum. All cells were assayed for mycoplasma species by aerobic and anaerobic techniques (Flow Laboratories, Rockville, Md.) and found to be negative. The following sets of cells were utilized.

(i) Uninfected cells. The sources of BALB/c 3T3 (1), NIH 3T3 (14), and SC-1 mouse embryo fibroblasts (11), permissive for both N- and B-tropic murine type C viruses, have been previously reported by the investigators who originally established these cell lines. Fischer rat embryo (FRE clone 2) cells, normal rat kidney (NRK) cells, and a virus-producing subclone of NRK cells (V-NRK) have also been previously described (32). A mink lung fibroblast cell (ccl 64) and a dog kidney cell line (ccl 34) were obtained from the American Type Culture Collection, Rockville, Md.

(ii) Sarcoma virus-transformed nonproducer cells. A canine cell nonproductively transformed by HaSV (designated Ha-MDCK), a mink cell nonproductively transformed by KiSV, and a dog cell nonproductively transformed by the p30+ strain of Moloney sarcoma virus (designated $S^{+}L^{-}$ dog) have been described in an earlier publication (29). A mink lung fibroblast nonproductively infected with the $S^{+}L^{-}$ strain of Moloney sarcoma virus, designated $S^{+}L^{-}$ mink (23), was also used.

(iii) Cells infected with ecotropic MuLV and biologically found not to contain SFFV. Three strains of N-tropic MuLV were used in these studies: (i) the Kirsten strain of murine type C virus (Ki-MuLV) (31) isolated from C3H mice; (ii) a strain of MuLV obtained from AKR 2B cells (AKR-MuLV) (26); and (iii) an N-tropic virus derived from the spleen of a BALB/c mouse (12), designated WN1802N.

Three sources of B-tropic MuLV were used and grown on SC-1 cells: (i) a B-tropic virus obtained from the spleen of a BALB/c mouse, designated WN1802B (12); (ii) a B-tropic virus cloned as previously described (38) from a stock of the B-tropic pseudotype of SFFV produced by Lilly and Steeves (19), designated MuLV clone 68; and (iii) a B-tropic MuLV designated B/T-LV clone A3, which was cloned from a stock of the Balb/Tennant leukemia virus (B/T-LV) obtained from the American Type Culture Collection.

Finally, three strains of ecotropic MuLV with NB-tropic host ranges were used. The Friend strain of MuLV (F-MuLV) and the Rauscher strain of MuLV (R-MuLV) were propagated on NRK and SC-1 cells after obtaining each MuLV free from SFFV in the original preparations by cloning on SC-1 cells as described below. An NB-tropic virus was obtained by forced passage of the WN1802B virus in NIH 3T3 cells by procedures which have been previously described (13); this virus was designated WN1802NB.

(iv) Cells producing ecotropic MuLV and biologically found to contain SFFV. Several BALB/c 3T3 clones used in these studies were obtained from a previously reported experiment in which an SFFV-containing nonproducer BALB/c 3T3 cell was isolated (38). These clones, previously referred to as 1902B clone 1, 4, etc., are designated Balb clone 1, 4, etc., in this paper. Balb clone 9 and Balb clone 13 are clones of BALB/c 3T3 cells that release both B-tropic MuLV and SFFV. Balb clone 4/F-MuLV was obtained by superinfecting Balb clone 4, an SFFV-containing nonproducer cell, with cloned F-MuLV. In contrast to these BALB/c 3T3 clones which release SFFV are uninfected BALB/c 3T3 cells which were infected with F-MuLV to produce a culture designated BALB/F-MuLV and Balb clone 7 and Balb clone 11, which are clones of BALB/c 3T3 cells that produce B-tropic MuLV in the absence of SFFV.

Other preparations of viruses that biologically cause erythroleukemia and were used in these studies include the following. (i) A preparation of the Friend virus complex that consisted of F-MuLV and the Friend strain of SFFV and caused anemia and erythroleukemia in susceptible mice was obtained from Charlotte Friend, Mount Sinai Hospital, New York, N.Y., and was propagated on SC-1 cells. (ii) A Rauscher erythroleukemia virus complex containing R-MuLV and the Rauscher strain of SFFV was originally obtained from University Laboratories Inc., Highland Park, N. J., and was supplied in the form of plasma from a BALB/c mouse infected with the Rauscher virus complex; this complex was propagated on SC-1 cells and designated SC-1/R. (iii) F69 virus, a complex containing B-tropic helper virus and the cloned Friend strain of SFFV, was obtained by inoculating SC-1 cells with terminal dilutions of

B-tropic Friend virus complex as previously described (38).

(v) Cells producing xenotropic MuLV and other type C viruses. Two strains of xenotropic virus, the Balb virus 2 and a strain of NZB virus, were obtained from Janet Hartley, National Institute of Allergy and Infectious Diseases, Bethesda, Md. The ATS-124 xenotropic virus has been previously described (37). Each of these xenotropic viruses was propagated on mink lung fibroblasts. The source of the woolly leukemia virus, which was also grown on mink lung fibroblasts, has been described in a previous publication (32).

Virus assays. (i) In vivo assays for SFFV. SFFV was assayed by the spleen focus formation assay in weanling female BALB/c mice as previously described (2). Inbred BALB/c mice used for these assays were obtained from The Small Animal Facility at the National Institutes of Health, Bethesda, Md., housed in disposable plastic cages, and given food and water ad libitum.

(ii) Cell culture assays for MuLV. Replicating type C MuLV's were assayed by either the XC plaque assay (27) on SC-1 mouse embryo fibroblasts or assays of the reverse transcriptase activity in supernatant media from either SC-1 cells or mink lung cells by methods previously described (38).

Virus cloning. To insure that the preparations of ecotropic viruses used in the current studies did not contain either xenotropic virus or SFFV, extensive cloning of each virus that has been subsequently indicated in this manuscript was performed. All ecotropic viruses were cloned on SC-1 mouse embryo fibroblasts by end point dilution in Microtest II culture plates (Falcon) as previously described (38). A virus was considered to be cloned if less than 10% of the total wells of the Microtest II culture plate were found to contain replicating type C viruses as detected by the reverse transcriptase assay. All ecotropic MuLV's used in these experiments were cloned in this way a single time, with the exception of F-MuLV, which was cloned three successive times in this manner. Similar end point dilution techniques on mink cells and S^+L^- mink cells (23) were used to obtain the xenotropic viruses that were used in these studies.

Isolation of nonproducer rat cells containing SFFV. To obtain rat cell clones that contained SFFV in the absence of replicating MuLV, an experiment similar to that which had been previously used (38) to isolate SFFV-containing BALB/3T3 nonproducer cells was carried out. Details of this experiment are given in Fig. 1. Three SFFV-containing nonproducer NRK clones, designated SFFV-NRK clones 1, 2, and 3, and one SFFV-containing FRE cell, designated SFFV-FRE clone 1, were chosen for use in subsequent studies.

Preparation of RNA. Total cellular RNA was extracted using a modification of the technique described by Glisson et al. (9). Each RNA preparation was treated with self-digested Pronase (100 μg/ml) and RNase-free DNase I (20 μg/ml; Sigma Chemical Co.) after isolation by cesium chloride centrifugation. Yields of approximately 1 to 3 mg of RNA per g of packed cells were obtained by this procedure.

Extraction of high-molecular-weight viral RNA used as template for exogenous reverse transcriptase reaction. High-molecular-weight (55 to 70*S*) viral RNA to be used as the template for the exogenous reverse transcriptase reaction was prepared from virus preparations banded a single time in sucrose density gradients. The viruses were disrupted at room temperature for 5 min in a 1.0-ml solution containing 1% SDS and 0.2% (vol/vol) diethylpyrocarbonate in TNE buffer (0.01 M Tris-hydrochloride [pH 7.2], 0.1 M NaCl, 0.001 M EDTA). The disrupted viruses were applied to a 36-ml 15 to 30% continuous sucrose gradient in TNE buffer which was then centrifuged at 16°C for 16 h at 16,000 rpm in an SW27 rotor. Fractions (1.0 ml) were collected from below, and the location of the 55 to 70*S* viral RNA was determined by reading the absorbancy at 260 nm on a Beckman model 25 spectrophotometer. Appropriate fractions that contained RNA were pooled and ethanol precipitated at −20°C. RNA was collected by centrifugation at 4°C for 1.0 h at 100,000 × *g*, resuspended in distilled water, and stored at −20°C prior to use.

Synthesis of [^{3}H]cDNA. Synthesis of viral-specific [^{3}H]cDNA probes from F-MuLV and from a B-tropic MuLV, WN1802B, was performed by the endogenous reverse transcriptase reaction in the presence of 60 μg of actinomycin D per ml, as previously described (3, 29). In the exogenous reverse transcriptase reaction, purified viral RNA from Balb clone 4/F-MuLV was used as a template for the partially purified avian myeloblastosis virus reverse transcriptase utilizing calf thymus DNA fragments as a primer (36). Avian myeloblastosis virus reverse transcriptase was partially purified by gel filtration as previously described (25), except that the dextran-polyethylene glycol step was omitted.

Fractionation of the cDNA probe. A [^{3}H]cDNA probe containing SFFV-specific sequences was obtained by hybridizing a probe made from viral RNA contained in virions released from Balb clone 4/F-MuLV to the appropriate RNA solution at 66°C for 24 h, as indicated in the Results, and subsequently fractionating the cDNA on a hydroxylapatite column containing 1.0 g of hydroxylapatite per mg of RNA used in the hybridization reaction mixture, as follows. [^{3}H]cDNA was loaded onto the column at room temperature in a buffer containing 0.025 M sodium phosphate (pH 6.8) and 0.4 M NaCl. After the sample had been loaded, the temperature on the column was raised to 66°C, and the unhybridized portion of cDNA was eluted at 66°C in buffer containing 0.12 M sodium phosphate, pH 6.8, and 0.4 M NaCl. The hybridized portion of the cDNA probe was then eluted at 85°C in a buffer containing 0.46 M sodium phosphate and no NaCl. The eluted fractions were pooled, treated with NaOH (final concentration, 0.5 N) for 4 h at 37°C, dialyzed extensively against distilled water, concentrated by lyophilization, and stored at −20°C prior to use.

Analytical hybridization reactions. RNA-[^{3}H]cDNA hybridization reactions were performed by procedures which have been previously described (3, 16) and assayed with the use of s1 nuclease. Briefly, each hybridization reaction was incubated

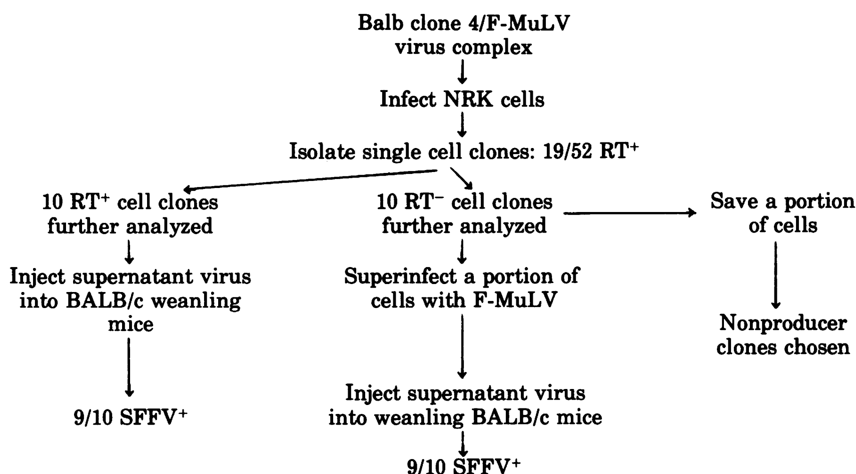

FIG. 1. *Schema used for the isolation of SFFV-containing rat nonproducer cells. The virus complex used to infect NRK cells in this experiment was obtained by superinfecting Balb clone 4 SFFV-containing nonproducer cells with cloned F-MuLV. By end point dilution, the resultant preparation was found to contain 10^4 to 10^5 XC plaque-forming viruses per ml of culture fluid and an excess of SFFV over helper virus. The isolation of NRK cells containing SFFV in the absence of F-MuLV was carried out as follows. NRK cells (3×10^4) were seeded in a 35-mm Falcon culture dish in medium containing 4 μg of Polybrene per ml and inoculated 24 h later with 0.5 ml of a filtered, undiluted portion of a 24-h harvest of supernatant media from Balb clone 4/F-MuLV. Six hours after infection, cells were trypsinized and redeposited in the wells of Falcon Microtest II culture plates at cell concentrations calculated to yield single cell clones. Fifty-two single cell clones were obtained and analyzed as described in reference 38 and as shown above. RT, Reverse transcriptase.*

at 66°C for 24 to 36 h and contained in 0.05 ml: 0.02 M Tris-hydrochloride (pH 7.5), 0.75 M NaCl, 0.001 M EDTA, 0.1% SDS, 1.0 μg of carrier calf thymus DNA, 10 μg of carrier yeast RNA, the indicated RNA, and 1,000 to 2,000 trichloroacetic acid-insoluble cpm of [^{3}H]cDNA (specific activity, 2×10^7 cpm/μg). Hybridization results were analyzed as a function of RNA concentration and time and expressed as C_rt (mol·s/liter).

RESULTS

Detection of F-MuLV sequences in SFFV-containing rat nonproducer cells. Earlier studies have shown that all replication-defective murine FT$^+$ viruses contain sequences homologous to the helper virus present in the initial stocks of the sarcoma-leukemia virus mixture (28, 29). Therefore, since F-MuLV was the helper virus in the initial stocks of the Friend virus complex, we investigated whether or not F-MuLV sequences were contained in rat nonproducer cells containing SFFV. Total cellular RNAs from SFFV-NRK clones 1, 2, and 3 and NRK cells producing F-MuLV (F-MuLV/NRK) were hybridized to F-MuLV [^{3}H]cDNA probe made from F-MuLV grown on SC-1 cells (Fig. 2A). Approximately 70% of the F-MuLV [^{3}H]cDNA probe hybridized to F-MuLV/NRK cellular RNA at saturating levels of RNA, and this value was defined as 100% hybridization of the F-MuLV probe. A similar final extent of hybridization was obtained with RNA from SC-1 cells infected with F-MuLV (data not shown). F-MuLV [^{3}H]cDNA also hybridized to a significant degree to all three SFFV-containing NRK clones, and the RNA from each clone could clearly be distinguished from that of NRK cells producing endogenous rat viruses (V-NRK). After subtracting a minimal background level of hybridization to V-NRK cellular RNA, the final extent of hybridization of F-MuLV cDNA to RNA from SFFV-NRK clone 1 was approximately 50% of that obtained with RNA from F-MuLV/NRK. Interestingly, strikingly different levels of RNA sequences homologous to F-MuLV cDNA were noted in the three SFFV-NRK clones, as determined by C_rt analysis with each of the RNAs (Fig. 2A). The 1/2 C_rt value for SFFV-NRK clone 1 was approximately 20 mol·s/liter, and the 1/2 C_rt value for SFFV-NRK clone 3 was approximately 80 mol·s/liter; in contrast, the total cellular RNA from SFFV-NRK clone 2 failed to saturate the F-MuLV cDNA at a C_rt value of greater than 5,000 mol·s/liter.

These results demonstrate that rat cells nonproductively infected with the Friend strain of SFFV express RNA sequences that are homologous to a portion of sequences present in F-MuLV. To rule out the possibility that the partial hybridization detected in these NRK cells with F-MuLV [^{3}H]cDNA was due to the lack of

expression of additional F-MuLV sequences in the cellular RNA from these cells, we superinfected SFFV-NRK clone 1 with a heterologous virus, the woolly leukemia virus, and hybridized F-MuLV cDNA to high-molecular-weight viral RNA obtained from virus particles released after superinfection. Hybridization with F-MuLV sequences plateaued at saturating levels of viral RNA at a final hybridization equivalent to 50% of that which had been obtained with F-MuLV/NRK (data not shown). The results indicate that the genome of SFFV contains only a portion of the F-MuLV genome and that all of these F-MuLV-specific sequences are expressed in the RNA of the SFFV-containing nonproducer NRK cells.

Biological analysis of SFFV-NRK clones. Because different levels of sequences homologous to F-MuLV (and putatively contained in SFFV) were detected in the three SFFV-NRK clones, we attempted to correlate these levels with the relative amounts of SFFV that could

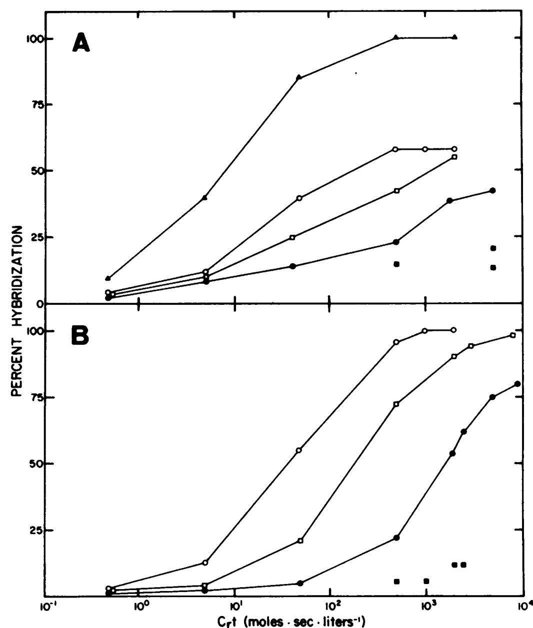

FIG. 2. *Hybridization to RNA of SFFV-NRK clones. (A) Hybridization with F-MuLV [³H]cDNA. Each hybridization reaction contained approximately 2,500 trichloroacetic acid-insoluble cpm of [³H]cDNA from F-MuLV that had been grown in SC-1 mouse cells. Conditions of hybridization and s1 assay are described in Materials and Methods. (B) Hybridization with SFFV [³H]cDNA. Each hybridization contained approximately 2,000 trichloroacetic acid-insoluble cpm of [³H]cDNA specific for SFFV (fraction III, Table 2). Symbols: ▲, RNA from F-MuLV/NRK; ○, SFFV-NRK clone 1; □, SFFV-NRK clone 3; ●, SFFV-NRK clone 2; ■, V-NRK.*

be rescued from each nonproducer cell by superinfection with F-MuLV. Therefore, we infected SFFV-NRK clones 1, 2, and 3 and control NRK cells with F-MuLV. Five days later, we harvested a 24-h collection of supernatant medium from each cell line, which we then assayed for F-MuLV by the XC plaque assay and for SFFV by the spleen focus formation assay. Table 1 shows a comparison of the relative SFFV titers to F-MuLV titers released from each of the superinfected cell lines after short-term infection. The ratio of SFFV to F-MuLV released from clone 1 (4.5×10^{-3}) was slightly greater than that from clone 3 (1.9×10^{-3}), and both of these were much greater than that from clone 2 (1.1×10^{-4}). These results indicate that greater numbers of SFFV particles per units of XC plaque-forming virus were rescued from clones 1 and 3 than from clone 2.

Analysis of high-molecular-weight RNA from SFFV-containing nonproducer mouse cells infected with helper virus. Because we intended to use high-molecular-weight viral RNA from a virus preparation containing SFFV and helper virus to make [³H]cDNA, we analyzed the high-molecular-weight viral RNA obtained from Balb clone 4 cells superinfected with a cloned B-tropic helper virus, WN1802B, and compared it to viral RNA obtained from normal BALB/c 3T3 cells infected with WN1802B, by hybridization across sucrose gradients with two different [³H]cDNA probes, as described in the legend to Fig. 3. To detect helper virus RNA, we used [³H]cDNA which was prepared from WN1802B grown in SC-1 cells; to detect RNA from SFFV, a [³H]cDNA probe prepared from F-MuLV grown on SC-1 cells was used, since we had shown that a portion of the SFFV genome could be detected by virtue of its homology to F-MuLV cDNA. The results of hybridization of these two probes to the high-molecular-weight viral RNA isolated from BALB/c 3T3 cells infected with WN1802B and from Balb clone 4 cells infected with WN1802B are shown in Fig. 3A and B, respectively. As shown in Fig. 3A, the [³H]cDNA made from the B-tropic MuLV detects a peak of high-molecular-weight RNA in the sucrose gradient which is relatively symmetrical and sediments at approximately 60 to 70*S*, as determined by labeled markers in parallel gradients. Consistent with our observation that the F-MuLV probe used in this experiment had only a 30% homology to RNA from WN1802B under our hybridization conditions (data not shown), the F-MuLV probe detected very little hybridization in the same region of this gradient. In contrast, when the same two probes were used to assay for hybridizable RNA in the high-

TABLE 1. *Biological and biochemical comparison of SFFV-containing NRK clones*[a]

Cell line	Virus titers after superinfection		Ratio of SFFV to F-MuLV	1/2 C_rt of RNA (mol·s / liter)
	F-MuLV titer (XC PFU/ml)	SFFV titer (FFU/ml)		
SFFV-NRK clone 1	2.9×10^4	1.3×10^2	4.5×10^{-3}	2.0×10^1
SFFV-NRK clone 2	5.9×10^4	6.6×10^0	1.1×10^{-4}	$>1.0 \times 10^3$
SFFV-NRK clone 3	7.3×10^4	1.4×10^2	1.9×10^{-3}	8.0×10^1
NRK	1.1×10^5	0	0	0

[a] Each cell line was seeded at 3×10^5 cells in 100-mm Falcon culture dishes in medium containing 4 μg of Polybrene per ml and was superinfected 24 h later with 10^5 XC PFU of F-MuLV. Media were changed on day 4, and on the following day, a 24-h harvest of medium from each cell line was collected and stored at −70°C in 1.0-ml portions. A portion of each culture was then assayed for F-MuLV by XC assay on SC-1 cells and for SFFV by the spleen focus formation assay. Three weanling BALB/c female mice were used for each assay. Titers are expressed as XC PFU per milliliter and FFU per milliliter, respectively. The ratio of relative SFFV to F-MuLV titers from each clone was compared to the 1/2 C_rt of RNA from each clone as detected with fraction III SFFV-specific cDNA. 1/2 C_rt values were calculated from the hybridization kinetics shown in Fig. 2B.

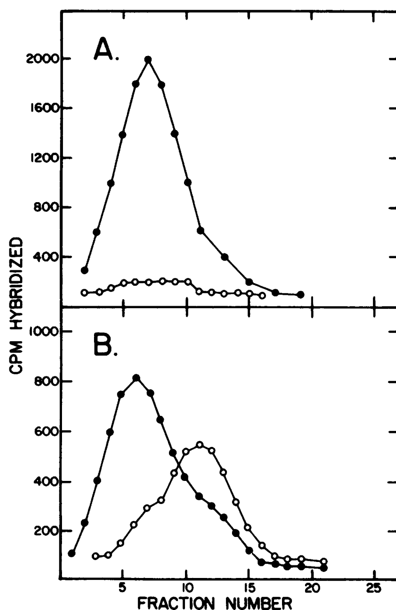

FIG. 3. *Hybridization to high-molecular-weight viral RNA from BALB/c 3T3 cells and Balb clone 4 cells after superinfection with B-tropic MuLV. Two sets of flasks (75 cm²) containing approximately 5 × 10⁶ cells per flask were fed with 10 ml of fresh medium per flask. One set of flasks contained BALB/c 3T3 cells that were infected with B-tropic MuLV WN1802B, and the other set contained Balb clone 4 cells that were superinfected with the same B-tropic MuLV. Eight hours after the fresh medium had been added, approximately 60 ml of culture medium was collected from each set of flasks. The fluid was clarified at 4°C at 3,000 rpm for 10 min in a PR6000 centrifuge and concentrated for 3 h at 100,000 × g at 4°C. The pelleted virus was resuspended in 1.0 ml of TNE buffer (0.01 M Tris-hydrochloride [pH 7.2], 0.1 M NaCl, 0.001 M EDTA) containing 1.0% SDS and 0.2% (vol/vol) diethylpyrocarbonate. The disrupted virus was layered onto a 12.5-ml, 15 to 30% sucrose gradient in TNE buffer and centrifuged for 3.0 h at 16°C at 39,000 rpm in an SW41 rotor. Fractions (0.5 ml) were collected from below, and each fraction was concentrated by ethanol precipitation with 20 μg of carrier yeast rRNA. RNA from each fraction was resuspended in 0.1 ml of 0.01 M Tris-hydrochloride (pH 7.2). (A) BALB/c 3T3 cells superinfected with WN1802B. Portions (0.02 ml) of the concentrated RNA from the indicated fractions were hybridized to approximately 2,500 trichloroacetic acid-insoluble cpm of [³H]cDNA from either F-MuLV (○) or B-tropic MuLV WN1802B (●). Hybridization assays were for 24 h. (B) Balb clone 4 superinfected with WN1802B. Portions (0.01 ml) from each RNA fraction from Balb clone 4/WN1802B culture were hybridized to the same [³H]cDNA's as in (A).*

molecular-weight region of the gradient obtained from Balb clone 4/WN1802B culture (Fig. 3B), a different profile was obtained. Again, the WN1802B probe detected sequences in the high-molecular-weight region of this gradient as well as a slight shoulder on the lighter side of the 70*S* peak; with the F-MuLV cDNA, a separate peak of hybridization was obtained on the right-hand shoulder of the high-molecular-weight viral RNA, indicating that the RNA of SFFV sedimented more slowly in the sucrose gradient than did the RNA of the helper MuLV which was used to pseudotype it. These results were consistent with previous observations that: (i) the RNA subunit of SFFV is smaller than that of its accompanying helper virus (20) and that (ii) the RNA dimers that were isolated from virus preparations containing both helper MuLV and SFFV and differ in molecular weight exist predominantly as homodimers in high-molecular-weight RNA (6). Furthermore, these results indicated that, in gradients prepared from cultures releasing both SFFV and helper virus, the lighter region of high-molecular-weight viral RNA would be enriched for SFFV RNA relative to helper virus RNA.

Preparation of SFFV-specific cDNA probe. To determine whether a set of nucleotide sequences distinct from F-MuLV sequences was present in SFFV, 55 to 70*S* viral RNA prepared from Balb clone 4 cells that had been rescued with F-MuLV was used to make a [^{3}H]cDNA probe. Based on the experiments with the B-tropic pseudotype of SFFV described in the preceding section, sucrose gradient fractions representing the slowest migrating one-third of the high-molecular-weight RNA from this culture were pooled, and the RNA contained in these fractions was utilized as a template for the avian myeloblastosis virus reverse transcriptase to prepare [^{3}H]cDNA. The [^{3}H]cDNA probe prepared from this viral RNA was tested in hybridizations as shown in Table 2. With unfractionated [^{3}H]cDNA (fraction I), approximately 50 to 55% of the input counts hybridized to RNA from F-MuLV/SC-1 or F-MuLV/NRK cells, 55 to 60% hybridized to RNA from SFFV NRK clone 1 and Balb clone 4, and 66% hybridized to Balb clone 4/F-MuLV. To remove F-MuLV sequences contained in this [^{3}H]cDNA, the fraction I probe was hybridized to cellular RNA from NRK cells producing F-MuLV to a final C_rt value of 1,700 mol·s/liter, and the cDNA was passed over a hydroxylapatite column as described in Materials and Methods. [^{3}H]cDNA eluting at 0.12 M sodium phosphate (unhybridized cDNA) was designated fraction II, and the results of hybridizations with this cDNA are also shown in Table 2. Fraction II probe hybridized to a much greater extent to the RNA from SFFV-containing nonproducer cells, Balb clone 4 (31%) and SFFV-NRK clone 1 (33%), compared to the RNA from SC-1 cells producing F-MuLV (10%) or NRK cells producing F-MuLV (5%), thus detecting RNA sequences that were contained in SFFV but were not present in F-MuLV. To further enrich fraction II cDNA for the SFFV-specific sequences, this probe was hybridized to saturating levels of SFFV-NRK clone 1 cellular RNA and again passed over a hydroxylapatite column. The cDNA sequences which eluted at 0.46 M sodium phosphate (hybridized cDNA) were collected and designated fraction III. The results of hybridization studies with this probe are also shown in Table 2; 71% of fraction III cDNA hybridized to RNA from SFFV-NRK clone 1, whereas only 7% hybridized to RNA from either NRK cells or SC-1 cells producing F-MuLV. The incomplete hybridization (57%) to Balb clone 4 cellular RNA in this particular study did not indicate a final value, since the 1/2 C_rt in this nonproducer cell was much lower than that of SFFV-NRK clone 1, and thus the RNA failed to saturate the probe, based on C_rt analysis (data not shown). These results indicated that SFFV contained a set of nucleotide sequences which was distinct from the sequences

TABLE 2. *Preparation of SFFV-specific [^{3}H]cDNA* [a]

Cellular RNA	Hybridization of [^{3}H]cDNA in:					
	Fraction I		Fraction II		Fraction III	
	cpm	% Hybrid	cpm	% Hybrid	cpm	% Hybrid
F-MuLV/NRK	1,570	52	98	5	133	7
F-MuLV/SC-1	1,610	54	196	10	150	7
SFFV-NRK clone 1	1,750	58	650	33	1,423	71
Balb clone 4	1,711	57	618	31	1,135	57
Balb clone 4/F-MuLV	1,982	66	925	46	1,450	72
SC-1	NT[b]		117	6	45	2
V-NRK[c]	NT		40	2		<1

[a] A [^{3}H]cDNA that was prepared from Balb clone 4/F-MuLV as described in Materials and Methods represents fraction I. Fraction II was obtained by hybridizing fraction I (approximately 10×10^6 trichloroacetic acid-insoluble cpm) to 3 mg of F-MuLV/NRK cellular RNA to a C_rt of 1,700 mol·s/liter and obtaining the portion of [^{3}H]cDNA that had eluted from hydroxylapatite with 0.12 M sodium phosphate. Approximately 2×10^6 trichloroacetic acid-insoluble cpm was obtained as fraction II. Fraction III was obtained by hybridizing fraction II to 3 mg of cellular RNA from SFFV-NRK clone 1 to a C_rt of 5,000 mol·s/liter and recovering the portion of [^{3}H]cDNA that had been eluted on hydroxylapatite with 0.46 M sodium phosphate as described in Materials and Methods. Approximately 800,000 trichloroacetic acid-insoluble cpm was recovered as fraction III. Each fraction was tested in hybridizations to C_rt values of 5,000 mol·s/liter of the indicated RNAs and assayed with s1 nuclease as described in Materials and Methods. Assays with fraction I contained approximately 3,000 trichloroacetic acid-insoluble cpm, and assays with fraction II and III contained 2,000 trichloroacetic acid-insoluble cpm.

[b] NT, Not tested.

[c] V-NRK, a virus-producing subclone of NRK cells.

contained in F-MuLV. Based on a comparison of the recoveries of the trichloroacetic acid-insoluble counts per minute in fractions III and I, these sequences represented approximately 10% of the initial cDNA prepared from the purified viral RNA with the avian myeloblastosis virus reverse transcriptase.

Specificity of hybridization of the SFFV [^{3}H]cDNA probe. To test the specificity of the SFFV cDNA probe, cellular RNA from SFFV-NRK clones 1, 2, and 3, which had earlier been shown to contain different levels of sequences homologous to F-MuLV and different levels of SFFV by biological assay, were tested for their ability to hybridize to fraction III probe. The results of this analysis are shown in Fig. 2B. The SFFV-specific probe detected somewhat higher levels of SFFV-specific sequences in RNA from SFFV-NRK clone 1 compared to that in SFFV-NRK clone 3, and cellular RNA from SFFV-NRK clone 2 failed to saturate the cDNA probe at a C_rt value of 10^4 mol·s/liter. These results closely paralleled those obtained in hybridization studies with F-MuLV cDNA (Fig. 2A). Furthermore, there was an excellent correlation between the relative levels of SFFV-specific sequences detected with SFFV cDNA by C_rt analysis and the relative biological titers of SFFV that could be rescued from each clone by superinfection with F-MuLV (Table 1).

To further test the specificity of the SFFV cDNA, a series of cells which had been well characterized biologically for SFFV activity by the in vivo focus formation assay were tested for the presence of the SFFV-specific sequences. The results of this experiment are shown in Table 3. Eight pairs of cells of both mouse and rat origin were divided into three series of cells and assayed for SFFV-specific sequences. (i) The first series of cells that were assayed for SFFV-specific sequences were BALB/c 3T3 clones. Balb clone 4 SFFV-containing nonproducer cells and uninfected BALB/c 3T3 cells were superinfected with F-MuLV and tested by the spleen focus formation assay and by hybridization. In addition, Balb clone 9 and Balb clone 13, both of which release high levels of SFFV, and Balb clone 7 and Balb clone 11, which biologically release only helper MuLV, were tested. In this series of Balb clones, the SFFV-specific sequences were detected in the RNA from cell clones releasing SFFV, namely Balb clone 4/F-MuLV, Balb clone 9, and Balb clone 13. In contrast, SFFV sequences were not detected in cellular RNA from the three Balb clones producing only helper virus. (ii) The second series was those viruses grown on SC-1 cells, and a similar correlation was obtained. The culture that was designated F-69/SC-1, which released cloned SFFV and helper virus, hybridized well to the SFFV-specific probe, whereas helper virus (MuLV clone 68) cloned from this virus complex failed to hybridize to fraction III cDNA. RNA sequences homologous to SFFV cDNA were also detected in a preparation of the Friend virus complex grown on SC-1 cells (FVA/SC-1) as well as in a preparation of the Rauscher murine erythroleukemia virus grown on SC-1 cells (SC-1/R), whereas helper viruses cloned from these preparations were negative for SFFV sequences. Although the titers of SFFV measured biologically in these two virus preparations grown in cell culture were quite low, approximately 80% of the sequences contained in SFFV cDNA were detected in each virus preparation. (iii) The third series consists

TABLE 3. *Specificity of hybridization of SFFV [^{3}H]cDNA for RNA from cells producing and not producing SFFV*[a]

Cellular RNA	Approximate titers of virus		% Hybridization of fraction III SFFV cDNA
	Helper virus titer (XC PFU/ml)	SFFV titer (FFU/ml)	
Balb clone 4/F-MuLV	$10^{6.3}$	$>10^5$	100
BALB/F-MuLV	$10^{6.0}$	0	8
Balb clone 9	$10^{6.5}$	$>10^4$	100
Balb clone 7	$10^{6.0}$	0	5
Balb clone 13	$>10^{6.0}$	$10^{4.5}$	100
Balb clone 11	$>10^{6.0}$	0	5
F69/SC-1	$10^{5.8}$	$10^{3.8}$	84
MuLV clone 68/SC-1	$10^{6.5}$	0	9
FVA/SC-1[b]	$10^{5.6}$	$10^{2.2}$	80
F-MuLV/SC-1	$10^{5.6}$	0	7
SC-1/R	$10^{6.2}$	$10^{1.0-2.0}$	77
R-MuLV/SC-1	$10^{6.2}$	0	6
SFFV-NRK clone 1/F-MuLV	$10^{4.8}$	10^4	100
F-MuLV/NRK	$10^{4.5}$	0	7
SFFV-FRE clone 1/F-MuLV	$10^{4.2}$	$10^{3.5}$	100
FRE clone 2/F-MuLV	$10^{4.5}$	0	7

[a] RNA from a series of cells producing type C viruses was prepared and hybridized to the fraction III SFFV cDNA. The viruses in each culture were assayed in weanling BALB/c mice for SFFV by the spleen focus-forming assay and assayed for helper virus by the XC plaque test on SC-1 mouse cells as described in Materials and Methods. Each RNA was hybridized to fraction III cDNA at a C_rt of 5,000 mol·s/liter. Each hybridization assay contained approximately 1,200 trichloroacetic acid-insoluble cpm of fraction III [^{3}H]cDNA, and the value of 930 cpm which hybridized to RNA from Balb clone 4/F-MuLV was defined as 100% hybridization.

[b] FVA, Friend virus complex.

of rat cells. SFFV-containing rat nonproducer cells, SFFV-NRK clone 1 and SFFV-FRE clone 1, when superinfected with F-MuLV, biologically released high titers of SFFV. The RNA from these cells also contained the SFFV-specific sequences, but NRK or FRE cells producing F-MuLV did not.

In summary, the results shown in Table 3 indicate a high degree of specificity of the SFFV-specific probe for the sequences found in eight independently derived clones of BALB cells, SC-1 cells, or rat cells that release viruses which have been well characterized for their ability to induce rapid erythroleukemia when inoculated into susceptible mice.

Presence of SFFV-related sequences in other murine type C viruses. The SFFV probe was next tested for the presence of related sequences in other preparations of cloned MuLV's and sarcoma viruses (MuSV's) in our laboratory. The results of these hybridizations are shown in Table 4 (experiment A). Sequences homologous to SFFV cDNA were not detected in three strains of N-tropic MuLV, namely, N-tropic AKR virus, Ki-MuLV, and WN1802N; nor were these sequences detected in three strains of B-tropic MuLV, namely, WN1802B, MuLV clone 68, and cloned Balb/Tennant leukemia virus, B/T-LV clone A3; similarly, SFFV sequences were not detected in three strains of NB-tropic virus, namely, F-MuLV, R-MuLV, and WN1802NB. Strikingly, however, sequences related to SFFV cDNA were found in cultures of three different strains of xenotropic virus growing in mink cells, namely, Balb virus 2, ATS 124, and an NZB virus; but these sequences were not found in normal mink cells or mink cells infected with woolly leukemia virus. At saturating levels of RNA, approximately 50% of the final extent of hybridization was achieved with RNA from each of the xenotropic virus-producing mink cell cultures as compared to SFFV-NRK clone 1. Finally, the sequences were not found in RNA of nonproducer cells transformed by KiSV, HaSV, or a P30+ strain of the Moloney MuSV (S^+L^- dog).

To further elucidate the nature of the hybridization of the SFFV-specific cDNA to the RNA from cells producing X-tropic viruses, a mixing experiment was performed as shown in experiment B of Table 4. When a mixture of RNA from ATS 124-infected mink cells and SFFV-NRK clone 1 was hybridized to the SFFV probe, the final extent of hybridization was no greater than that which had been obtained with RNA from SFFV-NRK clone 1 alone, indicating that the set of sequences that were detected in ATS 124 with the SFFV cDNA is a subset of the sequences that were detected in the SFFV-NRK clone 1 cells. Thus, the results indicate that the sequences contained in SFFV which are not part of the F-MuLV genome are related to sequences found in three separate strains of murine xenotropic type C virus but share no homology with RNA sequences contained in several strains of cloned ecotropic MuLV. Whether the partial hybridization (approximately 50%) of SFFV-specific cDNA to RNA from cells infected with xenotropic virus results

TABLE 4. *Hybridization of SFFV cDNA to various ecotropic and xenotropic MuLV's and MuSV's*[a]

Expt	Source of RNA	Host range of virus	[^{3}H]cDNA hybridized	
			cpm	%
A	SFFV-NRK clone 1		733	100
	Ecotropic MuLV			
	NIH 3T3		46	<5
	NIH 3T3/AKR-MuLV[b]	N-tropic	72	8
	NIH 3T3/Ki-MuLV	N-tropic	54	<5
	NIH 3T3/WN1802N	N-tropic	66	<5
	SC-1		54	<5
	SC-1/WN1802B	B-tropic	46	<5
	SC-1/MuLV clone 68	B-tropic	86	7
	SC-1/BTLV clone A3	B-tropic	83	7
	SC-1/F-MuLV	NB-tropic	98	8
	SC-1/R-MuLV	NB-tropic	103	11
	SC-1/WN1802NB	NB-tropic	78	<5
	Xenotropic MuLV			
	Mink		46	<5
	Mink/Balb virus 2	X-tropic[c]	330	46
	Mink/ATS 124	X-tropic	382	50
	Mink/NZB	X-tropic	349	47
	Mink/WoLV[d]		46	<5
	MuSV			
	Ki-Mink		38	<5
	Ha-MDCK[e]		38	<5
	S^+L^- Dog		35	<5
B	SFFV-NRK clone 1 (50 μg)			1,609
				1,635
	Mink/ATS 124 (100 μg)			946
				925
	SFFV-NRK clone 1 (50 μg) + Mink/ATS 124 (100 μg)			1,645
				1,647

[a] Each hybridization in experiment A contained approximately 1,000 trichloroacetic acid-insoluble cpm of fraction III cDNA, and those in experiment B contained 2,200 trichloroacetic acid-insoluble cpm of fraction III cDNA. All values represent the extent of hybridization at C_rt values of 5,000 mol·s/liter. Each RNA that failed to hybridize to SFFV fraction III cDNA was tested with a cDNA from either B-tropic MuLV WN1802 or F-MuLV to insure that the RNA preparations contained high levels of RNA of the indicated viruses.

[b] AKR-MuLV, a strain of MuLV that had been obtained from AKR 2B cells.

[c] X-tropic, Xenotropic host range.

[d] WoLV, Woolly leukemia virus.

[e] Ha-MDCK, a canine cell line that is transformed by HaSV.

from either (i) diffuse homology between the cDNA and RNA with areas of base pair mismatching or (ii) complete homology between only a portion of the SFFV-specific cDNA to xenotropic virus RNA, with an extra set of SFFV-specific cDNA sequences not present in xenotropic virus, is unresolved. Further studies to investigate this are now in progress in our laboratory.

Protein analysis of SFFV-NRK clones. Because the SFFV-NRK nonproducer cells contained sequences homologous to F-MuLV as well as a set of SFFV-specific sequences which have homology to sequences found in cultures of xenotropic viruses, the rat nonproducer cells were analyzed by radioimmunoassay for certain type C viral proteins. Three assays were performed: (i) a type-specific assay for the p12 of F-MuLV; (ii) a group-specific assay for the p30 of MuLV's; and (iii) an interspecies assay for gp70 that is capable of detecting gp70 of either ecotropic or xenotropic mouse type C viruses. The results of such analyses are summarized in Table 5. The rat cells infected with F-MuLV (F-MuLV/NRK) reacted well in each radioimmunoassay. However, no immunoreactive protein was detected in SFFV-NRK clone 1 in any assay. Importantly, human cells infected with the Balb virus 2 strain of murine xenotropic virus reacted well in the p30 assay and gp70 assay. An analysis of the SFFV-NRK cell for the p12 protein of each of the xenotropic viruses has not yet been performed. However, the results would suggest that the SFFV-containing nonproducer NRK cells are not coinfected with a defective xenotropic virus in addition to SFFV, since proteins of the gag gene and envelope gene, gp70, are not detected in this cell. In the absence of a radioimmunoassay for viral reverse transcriptase, we cannot exclude the fact that the cells contain viral reverse transcriptase; however, no particles containing reverse transcriptase are released from the SFFV-containing NRK nonproducer cells (data not shown).

TABLE 5. *Immunological analysis of SFFV-NRK nonproducer cells*[a]

Cell tested	Levels of immunoreactive protein (ng/mg of cellular protein)		
	p12	p30	gp70
F-MuLV/NRK	2,100	5,120	3,800
SFFV-NRK clone 1	<20	<20	<20
Balb virus 2/A673	NT[b]	2,800	3,300
V-NRK[c]	<20	<20	<20

[a] The p12 and gp70 proteins of Friend type C virus were the kind gift of D. Bolognesi, Duke University Medical Center, Durham, N.C. The p30 was obtained from Rauscher type C virus, and its purification has been described (22). The assay for Friend p12 used a 1:1,000 dilution of a goat serum that had been prepared against F-MuLV as the primary antibody; the assay for p30 used a 1:100,000 dilution of a goat serum that had been made against Moloney MuLV; and the assay for gp70 used a 1:5,000 dilution of a goat serum that had been made against feline leukemia virus (22). Each assay contained 50,000 cpm of ^{125}I-labeled antigen and primary serum as indicated. Competition assays were performed using excess second antibody and measuring ^{125}I-labeled antigens in pellets as previously described (32). A dilution of primary antibody which gave a 20 to 30% precipitation of each labeled antigen in the absence of putative competing antigen was chosen. Cell lines tested are described in Materials and Methods; a culture of human cells producing Balb virus 2 xenotropic virus (Balb virus 2/A673) was obtained from Electronucleonics, Bethesda, Md.

[b] NT, Not tested.

[c] V-NRK, a virus-producing subclone of NRK cells.

DISCUSSION

Historically, the Friend and Rauscher strains of murine erythroleukemia virus represent two independent virus isolates which are capable of causing acute leukemia. In 1957, the Friend strain (8) was isolated from the leukemic spleen of a 14-month-old Swiss mouse that had been inoculated at birth with cell-free extracts of Erlich ascites carcinoma cells; the Rauscher strain (24) was isolated in 1962 by passage of filtrates of leukemic mouse tissues in newborn BALB/c mice. The diseases induced by each of these viruses are pathologically quite similar in that each is characterized by rapid splenomegaly and fatal erythroleukemia (8, 24, 33). Each virus complex contains a replication-defective RNA tumor virus that is the cause of the rapid leukemogenicity of each preparation and which is designated SFFV (2, 33). Therefore, the current studies have compared by molecular hybridization the genomes of these two viruses, applying methods previously used to study replication-defective FT^+ viruses.

To analyze the genome of SFFV, we isolated a series of rat and mouse nonproducer cells that contain the Friend strain of SFFV in the absence of replicating MuLV. Since both the Friend and Rauscher strains of SFFV were derived from mice, we considered it mandatory to isolate SFFV in nonproducer cells of a heterologous (rat) species, thus allowing us to distinguish the nucleic acid sequences present in SFFV from those of either contaminating MuLV or potentially related endogenous sequences contained in mouse cells. Using SFFV-containing rat nonproducer cells, we have found that the Friend strain of SFFV is com-

prised of two sets of distinguishable nucleic acid sequences. One set is homologous to part of F-MuLV, and the other set, which is SFFV specific, is not contained in either of the helper viruses, F-MuLV or R-MuLV. These SFFV-specific sequences are detected in both the Friend and the Rauscher strains of erythroleukemia-inducing virus. Thus, a highly related set of sequences has been acquired in two distinct isolates of murine erythroleukemia virus; in the case of Friend virus, these SFFV-specific sequences were apparently acquired as a result of the replication of F-MuLV in newborn Swiss mice, whereas in the case of Rauscher virus, they were apparently acquired as a result of the replication of R-MuLV in newborn BALB/c mice. Since these SFFV-specific sequences had been acquired in two independent cases, we surveyed for the presence of SFFV-related sequences in other preparations of murine type C viruses. The SFFV-specific sequences were not detected in several strains of cloned murine ecotropic viruses derived from several mouse strains; however, SFFV-specific cDNA partially hybridized to RNA sequences in three separate isolates of xenotropic viruses derived from three strains of mice. Thus, the Friend and Rauscher strains of SFFV seem to represent recombinants between a portion of the ecotropic MuLV present in the initial virus preparations and a class of endogenous mouse type C viral sequences which are highly related to sequences contained in xenotropic viruses. Furthermore, our results suggest that murine xenotropic viruses contain a discrete set of RNA sequences that are not found in any naturally occurring MuLV's that we tested.

The SFFV genome, as characterized in the current studies, is analogous to the genomes of the Kirsten and Harvey strains of replication-defective FT$^+$ virus which have been characterized in earlier studies (30-32). Each of these MuSV's was isolated by passage of a replicating MuLV in newborn rats (13, 15), and hybridization studies have shown that each was derived by recombination between sequences contained in replicating MuLV and sequences contained in a distinct class of rat endogenous type C viral information (32). The endogenous rat genetic information contained in each MuSV has been defined as the sarc gene for these viruses, since it is contained in both KiSV and HaSV but is not contained in the helper viruses, Ki-MuLV and the Moloney strain of MuLV, respectively, that were used to isolate FT$^+$ virus by passage in rats. Thus, a plausible interpretation of the current data characterizing the SFFV genome is that the xenotropic virus-related sequences contained in SFFV are analogous to the endogenous rat type C "sarc" sequences contained in KiSV and HaSV, and that whatever the function(s) of the sarc sequences is in KiSV- and HaSV-induced fibroblast transformation, the SFFV-specific sequences have a similar function(s) in the leukemogenic potential of SFFV.

This model suggests several questions about the SFFV-specific sequences contained in the Friend and Rauscher murine erythroleukemia viruses. Firstly, do the xenotropic-related sequences code for a protein that accounts for the rapid leukemogenicity of SFFV, and can they thus be referred to as the "leuk" gene of SFFV, analogous to the more rigorously defined sarc gene of avian sarcoma viruses (35, 39, 40)? Or, alternatively, do the newly acquired sequences contained in SFFV cause this virus to integrate into DNA of infected cells at a location different from the usual site(s) of integration of replicating MuLV, thus altering the pathogenic potential of MuLV sequences by virtue of their proximity to unidentified specific cellular genes? Secondly, since xenotropic viruses are found in virtually all strains of mice that have been examined (17), do xenotropic-related genes such as those contained in SFFV play a role in other types of murine leukemia? For example, do other ecotropic MuLV, such as AKR-MuLV (10) or the radiation leukemia virus (18), produce leukemia as a consequence of either induction of or recombination with some class of sequences related to xenotropic viruses? Finally, since endogenous type C viral genes are known to be present in a variety of vertebrate species (4), is it possible that specific endogenous type C viral genes might, under certain circumstances, play a role in naturally occurring leukemias in vertebrate species other than mice? Thus, the current experiments on the genome of SFFV and recent studies on KiSV and HaSV make it necessary to reexamine the role of endogenous xenotropic viruses in naturally occurring leukemias and solid tumors in rodent species, and our current studies are directed toward testing predictions of the proposed model for murine virus-induced leukemia.

ACKNOWLEDGMENTS

We gratefully acknowledge the clerical assistance of Joan Todd and Audrey Boyle.

This work has been supported by the Virus Cancer Program of the National Cancer Institute.

LITERATURE CITED

1. **Aaronson, S. A., and G. J. Todaro.** 1968. Development of 3T3-like lines from BALB/c mouse embryo cultures: transformation susceptibility to SV40. J. Cell Physiol. 72:141-148.
2. **Axelrad, A. A., and R. A. Steeves.** 1964. Assay for Friend leukemia virus: rapid quantitation method

based on enumeration of macroscopic spleen foci in mice. Virology 24:513-518.
3. **Benveniste, R., and E. M. Scolnick.** 1973. RNA in mammalian sarcoma transformed nonproducer cells homologous to murine leukemia virus RNA. Virology 51:370-382.
4. **Benveniste, R. E., and G. J. Todaro.** 1974. Evolution of C-type viral genes: inheritance of exogenously acquired viral genes. Nature (London) 252:456-459.
5. **Birnsteil, M. I., B. H. Sells, and I. F. Purdon.** 1972. Kinetic complexity of RNA molecules. J. Mol. Biol. 63:21-39.
6. **Dube, S., H. J. Kung, W. Bender, N. Davidson, and W. Ostertag.** 1976. Size, subunit composition, and secondary structure of the Friend virus genome. J. Virol. 20:264-272.
7. **Frankel, A. E., R. L. Neubauer, and P. J. Fischinger.** 1976. Fractionation of DNA nucleotide transcripts from Moloney sarcoma virus and isolation of sarcoma virus-specific complementary DNA. J. Virol. 18:481-490.
8. **Friend, C.** 1957. Cell-free transmission in adult Swiss mice of a disease having the character of a leukemia. J. Exp. Med. 105:307-318.
9. **Glissin, V., R. Crkvenjakov, and C. Byers.** 1974. Ribonucleic acid isolation by cesium chloride centrifugation. Biochemistry 13:2633-2637.
10. **Gross, L.** 1970. Oncogenic viruses, 2nd ed., p. 286-469. Pergamon Press Ltd., New York.
11. **Hartley, J. W., and W. P. Rowe.** 1975. Clonal cell lines from a feral mouse embryo which lack host-range restrictions for murine leukemia viruses. Virology 65:128-134.
12. **Hartley, J. W., W. P. Rowe, and R. J. Huebner.** 1970. Host-range restriction of murine leukemia viruses in mouse embryo cell culture. J. Virol. 5:221-225.
13. **Harvey, J. J.** 1964. An unidentified virus which causes the rapid production of tumors in mice. Nature (London) 204:1104-1105.
14. **Jainchill, J. F., S. Aaronson, and G. J. Todaro.** 1969. Murine sarcoma and leukemia viruses: assay using clonal lines of contact-inhibited mouse cells. J. Virol. 4:549-553.
15. **Kirsten, W. H., and L. A. Mayer.** 1961. Morphologic responses to a murine erythroblastosis virus. J. Natl. Cancer Inst. 39:311-335.
16. **Leong, J. A., A. C. Garapin, H. Jackson, L. Fanshier, W. Levinson, and J. M. Bishop.** 1972. Virus-specific ribonucleic acid in cells producing Rous sarcoma virus: detection and characterization. J. Virol. 9:891-903.
17. **Levy, J. A.** 1974. Xenotropic viruses: murine leukemia viruses associated with NIH/Swiss, NZB, and other mouse strains. Science 181:1151-1153.
18. **Lieberman, M., and H. S. Kaplan.** 1959. Leukemogenic activity of filtrates from radiation-induced lymphoid tumors of mice. Science 130:387-388.
19. **Lilly, F., and R. A. Steeves.** 1973. B-tropic Friend virus: a host range pseudotype of spleen focus-forming virus (SFFV). Virology 55:363-370.
20. **Maisel, J., V. Klement, M. M.-C. Lai, W. Ostertag, and P. Duesberg.** 1973. Ribonucleic acid components of murine sarcoma and leukemia viruses. Proc. Natl. Acad. Sci. U.S.A. 70:3536-3540.
21. **Moloney, J. B.** 1966. A virus-induced rhabdomyosarcoma of mice. Natl. Cancer Inst. Monogr. 22:139-142.
22. **Parks, W. P., and E. M. Scolnick.** 1972. Radioimmunoassay of mammalian type-C viral proteins: interspecies antigenic reactivities of the major internal polypeptide. Proc. Natl. Acad. Sci. U.S.A. 69:1766-1770.
23. **Peebles, P. T.** 1975. An *in vitro* focus induction assay for xenotropic murine leukemia virus, feline leukemia virus C, and the feline-primate viruses RD114/CCC/M-7. Virology 67:288-291.
24. **Rauscher, F. J., Jr.** 1962. A virus-induced disease of mice characterized by erythropoiesis and lymphoid leukemia. J. Natl. Cancer Inst. 29:515-543.
25. **Ross, J., E. M. Scolnick, G. J. Todaro, and S. A. Aaronson.** 1971. Separation of murine cellular and murine leukemia virus DNA polymerases. Nature (London) New Biol. 231:163-167.
26. **Rowe, W. P., J. W. Hartley, M. R. Lander, W. E. Pugh, and N. Teich.** 1971. Noninfectious AKR mouse embryo cell lines in which each cell has the capacity to be activated to produce infectious murine leukemia virus. Virology 46:866-876.
27. **Rowe, W. P., W. E. Pugh, and J. W. Hartley.** 1970. Plaque assay techniques for murine leukemia viruses. Virology 42:1136-1139.
28. **Scolnick, E. M., R. J. Goldberg, and W. P. Parks.** 1975. A biochemical and genetic analysis of mammalian RNA containing sarcoma viruses. Cold Spring Harbor Symp. Tumor Viruses 39:885-895.
29. **Scolnick, E. M., R. Howk, A. Anisowicz, P. Peebles, C. Scher, and W. 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.
30. **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.
31. **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.
32. **Scolnick, E. M., D. Williams, J. Maryak, W. Vass, R. Goldberg, and W. 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.
33. **Steeves, R. A.** 1975. Spleen focus-forming virus in Friend and Rauscher leukemia virus preparations. J. Natl. Cancer Inst. 54:289-294.
34. **Steeves, R. A., R. J. Eckner, M. Bennett, E. A. Mirand, and P. J. Trudel.** 1971. Isolation and characterization of a lymphatic leukemia virus in the Friend virus complex. J. Natl. Cancer Inst. 46:1209-1217.
35. **Stehelin, D., H. E. Varmus, J. M. Bishop, and P. K. Vogt.** 1976. DNA related to the transforming genes of avian sarcoma virus is present in normal avian DNA. Nature (London) 260:170-173.
36. **Taylor, J., R. Illmensee, and J. Summers.** 1976. Efficient transcription of RNA into DNA by avian sarcoma virus polymerase. Biochim. Biophys. Acta 442:324-330.
37. **Todaro, G. J, P. Arnstein, W. P. Parks, E. H. Lennette, and R. J. Huebner.** 1973. A type-C virus in human rhabdomyosarcoma cells after inoculation into antithymocyte serum-treated NIH Swiss mice. Proc. Natl. Acad. Sci. U.S.A. 70:859-862.
38. **Troxler, D. H., W. P. Parks, W. C. Vass, and E. M. Scolnick.** 1977. Isolation of a fibroblast nonproducer cell line containing the Friend strain of the spleen focus-forming virus. Virology 76:602-615.
39. **Vogt, P. K.** 1971. Spontaneous segregation of nontransforming viruses from cloned sarcoma viruses. Virology 46:939-946.
40. **Wang, L. H., P. Duesberg, K. Beemon, and P. 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.