JOURNAL OF VIROLOGY, May 1983, p. 606–613
0022-538X/83/050606-08$02.00/0

Vol. 46, No. 2

The Parodi-Irgens Feline Sarcoma Virus and Simian Sarcoma Virus Have Homologous Oncogenes, but in Different Contexts of the Viral Genomes

PETER BESMER,[1]* HARRY W. SNYDER, JR.,[1] JOHN E. MURPHY,[1] WILLIAM D. HARDY, JR.,[1] AND ANDRE PARODI[2]

Memorial-Sloan-Kettering Cancer Center, New York, New York 10021,[1] and Ecole Nationale Veterinaire, 94702 Maisons-Alfort, France[2]

Received 2 December 1982/Accepted 16 February 1983

We have identified the oncogene and the putative transforming protein of the Parodi-Irgens feline sarcoma virus (PI-FeSV). The PI-FeSV is defective and needs a helper virus for its replication. The v-*onc* sequences in the PI-FeSV were found to be related to the v-*sis* sequences of the simian sarcoma virus (SSV). PI-FeSV nonproducer cells express two viral RNAs, a 6.8- and a 3.3-kilobase RNA. The 6.8-kilobase RNA contains *gag*, *sis*, and *env* sequences but lacks the *pol* gene. The 3.3-kilobase RNA, on the other hand, contains only *env* sequences. We have detected one feline leukemia virus-related protein product in these cells, namely, a 76-kilodalton protein which contains determinants of the feline leukemia virus *gag* proteins p15 and p30. The v-*sis* sequences in the PI-FeSV have been located near the 5′ end of the viral genome. Taken together, these results imply that the p76 protein contains both feline leukemia virus *gag* and *sis* sequences and probably is the transforming protein of this virus. In contrast, in SSV the *sis* sequences are located towards the 3′ end of the viral genome, and the *sis* protein is thought to be expressed via a subgenomic RNA. PI-FeSV and SSV therefore use different schemes to express their *onc*-related sequences. The v-*sis* sequences in the PI-FeSV contain restriction sites which reflect the different origin of the v-*sis* sequences in the PI-FeSV and SSV. The homologous oncogenes of the PI-FeSV and SSV thus were transduced by two different retroviruses, feline leukemia virus and the simian sarcoma-associated virus, apparently from the genomes of different species.

Acute transforming retroviruses have been isolated from a number of vertebrate species, from naturally occurring tumors of animals with frequent retrovirus infections or after in vivo passage of leukemia viruses in laboratory animals. It is now widely believed that the oncogenes (v-*onc* sequences) of the acute transforming retroviruses have been acquired by transduction of cellular genes (5, 44). Such cellular homologs are highly conserved in the animal kingdom; thus, they probably serve important functions in cellular physiology or differentiation. To date, 16 different retroviral oncogenes have been identified in at least 31 virus strains. Of these 31, 18 were isolated from chickens, 1 was isolated from a turkey, and 12 were isolated from various mammals. Several retroviral oncogenes are shared by more than one virus strain isolated from the same species, but only twice have homologous v-*onc* sequences been found in viruses isolated from different species (1, 38).

onc sequences are arranged in different ways in the genomes of acute transforming retroviruses; thus, different strategies are used for their expression (19, 36, 44). In most cases, the primary translation products of the *onc* sequences are protein fusions with various viral gene products at their N-terminus (6, 42, 45). Less frequently, the v-*onc* translation products derive entirely from the v-*onc* sequences, as is the case for Rous avian sarcoma virus, Harvey murine sarcoma virus, and Kirsten murine sarcoma virus (8, 44). Most viruses with homologous v-*onc* sequences have a similar oncogenic spectrum, and their v-*onc* sequences are expressed in analogous fashion (16).

Feline leukemia virus (FeLV) is horizontally transmitted between domestic cats and has been shown to be the etiological agent for lymphosarcoma and a variety of other feline diseases (18, 44). Five feline sarcoma viruses (FeSVs) have been isolated from naturally occurring FeLV-associated feline fibrosarcomas (22, 30, 44). Three of them, the GA-, the ST-, and the SM-FeSV, have been characterized extensively (10, 12, 37). The ST- and the GA-FeSV contain the

oncogene *fes* (13), which is related to the avian oncogene *fps* (38). The SM-FeSV contains the oncogene *fms* (10). The Parodi-Irgens FeSV (PI-FeSV) was isolated in 1973 from an 8-month-old FeLV-infected pet cat with a naturally occurring multicentric fibrosarcoma (22). Simian sarcoma virus (SSV) was isolated from a naturally occurring sarcoma of a pet woolly monkey and is the only acute transforming retrovirus which derived from a primate (43). SSV originated by recombination of the simian sarcoma-associated virus and woolly monkey c-*sis* sequences (33, 48). In the present communication, we report that the oncogene of the PI-FeSV is homologous to the SSV oncogene *sis*, that the *sis* sequences in PI-FeSV and SSV are in different contexts of the viral genomes, and that PI-FeSV specifies a *gag* polyprotein.

MATERIALS AND METHODS

Cells and viruses. NIH 3T3 (23), BALB 3T3 (21), FRE-3a rat (obtained from J. R. Stephenson), mink CCL64 (20), and FG10 S+L− (2) cells were grown in Dulbecco modified Eagle medium supplemented with 10% calf serum, and feline embryo fibroblasts (FEF) were grown in McCoy medium supplemented with 15% fetal calf serum.

The isolation of the PI-FeSV has been described in a previous report. Cloned Moloney murine leukemia virus (Mo-MuLV) and amphotropic murine leukemia virus (292 amph-MuLV [14])-producing cell lines originated from D. Baltimore's laboratory. Transformation assays were performed according to standard procedures. Transformed cells were cloned in 0.25% agarose or 0.3% agar by the endpoint dilution method. amph-MuLV and Mo-MuLV titers were determined by using FG10 S+L− indicator cells, and reverse transcriptase assays were performed as described previously (3).

Analysis of integrated provirus genome. High-molecular-weight DNA from PI-FeSV-infected and uninfected cells was prepared by the method of Steffen et al. (41). For gel analysis, 10 μg of DNA was digested with restriction enzymes according to the supplier's instructions. Restriction enzymes were purchased from New England Biolabs and from Bethesda Research Laboratories. Digested DNA samples were fractionated by electrophoresis in 1% agarose (Seakem) in Tris-acetate pH 7.9 (40 mM Tris [pH 7.9]–5 mM sodium acetate–1 mM EDTA). The DNA was denatured and transferred to nitrocellulose as described by Southern (40). Baked nitrocellulose filters were prehybridized in 6× SSCPE (0.72 M NaCl–0.09 M sodium citrate–0.075 mM H_2PO_4–0.0006 M EDTA [pH 7.2]), 50% formamide (vol/vol), 5× Denhardt solution, 0.25 mg of yeast RNA per ml, 250 μg of denatured salmon sperm DNA per ml, and 0.1% SDS at 42°C for 12 h. Hybridization was done in 6× SSCPE, 50% formamide, 1× Denhardt solution, 0.25 mg of yeast RNA per ml, 0.125 mg of salmon sperm DNA per ml, 0.1% sodium dodecyl sulfate (SDS), and 10 ng of ^{32}P-labeled hybridization probe per kilobase pair per ml (5×10^8 cpm/μg) at 42°C for 48 h. Low-stringency hybridizations were done at 35°C. The blots were washed three times for 30 min each at 25°C in 2× SSC–0.1% SDS (pH 7.4), twice at 65°C, and, for hybridization with FeLV probes, twice at 66°C in 0.2× SSC–0.1% SDS (SSC: 0.15 M NaCl plus 0.015 M sodium citrate). For low-stringency hybridizations, the blots were washed four times at 25°C and three times at 55°C in 2× SSC–0.1% SDS. After blotting, dry filters were exposed at −70°C with Kodak XAR-5 film and intensifying screens.

Analysis of cytoplasmic RNAs by blot hybridization procedures. Cytoplasmic polyadenylated [poly(A)$^+$] RNA from nonproducer cells was prepared by the urea-SDS method as described previously (4). RNA (5 μg) was denatured with glyoxal and dimethyl sulfoxide and then was fractionated on a 1% agarose gel in 10 mM phosphate (pH 7.0) according to the method of McMaster and Carmichael (26). The RNA was transferred to diazophenyl-thioether paper, and the RNA-containing filters were hybridized with ^{32}P hybridization probes as described previously (4). For hybridizations with FeLV hybridization probes, the filters were washed twice each at 66°C for 20 min in 0.2× SSCPE–0.1% SDS. For low-stringency hybridizations, the filters were washed twice in 0.2× SSCPE–0.1% SDS at room temperature.

Hybridization reagents. The viral insert of FeLV B was subcloned into the EcoRI site in the chloramphenicol resistance gene of the plasmid pBR325 (pB-FeLV-B). The v-*fes* sequences in the ST-FeSV DNA genome contain several *Pst*I restriction sites (37). Two *Pst*I fragments, 0.5 and 0.55 kilobase pairs (kb), respectively (2.3 to 2.8 and 2.8 to 3.45 kb on the ST-FeSV map), contain 80% of the v-*fes* sequences. A recombinant plasmid, pB-v-*fes*, was derived which contains both the 0.5- and the 0.55-kb *Pst*I fragments of ST-FeSV. This plasmid was designated pB-v-*fes*. Recombinant plasmids containing fragments of the SSV genome were obtained from K. Robbins and S. A. Aaronson (31). The two *sis*-specific fragments *Sac*I-*Xba* (1.05 kb) and *Pst*I-*Xba* (1.0 kb) were prepared by double digestion with the enzymes *Sac*I and *Xba* and *Pst*I-*Xba*, respectively, from the *Sac*I-*Eco*RI and the *Pst*I SSV plasmids. Plasmids were prepared essentially by the cleared lysate method of Rambach and Hogness (29) and centrifugation in CsCl-ethidium bromide gradients.

The following DNAs were used as hybridization reagents: plasmid pBFeLV-B as an FeLV rep probe; plasmid pB-v-*fes* as a v-*fes*-specific probe; the 1.05- and the 1.0-kb *Sac*I-*Xba* and *Pst*I-*Xba* v-*sis* fragments as v-*sis* probes; the FeLV *Bgl*II-*Bam*HI fragment (1.13 to 2.1 kb of FeLVb) which contains the coding sequences for the FeLV *gag* proteins p12 and part of p27 as 5′ *gag*-specific probe; the FeLV *Bam*HI-*Bgl*II fragment (2.1 to 4.6 kb of FeLV-B) which contains the coding sequences for the C-terminal half of p27, p19, and part of the *pol* gene as 3′ *gag-pol*-specific probe; and the FeLV *Hin*dIII fragment (5.4 to 7.2 kb of FeLVB) as *env*-specific probe. DNA probes were labeled by nick translation according to the method of Maniatis et al. (25) to a specific activity of 5×10^8 cpm/μg, using [^{32}P]TTP at 3,000 Ci/mmol.

RESULTS

Biological properties of the PI-FeSV. Feline embryo fibroblasts infected with the PI-FeSV complex were obtained from Andre Parodi. Virus stock derived from FEF cells infected with

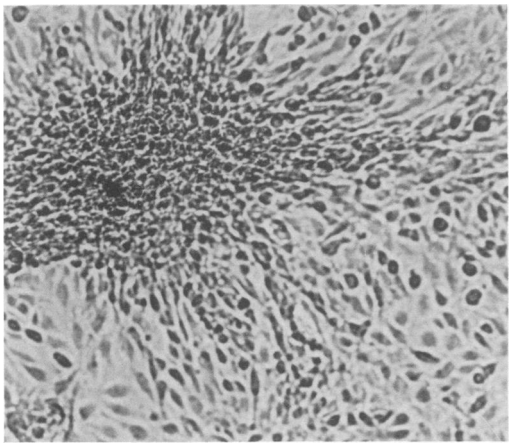

FIG. 1. Morphology of FRE cells transformed by the PI-FeSV: focus induced by PI-FeSV (amph-292-MuLV) 12 days after infection (×32).

PI-FeSV contained 3×10^2 focus-forming units per ml of PI-FeSV and a 1×10^3 to 5×10^3-fold excess of a nontransforming helper virus. The helper virus in the PI-FeSV stock, designated PI-FeSV-associated virus, was isolated by end-point dilution on FEF cells. Interference tests done by O. Jarrett in Glasgow indicated that the PI-FeSV-associated virus belongs to the FeLV-B subgroup (34, 35). To extend the host range of the PI-FeSV complex, amphotropic murine leukemia virus pseudotypes were made of the PI-FeSV, which then were used to infect heterologous cells. Foci of transformed cells were detected on BALB 3T3, NIH 3T3 mouse, and FRE rat cells, but not on mink CCL64 cells, a cell line commonly used in transformation assays. A focus of FRE-3a rat cells transformed by PI-FeSV is shown in Fig. 1. Foci are compact and mounded and consist of nonrefractile cells. Foci induced by the PI-FeSV are easily distinguished from those induced by most other acute transforming retroviruses on the basis of their characteristic focus morphology. To clone the PI-FeSV, FRE cells were infected with PI-FeSV amph-MuLV pseudotypes and transferred into soft agar, and colonies of transformed cells were picked and grown up to mass culture for analysis. Of the transformants, 75% were found to be virus nonproducers, that is, they did not release virus particles detectable by the reverse transcriptase assay; nor, when tested, did they release infectious virus. Superinfection of the NP transformants with amph-MuLV or with Mo-MuLV produced 10^3 to 10^4 focus-forming units of PI-FeSV per ml, as determined by the focus assay. The morphology of the foci induced by the rescued sarcoma virus was identical to that induced by the original PI-FeSV complex. These results demonstrate the replication-defective nature of the PI-FeSV.

Identification of the PI-FeSV v-*onc* sequences. To characterize the v-*onc* sequences of the PI-FeSV, we first analyzed the integrated PI-FeSV genome in the cellular DNA of rat NP cells. High-molecular-weight DNA from PI-FeSV-infected rat cells (RP-5 cells) and from uninfected rat cells was digested with the restriction enzymes *Eco*RI, *Hin*dIII, and *Sac*I and analyzed by blot hybridization. The following FeLV-related restriction fragments were detected: *Eco*RI, 11 and 7.1 kb; *Hin*dIII, 9.6 and 6 kb; and *Sac*I, 5.1, 3.9 (faint), and 2.2 kb (Fig. 2A). With a *gag*-specific probe, the 7.1-kb *Eco*RI, the 9.6-kb *Hin*dIII, and the 5.1-kb *Sac*I fragments were detected, and with an *env*-specific probe, the 11-kb *Eco*RI and the 5.2-kb *Sac*I fragments were detected (data not shown). Furthermore, two *Eco*RI fragments each, of various sizes, were found in the DNAs of five different PI-FeSV NP cell lines. The simplest interpretation of these results is that the NP cell lines contained one PI-FeSV provirus and that the provirus contained one site for the restriction endonuclease *Eco*RI and two sites each for *Hin*dIII and *Sac*I.

To characterize the v-*onc* sequences of the PI-FeSV, we undertook to determine whether they were related to any of the known retroviral oncogenes. Two distinct oncogenes have been identified in acute feline retroviruses, the v-*fes* gene in the GA- and the ST-FeSVs, and the v-*fms* gene found in the SM-FeSV (19). The v-*fes* gene is homologous with the avian oncogene v-*fps*, which is found in the avian sarcoma virus FSV, PRCII, PRCIV, UR1, and 16L (28, 44). Because v-*fes* and v-*fps* are the most abundant oncogenes in viruses isolated from cats and chickens, we first determined whether the transforming sequences of the PI-FeSV were related to v-*fes*. A duplicate DNA blot containing DNA from PI-FeSV-infected rat cells and from uninfected rat cells (Fig. 2A) was hybridized with a v-*fes* probe to determine whether the fragments containing PI-FeSV sequences identified by the FeLV *rep* probe also contained v-*fes* sequences. Hybridization with the *fes* hybridization probe revealed only rat c-*fes* sequences that were present in the DNA samples of both infected and uninfected rat cells (Fig. 2C). Since no *fes* sequences were found to be associated with FeLV sequences, the PI-FeSV did not contain a *fes* oncogene.

We then determined the relationship of the PI-FeSV v-*onc* with the *sis* sequences of SSV. The woolly monkey-derived SSV is the only acute transforming virus which contains v-*sis* (33, 48). The v-*sis* sequences in SSV constitute a 1-kb segment which has been mapped toward the 3′ end of the viral genome (15, 33; see Fig. 6). A

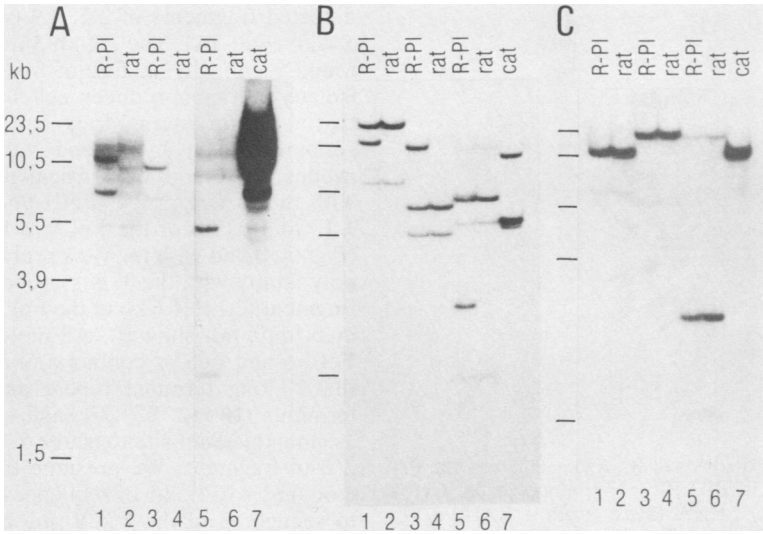

FIG. 2. Identification of *sis* sequences in the integrated PI-FeSV provirus by Southern blot analysis. DNA from RP-5 cells (R-PI) and from uninfected rat cells was digested with the restriction endonucleases *Eco*RI, *Hin*dIII, and *Sac*I. The DNAs were fractionated in 1% agarose gels and transferred to nitrocellulose paper. The filters were hybridized with the following ^{32}P-labeled hybridization probes: (A) $FeLV_{rep}$, (B) *sis*, and (C) *fes*. The DNAs analyzed were as follows: R-PI-DNA digested with *Eco*RI (lane 1), *Hin*dIII (lane 3), *Sac*I (lane 5), rat DNA digested with *Eco*RI (lane 2), *Hin*dIII (lane 4), *Sac*I (lane 6), FLF-3 cat DNA digested with *Eco*RI (lane 7). Migration of *Bam*HI-digested charon 4a DNA fragments is indicated at the left in kilobase pairs (kb).

duplicate DNA blot containing DNA from PI-FeSV-infected rat cells and from uninfected rat cells, as described above, was hybridized with a v-*sis* probe (*Sac*I-*Xba* restriction fragment, 4.0 to 5.0 kb of the SSV map [Fig. 2B]). Under relaxed hybridization conditions, the v-*sis* probe detected rat c-*sis* sequences which were present in the DNAs of both the uninfected and the infected rat cells and DNA restriction fragments which were detected only in the DNA of the PI-FeSV infected cells, namely, an 11- and a 7.1-kb *Eco*RI fragment, a 9.6-kb *Hin*dIII fragment, and a 5.1- and a 2.2-kb *Sac*I fragment. When comparing the sizes of the restriction fragments detected by the FeLV probe and those detected by the v-*sis* probe, it is apparent that all of the v-*sis*-containing fragments also contained FeLV sequences, demonstrating that the FeLV and the v-*sis* sequences are linked in the PI-FeSV genome. The size of the c-*sis*-containing restriction fragments in *Eco*RI-cleaved FRE cat DNA and cat DNA (Fig. 2, lanes 2 and 7) is in agreement with data published by others (33) indicating that our *sis* hybridization probe is authentic.

The PI-FeSV v-*sis* sequences detected by the SSV v-*sis* *Sac*I-*Xba* probe contained sites for the restriction enzymes *Eco*RI and *Sac*I (Fig. 2B, lanes 1 and 5), and the cellular homolog of v-*sis* in the cat genome contained two internal *Eco*RI sites generating fragments of 4.0, 4.3, and 8.0 kb (Fig. 2B, lane 7). The SSV v-*sis* insert, on the other hand, does not contain sites for *Eco*RI and *Sac*I, and the woolly monkey and the human homologs of v-*sis* do not contain internal *Eco*RI sites (33). These observations suggest that the PI-FeSV v-*sis* sequences are more closely related to the cat c-*sis* than to primate c-*sis* sequences, and they are consistent with a feline origin of PI-FeSV v-*sis*.

Transcriptional and transitional products of the PI-FeSV genome. We have analyzed viral transcription in a BALB-PI-FeSV NP cell line, which contains a single provirus (data not shown) by blotting analysis. Hybridization of ^{32}P-labeled DNA fragments corresponding to various regions of the FeLV-B genome to blots containing cytoplasmic poly(A)$^+$ RNA is shown in Fig. 3. Two RNA species were detected with the $FeLV_{rep}$ probe, a 6.8- and a 3.3-kb RNA. The same two RNAs were also detected with an *env*-specific probe (5.4 to 7.2 kb of the FeLV B genome). The 5′-*gag* and the 3′-*gag-pol* probes, 1.1 to 2.1 kb and 2.1 to 4.6 kb of the FeLV-B genome, respectively, and the v-*sis* probe, reacted only with the 6.8-kb RNA; however, hybridization of the 3′ *gag-pol* probe with the 6.8-kb RNA was very weak, indicating a minor homology of 0.1 to 0.2 kb. The 6.8-kb RNA which presumably is the genome of the PI-FeSV thus contains *gag*, *sis*, and *env* sequences but lacks most of the *pol* gene. The 3.3-kb RNA only

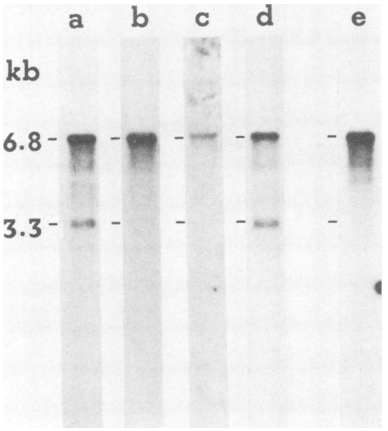

FIG. 3. Blot analysis of RNAs specified by the PI-FeSV genome in BALB/c nonproducer cells. Cytoplasmic poly(A)$^+$ RNA (5 μg) from a BALB/c PI-FeSV nonproducer cell line was denatured with dimethyl sulfoxide, glyoxal fractionated in a 1% agarose gel, and transferred to diazophenyl thioether paper. The blot was hybridized with the following ^{32}P-labeled hybridization probes: (a) FeLV$_{rep}$, (b) FeLV 5′ *gag*, (c) FeLV 3′ *gag-pol*, (d) FeLV *env*, and (e) *sis*.

contains *env* sequences, suggesting that it is a subgenomic RNA. Our results imply that the 6.8-kb RNA is the mRNA for the synthesis of the *sis* protein of the PI-FeSV.

To identify PI-FeSV-specific proteins in transformed nonproducer rat cells, immunoprecipitation assays were performed. Cellular proteins were radiolabeled by incubating cells in the presence of [^{35}S]methionine and then were subjected to immunoprecipitation from cell extracts with antisera to FeLV structural proteins. A 76,000-dalton protein was precipitated with rabbit antiserum against FeLV p27 (Fig. 4). The same protein was also detected by immunoprecipitation with anti-FeLV p15 but not with normal rabbit serum or rabbit anti-FeLV gp70 serum (data not shown). The 76,000-dalton protein was associated with PI-FeSV infection in five of five rat nonproducer clones analyzed, and it was not found in uninfected FRE cells (data not shown).

Mapping of the *sis* sequences in the PI-FeSV genome. The 1.05-kb segment of v-*sis* sequences in SSV has been localized towards the 3′ end of the viral genome (15, 31). To determine the location of v-*sis* in the PI-FeSV genome, Southern blots containing cellular DNA of PI-FeSV NP cells and of uninfected FRE rat cells were hybridized with probes specific for different regions of the FeLV genome and with the SSV v-*sis* hybridization probe (Fig. 5). Upon hybridization of DNA digested with the restriction enzyme *Sac*I, the FeLV$_{rep}$ hybridization probe detected fragments of 2.2, 3.9 (very faint), and 5.1 kb (Fig. 5A). The 2.2-kb *Sac*I fragment was found identically in five of five independently isolated rat nonproducer cell lines, indicating that it was an internal fragment of the PI-FeSV genome. When hybridized with the various probes, the 2.2-kb *Sac*I fragment was detected with the 5′ *gag* probe (*Bgl*II-*Bam*HI fragment; 1.13 to 2.09 kb of the FeLV-B restriction map [Fig. 5C]) and with the v-*sis* probe (Fig. 5B), but only faintly with the 3′ *gag* probe (*Bam*HI-*Bgl*II fragment; 2.1 to 4.6 kb of the FeLV B restriction map [data not shown]). All molecularly cloned FeLVs and FeSVs contain a *Sac*I site between the 5′ long terminal repeat and the *gag* N-terminus (10, 12, 27, 37), and by analogy, we assume this *Sac*I site to be the 5′ terminus of the 2.2-kb fragment. We presume this fragment to contain 1.4 to 1.5 kb of sequences corresponding to sequences of the FeLV *gag* genes p15, p12, and p27 (17), and 0.7 to 0.8 kb of the v-*sis* gene. Therefore, in contrast to SSV, the PI-FeSV *sis* sequences are located near the 5′ end of the viral genome.

DISCUSSION

The studies reported here indicate that PI-FeSV and SSV were generated by transduction of the same or a closely related cellular gene from different species with FeLV and SSAV, respectively. The PI-FeSV is thought to be of

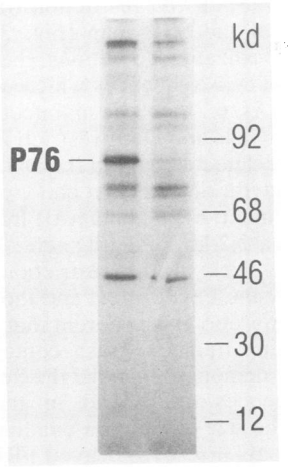

FIG. 4. SDS-polyacrylamide gel electrophoresis of [^{35}S]methionine-labeled proteins in an extract of FRE cells nonproductively transformed by PI-FeSV which are precipitable with 5 μl of rabbit anti-FeLV p27 serum (left lane) and normal rabbit serum (right lane). Proteins in *S. aureus*-bound immunoprecipitates were subjected to electrophoresis in a step gradient slab gel of 7.5 to 12.5% acrylamide and were visualized by autoradiography according to published protocols (39).

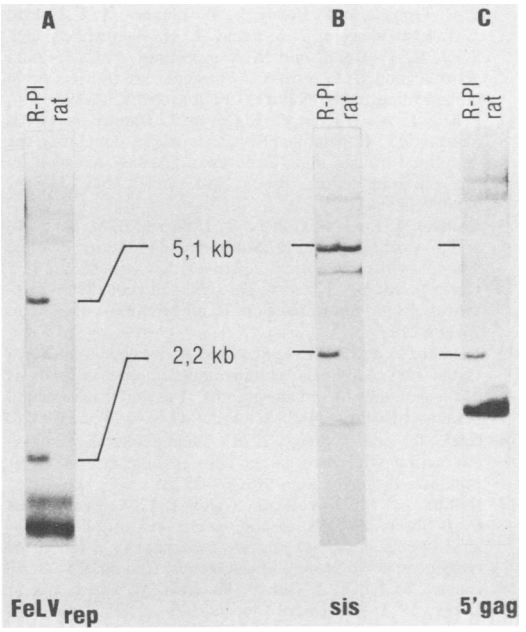

FIG. 5. Mapping of the *sis* sequences in the PI-FeSV genome. Southern blots with DNA from PI-FeSV-infected cells (R-PI, lane 1) and from uninfected rat cells (lane 2) digested with the restriction endonuclease *Sac*I were hybridized with the following ^{32}P-labeled hybridization probes: (A) FeLV$_{rep}$, (B) *sis*, and (C) FeLV 5′ *gag*.

feline origin for the following reasons: (i) the virus was isolated from a pet cat with multicentric fibrosarcoma; (ii) PI-FeSV derives from FeLV; and (iii) the v-*sis* sequences in the PI-FeSV contain restriction sites which are characteristic for the cat c-*sis* locus.

To date, at least 16 different retroviral oncogenes are known which can be distinguished by their nucleic acid sequence (44). Viruses isolated independently from the same species are frequently found to contain related oncogenes. Only twice has homology been reported between retroviral oncogenes from different species (1, 38). The results presented here and the recent finding that there is homology between the Abelson-MuLV v-*abl* sequences and those of another new FeSV isolate (P. Besmer and W. D. Hardy, manuscript in preparation) provide further evidence for the transduction of homologous cellular genes from different species by retroviruses. The increasing number of reisolations of oncogenes in viruses originating from the same species, as well as in viruses from different species, as reported here, is in agreement with the idea that the number of cellular oncogenes that can be transduced by retroviruses is limited.

Our analysis of the structure of the PI-FeSV genome shows that the v-*sis* sequences in this virus are located toward the 5′ end of the viral genome. The boundary between the FeLV 5′ and the v-*sis* sequences predicts that the 76,000-dalton protein we have identified is a *gag-sis* fusion protein (Fig. 6). We presume, therefore, that the *gag-sis* 76,000-dalton fusion protein is the transforming protein of the PI-FeSV. By contrast, in SSV the v-*sis* sequences are located toward the 3′ end of the viral genome, and they are expressed via a subgenomic message (11). The recently published nucleic acid sequence of v-*sis* predicts a 25,000-dalton *sis* protein which contains a short N-terminal viral *env* leader sequence (9, 32). Thus, it appears that the *sis* protein sequences of the PI-FeSV and of SSV are expressed in different contexts of the retroviral genome. In most acute transforming retroviruses which contain homologous *onc* sequences, these sequences are expressed in analogous fashion (16). The PI-FeSV and SSV thus provide an example of homologous oncogenes being expressed with different strategies. Similarly, different strategies have been reported for the expression of the v-*myb* gene in the avian retroviruses AMV and E26 (7).

Our tissue culture characterization of the PI-FeSV showed that the PI-FeSV and SSV displayed indistinguishable behavior. The same focus morphology is observed with transformation induced by SSV and PI-FeSV, and mink cells support the replication of both viruses but are not transformed by either (20; P. Besmer and J. E. Murphy, unpublished data). SSV has been shown to induce well-differentiated fibrosarcomas and fibromas in marmosets when administered intramuscularly (47) and gliomas when injected intracerebrally (46). Only fibrosarcomas were obtained upon injection of kittens with the PI-FeSV; no intracerebral inoculations, however, have been performed with this virus (22). Thus, whatever the mode of expression, the v-

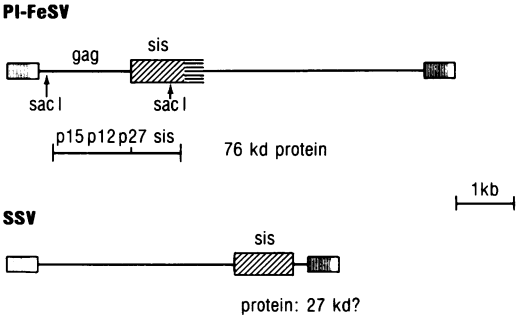

FIG. 6. Physical maps of PI-FeSV and SSV. The positions of the *sis* sequences are indicated by cross-hatched lines.

sis product appears to act similarly in both SSV and PI-FeSV.

Three different subgroup FeLVs, A, B, and C, have been isolated from FeLV-infected pet cats (34, 35, 38). All isolates of FeLV-B and FeLV-C have been shown also to contain FeLV-A (24). Thus, although we have isolated a FeLV-B subgroup virus by endpoint dilution from the PI-FeSV complex, we cannot rule out the possibility that an FeLV-A virus was involved in the generation of the PI-FeSV.

ACKNOWLEDGMENTS

We thank C. J. Sherr for the recombinant phages λ-FeLV-B and λ-ST-FeSV and for hybridizing a Southern blot with his *fms*-specific probe. K. C. Robbins, Yen Li, S. P. Goff, D. Dina, and R. Ellis provided the *onc* probes used in this study. We thank E. Fleissner and the members of the Viral Oncology Laboratory for discussion, and E. Enea and A. Bothwell for their comments on this manuscript. We also thank Peter Bergold and Lucy Li for subcloning fragments of the FeLV-B genome, Nancy Hopkins for her help with DNA transfections early in this project, and O. Jarrett for the subgroup determination of the PIAV.

This work was supported by American Cancer Society grant MV-82 to P.B. and by Public Health Service grant CA-16599 to H.S. and core grant CA-08748 to P. Marks from the National Cancer Institute. H.W.S. is a Scholar of the Leukemia Society of America, Inc.

LITERATURE CITED

1. **Andersen, P. R., S. G. Devare, S. R. Tronick, R. W. Ellis, S. A. Aaronson, and E. M. Scolnick.** 1981. Generation of Balb-MusV and Ha-MuSV by type C virus transduction of homologous transforming genes from different species. Cell **26**:129–131.
2. **Bassin, R. H., N. Tuttle, and P. J. Fischinger.** 1971. Rapid cell culture assay technique for murine leukemia viruses. Nature (London) **269**:56.
3. **Besmer, P., and D. Baltimore.** 1977. Mechanism of restriction of ecotropic and xenotropic murine leukemia viruses and formation of pseudotypes between the two viruses. J. Virol. **21**:965–973.
4. **Besmer, P., H. Fan, M. Paskind, and D. Baltimore.** 1979. Isolation and characterization of a mouse cell line containing a defective Moloney murine leukemia virus genome. J. Virol. **29**:1023–1034.
5. **Bishop, J. M.** 1981. Enemies within: the genesis of retrovirus oncogenes. Cell **23**:5–6.
6. **Bister, K., M. J. Hayman, and P. K. Vogt.** 1977. Defectiveness of avian myelocytomatosis virus MC29: isolation of long term nonproducer cultures and analysis of virus specific polypeptide synthesis. Virology **82**:431–448.
7. **Bister, K., M. Nunn, C. Moscovici, B. Perbal, M. A. Baluda, and P. H. Duesberg.** 1982. Acute leukemia viruses E26 and avian myeloblastosis virus have related transformation specific RNA sequences, but different genetic structures, gene products and oncogenic properties. Proc. Natl. Acad. Sci. U.S.A. **79**:3677–3681.
8. **Dahr, R., R. W. Ellis, T. Y. Shih, S. Oroszlan, J. Maizel, D. Lowy, and E. M. Scolnick.** 1982. Nucleotide sequence of the p21 transforming protein of Harvey murine sarcoma virus. Science **217**:934–937.
9. **Devare, S. G., E. P. Reddy, K. C. Robbins, P. R. Andersen, S. R. Tronick, and S. A. Aaronson.** 1982. Nucleotide sequence of the transforming gene of simian sarcoma virus. Proc. Natl. Acad. Sci. U.S.A. **79**:3179–3182.
10. **Donner, L., L. A. Fedele, C. F. Garon, S. J. Anderson, and C. J. Sherr.** 1982. McDonough feline sarcoma virus: characterization of the molecularly cloned provirus and its feline oncogene (V-*fms*). J. Virol. **41**:489–500.
11. **Eva, A., K. C. Robbins, P. R. Andersen, A. Srinivasan, S. R. Tronick, E. P. Reddy, N. W. Ellmore, A. T. Galem, J. A. Lautenberger, T. S. Papas, E. H. Westin, F. Wong-Staal, R. C. Gallo, and S. A. Aaronson.** 1982. Cellular genes analogous to retroviral onc genes are transcribed in human tumor cells. Nature (London) **295**:116–119.
12. **Fedele, L. A., J. Even, C. F. Garon, L. Donner, and C. J. Sherr.** 1981. Recombinant bacteriophages containing the integrated transforming provirus of Gardner-Arnstein feline sarcoma virus. Proc. Natl. Acad. Sci. U.S.A. **78**:4036–4040.
13. **Frankel, A. E., J. H. Gilbert, K. J. Porzig, E. M. Scolnick, and S. A. Aaronson.** 1979. Nature and distribution of feline sarcoma virus nucleotide sequences. J. Virol. **30**:821–827.
14. **Gardner, M. B.** 1978. Amphotropic and ecotropic type C viruses from wild mice. Curr. Top. Microbiol. Immunol. **70**:215–219.
15. **Gelman, E. P., F. Wong-Staal, R. A. Kramer, and R. C. Gallo.** 1981. Molecular cloning and comparative analysis of the genomes of simian sarcoma virus and its associated helper virus. Proc. Natl. Acad. Sci. U.S.A. **78**:3373–3378.
16. **Graf, T., and J. Beug.** 1978. Avian leukemia viruses: interaction with their target cells in vivo and in vitro. Biochim. Biophys. Acta **516**:269–299.
17. **Hampe, A., I. Laperotte, F. Galibert, L. A. Fedele, and C. J. Sherr.** 1982. Nucleotide sequences of feline retroviral oncogens (v-fes) provide evidence for a family of tyrosine-specific protein kinase genes. Cell **30**:775–785.
18. **Hardy, W. D., L. J. Old, P. W. Hess, M. Essex, and S. Cotter.** 1973. Horizontal transmission of feline leukemia virus. Nature (London) **244**:266–269.
19. **Hayward, W. S.** 1977. Size and genetic content of viral RNAs in avian oncovirus-infected cells. J. Virol. **24**:47–63.
20. **Henderson, I. C., M. M. Lieber, and G. Todaro.** 1974. Mink cell line MvlLU(CCL64). Focus formation and the generation of nonproducer transformed cell lines with murine feline sarcoma viruses. Virology **60**:282–287.
21. **Hopkins, N., P. Besmer, A. DeLeo, and L. W. Law.** 1981. High frequency cotransfer of the transformed phenotype and a tumor specific transplantation antigen by DNA from the 3-methylcholathrene induced Meth A sarcoma of Balb/c mice. Proc. Natl. Acad. Sci. U.S.A. **78**:7555–7559.
22. **Irgens, K., M. Wyers, A. Moraillon, A. Parodi, and V. Fortuny.** 1973. Isolement d'un virus sarcomatogene felin a partir d'un fibrosarcome spontane du chat: etude du pouvoir sarcomatogene in vivo. C. R. Acad. Sci. Paris. **26**:1783–1786.
23. **Jainchill, J. L., S. A. Aaronson, and G. Todaro.** 1969. Murine sarcoma and leukemia viruses: assay using clonal lines of contact-inhibited mouse cells. J. Virol. **4**:549–553.
24. **Jarrett, O.** 1980. p. 473–479. *In* W. D. Hardy, M. Essex, and A. J. McClelland (ed.), Feline leukemia virus subgroups. Elsevier/North Holland Publishing Co., Amsterdam.
25. **Maniatis, T., A. Jeffrey, and H. Vande Sande.** 1975. Chain length determination of small double and single stranded DNA molecules by polyacrylamide gel electrophoresis. Biochemistry **14**:3787–3794.
26. **McMaster, G., and G. Carmichael.** 1977. Analysis of single stranded and double stranded nucleic acids on acrylamide and agarose gels with glyoxal and acridine orange. Proc. Natl. Acad. Sci. U.S.A. **74**:4835–4838.
27. **Mullins, J. I., J. W. Casey, M. O. Nicholson, K. B. Burck, and N. Davidson.** 1981. Sequence arrangement and biological activity of cloned feline leukemia virus proviruses from a virus-productive human cell line. J. Virol. **38**:688–703.
28. **Neel, B. G., L. H. Wang, B. Mathey-Prevot, T. Hanafusa, H. Hanafusa, and W. S. Hayward.** 1982. Isolation of 16L virus: a rapidly transforming sarcoma virus from an avian leukosis virus induced sarcoma. Proc. Natl. Acad. Sci. U.S.A. **79**:5088–5092.
29. **Rambach, A., and D. Hogness.** 1977. Translation of *Drosophila melanogaster* sequences in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **74**:5041–5045.

30. **Rasheed, S., M. Barbacid, S. A. Aaronson, and M. B. Gardner.** 1982. Origin and biological properties of a new feline sarcoma virus. Virology **117**:238–244.
31. **Robbins, K. C., S. G. Devare, and S. A. Aaronson.** 1981. Molecular cloning of integrated simian sarcoma virus: genomic organization of infectious DNA clones. Proc. Natl. Acad. Sci. U.S.A. **78**:2918–2922.
32. **Robbins, K. C., S. G. Devare, E. P. Reddy, and S. A. Aaronson.** 1982. In vitro identification of the transforming gene product of simian sarcoma virus. Science **218**:1131–1133.
33. **Robbins, K. C., R. L. Hill, and S. A. Aaronson.** 1982. Primate origin of the cell-derived sequences of simian sarcoma virus. J. Virol. **41**:721–725.
34. **Sarma, P. S. and T. Log.** 1971. Viral interference in feline leukemia-sarcoma complex. Virology **44**:352–358.
35. **Sarma, P. S., and T. Log.** 1973. Subgroup classification of feline leukemia and sarcoma viruses by viral interference and neutralization tests. Virology **54**:160–169.
36. **Sheiness, D., B. Vennstrom, and J. M. Bishop.** 1981. Virus-specific RNAs in cells infected by avian myelocytomatosis virus avian erythroblastosis virus: modes of oncogene expression. Cell **23**:291–300.
37. **Sherr, C. J., L. A. Fedele, M. Oskarsson, J. Maizel, and G. Vande Woude.** 1980. Molecular cloning of Snyder-Theilen feline leukemia and sarcoma viruses: comparative studies of feline sarcoma virus with its natural helper virus and with Moloney murine sarcoma virus. J. Virol. **34**:200–212.
38. **Shibuya, M., T. Hanafusa, H. Hanafusa, and J. R. Stephenson.** 1980. Homology exists among the transforming sequences of the avian and feline sarcoma viruses. Proc. Natl. Acad. Sci. U.S.A. **77**:6536–6540.
39. **Snyder, H. W., Jr.** 1982. Biochemical characterization of protein kinase activities associated with transforming gene products of the Snyder-Theilen and Gardner-Arnstein strains of feline sarcoma virus. Virology **117**:165–172.
40. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:513–517.
41. **Steffen, D., S. Bird, W. P. Rowe, and R. A. Weinberg.** 1979. Identification of DNA fragments carrying ecotropic proviruses of AKR mice. Proc. Natl. Acad. Sci. U.S.A. **76**:4554.
42. **Stephenson, J. R., A. S. Kahn, A. H. Sliski, and M. Essex.** 1977. Feline oncornavirus-associated cell membrane antigen: evidence for an immunologically cross-reactive feline sarcoma virus coded protein. Proc. Natl. Acad. Sci. U.S.A. **74**:5608–5612.
43. **Theilen, G. H., D. Gould, M. Fowler, and D. L. Dungworth.** 1971. C-type virus in tumor tissue of a woolly monkey (Lagothrix spp.) with fibrosarcoma. J. Natl. Cancer Inst. **47**:881–889.
44. **Weiss, R., N. Teich, H. Varmus, and J. Coffin.** 1982. RNA tumor viruses. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
45. **Witte, O. N., N. Rosenberg, M. Paskind, A. Shields, and D. Baltimore.** 1978. Identification of an Abelson murine leukemia virus-encoded protein present in transformed fibroblasts and lymphoid cells. Proc. Natl. Acad. Sci. U.S.A. **75**:2488–2492.
46. **Wolfe, L. G., and F. Deinhardt.** 1978. Primates. Medicine (Baltimore) **10**:96–118.
47. **Wolfe, L. G., F. Deinhardt, G. H. Theilen, H. Robin, T. Kawakami, and L. K. Bustad.** 1971. Induction of tumors in marmoset monkeys by simian sarcoma virus type 1 (Lagothrix): a preliminary report. J. Natl. Cancer Inst. **48**:1115–1120.
48. **Wong-Staal, F., R. Dalla Favera, E. P. Gelman, V. Manzari, S. Szala, S. F. Josephs, and R. C. Gallo.** 1981. The v-sis gene of simian sarcoma virus is a new onc gene of primate origin. Nature (London) **294**:273–275.