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Nature and Distribution of Feline Sarcoma Virus Nucleotide Sequences

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The genomes of three independent isolates of feline sarcoma virus (FeSV) were compared by molecular hybridization techniques. Using complementary DNAs prepared from two strains, SM- and ST-FeSV, common complementary DNAs were selected by sequential hybridization to FeSV and feline leukemia virus RNAs. These DNAs were shown to be highly related among the three independent sarcoma virus isolates. FeSV-specific complementary DNAs were prepared by selection for hybridization by the homologous FeSV RNA and against hybridization by feline leukemia virus RNA. Sarcoma virus-specific sequences of SM-FeSV were shown to differ from those of either ST- or GA-FeSV strains, whereas ST-FeSV-specific DNA shared extensive sequence homology with GA-FeSV. By molecular hybridization, each set of FeSV-specific sequences was demonstrated to be present in normal cat cellular DNA in approximately one copy per haploid genome and was conserved throughout Felidae. In contrast, FeSV-common sequences were present in multiple DNA copies and were found only in Mediterranean cats. The present results are consistent with the concept that each FeSV strain has arisen by a mechanism involving recombination between feline leukemia virus and cat cellular DNA sequences, the latter represented within the cat genome in a manner analogous to that of a cellular gene.

Sarcoma viruses have been isolated from a number of mammalian species. Accumulating evidence indicates that these viruses contain genetic information of a type C RNA helper leukemia virus (1, 4, 5, 8, 14, 17, 20, 34) as well as some distinct cellular DNA sequences (3, 11, 23-25). These observations have led to the concept that sarcoma viruses have arisen in nature by a mechanism involving recombination between a replicating helper virus and some cellular gene(s).

In outbred cats, three independent naturally occurring sarcoma virus isolates have been reported. These include Snyder-Theilen (ST) (29) and Gardner-Arnstein (GA) (15) feline sarcoma virus (FeSV) strains. A more recent isolate by McDonough and co-workers (19) has been designated SM-FeSV by Sarma et al. (22). These viruses each cause nonproductive transformation of mammalian cells in vitro (22) and induce tumors, principally fibrosarcomas, in vivo. By molecular approaches analogous to those used to characterize murine sarcoma viruses (11, 14, 23, 24), we have studied the genomes of the three FeSV's and investigated their relationship to one another as well as to the cellular genome of their species of origin.

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MATERIALS AND METHODS

Cells. Cells were grown in the Dulbecco-Vogt modification of Eagle medium supplemented with 10% heat-inactivated calf serum (Colorado Serum Co., Denver, Colo.). Cell lines included normal rat kidney (NRK) (9) and mink lung (Mv1Lu), from the American Type Culture Collection, Rockville, Md.; dog kidney (DK), feline embryo, and feline lymphoma FL74 (32) cell lines were also used. The isolation of SM-, ST-, and GA-FeSV nonproducer transformed NRK and Mv1Lu clonal lines has been reported (21).

Viruses. Early-passage stocks of SM-, ST-, and GA-FeSV (FeLV [feline leukemia virus]), respectively, were generously provided by P. Sarma, National Cancer Institute, Bethesda, Md., G. Theilen, University of California, Davis, Calif., and M. Gardner, University of Southern California, Los Angeles, Calif. $FeLA_A$, $FeLV_B$, and $FeLV_C$ subgroups were obtained from O. Jarrett, Glasgow, Scotland. These viruses were propagated in feline embryo, DK, or Mv1Lu cells. SM-FeSV (FeLV), used to prepare complementary DNA (cDNA), was obtained by propagating SM-FeSV

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(FeLV) in Mv1Lu cells. The viral supernatant had a titer of 3.7×10^4 focus-forming units per ml, or sarcoma virus titer, on mink cells, and 3.9×10^4 focus-forming units per ml, or leukemia helper virus titer, on S+L− murine sarcoma virus (MSV)-infected cat clone 81 cells (10a). The ST-FeSV (FeLV) virus used for cDNA preparation was produced from ST-FeSV (FeLV)-infected Mv1Lu cells. The virus mixture contained 3.2×10^6 focus-forming units per ml and 1.5×10^7 focus-inducing units per ml, or approximately a threefold excess of helper leukemia virus over sarcoma virus. The $FeLV_A$ and $FeLV_B$ subgroups were propagated in DK cells for production of cell pack for cellular RNA fractionations. The $FeLV_C$ subgroup was propagated in Mv1Lu cells for cellular RNA fractionation. The three FeLV subgroup-infected cell lines yielded supernatants of 10^5 to 10^6 focus-inducing units per ml per day. These feline sarcoma and leukemia bioassays were performed as described previously (21).

Preparation of FeSV cDNA. cDNA to the SM- and ST-FeSV (FeLV) was synthesized in an endogenous reaction utilizing sheared calf thymus DNA primer (2 mg/ml) and actinomycin D (100 μg/ml) (13, 31). The specific activity of a given cDNA was around 2×10^7 cpm/μg. Each cDNA was hybridized to ^{125}I-labeled 65S homologous viral RNA as previously described (7, 14). At a cDNA/RNA ratio of around 3:1, around 60 to 80% of the homologous viral RNA was protected from digestion with RNase T1.

cDNA fractionation scheme. Each FeSV (FeLV) total cDNA was hybridized to an appropriate RNA source, followed by fractionation of the annealed and unannealed portions on hydroxyapatite as previously described (14, 24). To select for FeSV sequences in each FeSV (FeLV) cDNA, around 5 μg of FeSV (FeLV) total cDNA was hybridized with 30 mg of cellular RNA from the homologous FeSV nonproducer transformed NRK line. After separation of the hybridized from the nonhybridized cDNA on hydroxyapatite, the hybridized portion, representing FeSV cDNA sequences, was sequentially hybridized to 30 mg of RNA of $FeLV_B$-infected DK cells and 30 mg of RNA of $FeLV_C$-infected Mv1Lu cells. The hybridizing portion from the first FeLV fractionation was designated SM- or ST-FeSV-common cDNA, and the cDNA that failed to hybridize to either FeLV RNA was designated SM- or ST-FeSV-specific cDNA. Around 78% of the unfractionated SM-FeSV (FeLV) and 50% of the unfractionated ST-FeSV (FeLV) cDNA hybridized with cellular RNA of the homologous FeSV-transformed nonproducer NRK line. Moreover, around 70% of each selected FeSV cDNA hybridized with RNA of $FeLV_B$-infected DK cells. Finally, approximately 30% of the remaining FeSV cDNA was further hybridized by $FeLV_C$ RNA. Hence, FeSV-common cDNA sequences represented around 55% of the SM-FeSV (FeLV) cDNA and 35% of the ST-FeSV (FeLV) cDNA. FeSV-specific cDNA's represented around 10 to 15% of the starting material.

Molecular hybridization. Viral and cellular RNAs were isolated according to methods previously described (16, 27). DNA was prepared from normal tissues of various species by a modification of the Thomas procedure (33). For cDNA-RNA hybridization, around 10^3 cpm of cDNA was incubated at 60°C with cellular RNA in a reaction containing 0.5 M NaCl to a C_rt of 10^4 mol·s/liter. Hybrids were assayed on hydroxyapatite as previously reported (11). For cDNA-DNA hybridization, the cDNA probe (10^3 cpm) was incubated at 70°C in a 100-μl reaction volume containing 2 mg of cellular DNA in 0.75 M NaCl. At varying times, samples were assayed on hydroxyapatite for hybrid formation (12). Unique-sequence cellular DNA reannealing was monitored for each hybridization. Thermal denaturation profiles were determined as previously reported (12).

RESULTS

Characterization of FeSV-specific and -common cDNA's. Each cDNA fraction was approximately 100 bases in length, based on cosedimentation with ^{32}P-labeled single-stranded lambda DNA markers in alkaline sucrose gradients. The SM-FeSV unfractionated and common cDNA's demonstrated $C_rt_{1/2}$ values of 0.05 mol·s/liter with SM-FeSV (FeLV) 65S RNA, whereas SM-FeSV-specific cDNA had a $C_rt_{1/2}$ of 0.1 mol·s/liter with the same viral RNA (Fig. 1). These data suggested that the viral mixture contained approximately equal amounts of sarcoma and leukemia virus RNA. ST-FeSV unfractionated and common cDNA's had $C_rt_{1/2}$ values of 0.05 mol·s/liter, whereas ST-FeSV-specific cDNA had a $C_rt_{1/2}$ of 0.15 mol·s/liter with the same ST-FeSV (FeLV) 65S RNA (Fig. 1). These findings suggested a 3:1 FeLV-to-FeSV RNA ratio in the ST-FeSV (FeLV) virus stock. None of the cDNA fractions appeared to represent a minor contaminant by C_rt analysis. Hybrids formed between the cDNA fractions and the respective FeSV (FeLV) 65S RNA demonstrated high T_m's (79 to 87°C), suggesting that the selected cDNA sequences were closely matched with sequences represented within the viral RNA (Fig. 2).

Relatedness of independent FeSV iso-

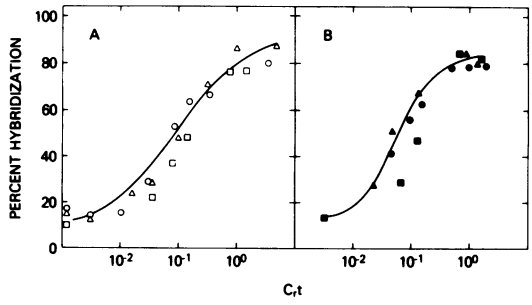

FIG. 1. *Association kinetics of SM- and ST-FeSV cDNA fractions using homologous 60-70S viral RNAs. Hybridization was performed as described in the text. (A) Hybridization of 60-70S SM-FeSV (FeLV) RNA to SM-FeSV (FeLV) DNA* (○), *SM-FeSV-common cDNA* (△), *and SM-FeSV-specific cDNA* (□). *(B) Hybridization of 60-70S ST-FeSV (FeLV) RNA to ST-FeSV (FeLV) cDNA* (●), *ST-FeSV-common cDNA* (▲), *and ST-FeSV-specific cDNA* (■).

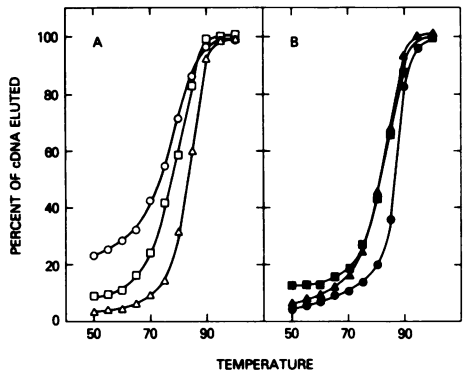

FIG. 2. *Thermal denaturization profiles of hybrids involving SM- or ST-FeSV cDNA fractions and their homologous 60-70S RNAs. Hybrids were subjected to increasing temperature and analyzed by hydroxyapatite as previously described (12). Symbols for A and B are the same as in the legend to Fig. 1.*

lates. The cDNA probes prepared from SM- and ST-FeSV were utilized to analyze nucleotide sequence relatedness among the three independent FeSV isolates. As shown in Table 1, SM-FeSV-specific cDNA exhibited extensive homology with cellular RNAs of SM-FeSV-transformed nonproducer Mv1Lu and NRK lines, but not with RNAs of the same lines transformed by either GA- or ST-FeSV. There was also little residual hybridization of SM-FeSV-specific cDNA by RNAs of cells infected with either of three FeLV subgroups.

ST-FeSV-specific cDNA was hybridized substantially by RNAs of Mv1Lu and NRK cells nonproductively transformed by ST-FeSV. As indicated in Table 1, ST-FeSV-specific cDNA was also hybridized to a large extent by cellular RNA of GA-FeSV nonproducer transformants as well. The same probe lacked significant homology with RNAs or uninfected or SM-FeSV-transformed parental lines or any of the three FeLV subgroups. These results indicated that sarcoma virus-specific sequences of ST- and GA-FeSV were related, yet readily distinguishable from those of SM-FeSV. In contrast, FeSV-common sequences were highly related among the three FeSV strains as well as to FeLV (Table 1).

Distribution of FeSV sequences among other type C viruses. SM- and ST-FeSV-specific cDNA's showed little homology to FeLV RNA (Table 1). Similarly, these cDNA's were not annealed by RNAs of several other helper viruses, including AKR, Moloney murine leukemia virus, HIX, simian sarcoma-associated virus (SSAV), and rat and mink endogenous viruses (Table 2). Finally, there was no significant hybridization of these probes by mammalian transforming virus RNAs, including those of Abelson lymphosarcoma virus, Moloney MSV, Harvey MSV, and simian sarcoma virus.

The FeSV-common cDNA's demonstrated significant (35 to 45%) hybridization to RNAs of Moloney murine leukemia, mink, and hamster viruses (Table 2). However, compared to a T_m of 83°C for hybrids of ST- or SM-FeSV-common cDNA's with FeSV (FeLV) viral RNA, the T_m's of hybrids formed with other viral RNAs were

TABLE 1. *Genetic relationships between independent FeSV isolates*[a]

RNA source	Percent hybridization with cDNA:			
	SM-FeSV-specific	ST-FeSV-specific	SM-FeSV-common	ST-FeSV-common
$FeLV_A$	15	9	81	85
$FeLV_B$	13	9	87	91
$FeLV_C$	7	8	90	84
NRK cells nonproductively transformed by:				
SM-FeSV	71	9	93	78
ST-FeSV	13	55	64	74
GA-FeSV	11	55	72	79
Uninfected control	6	3	11	12
Mv1Lu cells nonproductively transformed by:				
SM-FeSV	60	12	81	69
ST-FeSV	13	50	81	87
GA-FeSV	12	50	81	78
Uninfected control	4	8	18	13
None	9	7	15	10

[a] Hybridization was performed with 10^3 cpm of cDNA and 200 μg of cell RNA in 20 μl of 0.5 M NaCl at 60°C for 72 h at a C,t of 10^4 mol·s/liter; assayed on hydroxyapatite at 50°C and 0.14 to 0.3 M PB (11).

TABLE 2. *Relatedness of FeSV nucleotide sequences to other retroviral RNAs*[a]

RNA source[b]	Percent hybridization with cDNA:		
	SM-FeSV-specific	ST-FeSV-specific	SM-FeSV-common
Type C helper virus			
Mouse:			
M-MuLV	10	11	41
AKR	10	2	22
HIX	13	11	35
Rat: RaEV	3	3	20
Hamster:			
HaEV	9	7	35
Mink: MiEV	18	15	42
Cat: RD114	14	4	9
Woolly monkey: SSAV	7	7	15
Type C transforming virus			
RSV	15	7	14
KMSV	10	8	18
HMSV	6	3	11
SSV	11	6	22
Type B virus			
MMTV	8	7	16
None	9	7	15

[a] Hybridization was performed as described in the text at a C_rt of 10^4 mol·s/liter for cell RNAs. M-MuLV, MiEV, HaEV, HIX, AKR, and RSV were purified viral RNAs and were assayed at a C_rt of 10 mol·s/liter. Percent hybridization was assayed by hydroxyapatite at 50°C with 0.14 and 0.3 M PB washes followed by precipitation and counting.

[b] M-MuLV, Moloney murine leukemia virus; RaEV, HaEV, and MiEV, rat, hamster, and mink endogenous viruses, respectively; RSV, Rous sarcoma virus; KMSV and HMSV, Kirsten and Harvey MSV, respectively; SSV, simian sarcoma virus; MMTV, mouse mammary tumor virus.

significantly lower (67 to 69°C). These findings indicated that the hybridization observed was due to partially related nucleotide sequences. There was no detectable homology between the common cDNA's and RNAs of other type C viruses analyzed (Table 2).

Distribution of FeSV sequences among cellular DNAs of various species. To assess the relationship of FeSV to the feline cell genome, the various cDNA probes were hybridized to cellular DNAs of different feline as well as other mammalian species. As shown in Table 3, SM-FeSV-specific cDNA was hybridized to an extent of 100% by normal domestic cat kidney DNA. The $C_0t_{1/2}$ was 1,500 mol·s/liter, and the T_m of hybrids formed was 78°C. Cellular DNAs of other carnivores also exhibited levels of hybridization to SM-FeSV-specific cDNA that were significantly above background and associated with low but structured T_m's (71 to 74°C). Other mammalian cell DNAs, including calf, human, marmoset, and rat, did not hybridize to the SM-FeSV-specific cDNA significantly under the conditions utilized (0.14 M phosphate buffer [PB], 50°C on hydroxyapatite). In all cDNA-DNA hybridizations, the cellular DNA reannealed at least 75 to 80% with a $C_0t_{1/2}$ of 1,200 to 1,800 mol·s/liter and a unique sequence DNA T_m of 83 to 85°C. According to this standard (Fig. 3), SM-FeSV-specific DNA sequences were represented in only one to a few copies per haploid genome.

The behavior of the ST-FeSV-specific cDNA paralleled that of SM-FeSV-specific cDNA. Ex-

TABLE 3. *Presence of FeSV nucleotide sequences in cellular DNAs*[a]

Species	SM-FeSV-specific cDNA		ST-FeSV-specific cDNA		SM-FeSV-common cDNA	
	% Hybrid	T_m (°C)	% Hybrid	T_m (°C)	% Hybrid	T_m (°C)
SM-FeSV nonproducer	100	79	21	68	100	82
ST-FeSV nonproducer NRK	19	65	100	81	100	79
Domestic cat	100	78	97	76	100	78
Jungle cat	95	77	89	75	100	77
Sand cat	73	78	79	77	77	76
Lion	86	77	76	76	20	73
Cheetah	95	77	87	77	36	73
Mountain lion	81	76	87	77	20	72
Leopard	68	75	87	77	23	71
Fishing cat	84	77	76	77	28	71
Genet	70	73	74	74	15	76
Mink	41	74	58	74	28	74
Dog	43	71	39	74	20	71
Human	13	71	34	70	23	69
Marmoset	5	58	34	72	18	70
Calf	16	72	29	72	5	71
Rat	0	NT[b]	10	66	38	72
Chicken	0	NT	3	60	15	71
Salmon	0	NT	0	NT	0	NT
None	0	NT	0	NT	0	NT

[a] Molecular hybridization and T_m analysis were performed as described in the text. The results are normalized to values obtained with SM-FeSV-specific cDNA hybridization to the SM-FeSV (FeLV) nonproducer NRK cell DNA (71%) and with no DNA present (7%). ST-FeSV-specific cDNA hybridized maximally 48% with ST-FeSV (FeLV) nonproducer NRK cell DNA and 5% without DNA present. SM-FeSV-common cDNA hybridized maximally 74% with the SM-FeSV (FeLV) nonproducer NRK cell DNA, and hybridized 11% with no cell DNA present.

[b] NT, Not tested.

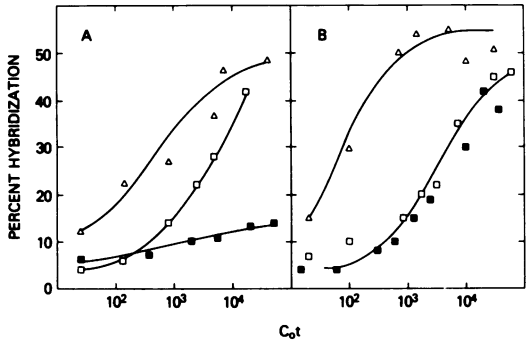

FIG. 3. *Comparison of nucleotide sequences homologous to SM-FeSV and ST-FeSV in DNAs and SM-FeSV nonproducer rat and normal cat cells. DNA-DNA hybridization was performed as described in the text. (A) Hybridization of SM-FeSV-common cDNA (△), SM-FeSV-specific cDNA (□), and ST-FeSV-specific cDNA (■) by cellular DNA or NRK cells nonproductively transformed by SM-FeSV. (B) Hybridization of these same cDNA probes by cellular DNA of normal cat kidney cells.*

tensive hybridization was observed with DNAs of various feline species and, to a lesser extent, with those of other carnivores (Table 3). Interestingly, ST-FeSV-specific sequences were slightly more conserved phylogenetically than those of SM-FeSV. Finally, ST-FeSV-specific sequences, as with SM-FeSV, were represented in approximately one copy per haploid genome in domestic cat DNA (Fig. 3).

Viral sequences related to those of the FeSV-common cDNA's were distributed quite differently from FeSV-specific sequences within mammalian cellular DNAs. SM-FeSV-common cDNA was hybridized 100% by normal domestic cat DNA with a T_m of 78°C (Table 3). Similar results were observed with ST-FeSV-common cDNA (data not shown). However, among DNAs of other feline species tested, only those of Mediterranean cats, jungle cats, and sand cats hybridized the FeSV-common cDNA's. Thus, all other Felidae DNAs tested, including those of lion, mountain lion, cheetah, fishing cat, and leopard, failed to hybridize SM- or ST-FeSV-common cDNA's, as did DNAs of other carnivores tested. Rat cellular DNA annealed 38% of the SM-FeSV-common cDNA with a T_m of 72°C for the hybrids formed. These later findings were consistent with the partial sequence homology observed between FeSV-common sequences and the endogenous rat type C virus (Table 2). Finally, in contrast to the single copy representation of FeSV-specific sequences within domestic cat cellular DNA, the FeSV-common cDNA's showed evidence of multiple copies (6 to 8 per haploid genome) (see Fig. 3). Multiple copies were also demonstrated within DNAs of the Mediterranean cat species tested.

FeSV expression in normal and transformed feline cells. The availability of SM- and ST-FeSV-specific cDNA made it possible to examine expression of each virus in normal and transformed feline cells. Feline embryo cells expressed undetectable levels of either SM- or ST-FeSV-specific RNA. Moreover, there was no evidence of expression of viral RNA homologous to FeSV-common cDNA (Table 4). We also examined a cultured feline lymphoma cell line, FL74, which is known to release a high-titered FeLV (32). FL74 cells are also indicator cells for detection of antibodies to the feline oncornavirus membrane antigen (FOCMA) (10), an antigen recently reported to be a product of the ST-FeSV genome (26, 28, 30). As shown in Table 4, FL74 cellular RNA contained around 100 viral RNA copies homologous to FeSV-common cDNA sequences, as might be expected, due to the FeLV-productive nature of the cells. However, these cells did not express detectable levels of RNA homologous to either SM- or ST-FeSV-specific cDNA's. As controls, the sensitivity and specificity for detection of the FeSV-specific sequences in FeSV nonproducer transformed NRK cells are also indicated in Table 4.

DISCUSSION

Accumulating evidence indicates that replication-defective mammalian transforming vi-

TABLE 4. *Presence of FeSV sequences in feline cellular RNA*[a]

Cell line	SM-FeSV-specific cDNA		ST-FeSV-specific cDNA		SM-FeSV-common cDNA[b]	
	% Hybrid	Copy no. per cell[c]	% Hybrid	Copy no. per cell	% Hybrid	Copy no. per cell
FEF, normal cat embryo cell	8	<1	7	<1	7	<1
FL74, FeLV-producing cat lymphoma cell	13	<1	14	<1	65	100
SM-FeSV nonproducer NRK	71	30	9	<1	93	30
ST-FeSV nonproducer NRK	12	<1	50	10	81	10
NRK, control rat	6	<1	3	<1	11	<1

[a] Hybridizations were carried out with 10^3 cpm of cDNA and 200 mg of cell RNA in 20 μl of 0.5 M NaCl at 60°C for times up to 72 h, with a C_rt of 10^4 mol·s/liter maximally. C_rt's for intermediate times were calculated, and a $C_rt_{1/2}$ was determined as described previously (18). Hybrids were assayed on hydroxyapatite at 50°C with 0.14 to 0.3 M PB, as described in Tables 2 and 3 and reference 11.

[b] ST-FeSV-common cDNA gave results similar to those with SM-FeSV-common cDNA in percent hybridization at both maximal C_rt and $C_rt_{1/2}$.

[c] Copy number of RNA molecules per cell was based on a single RNA molecule $C_rt_{1/2}$ of 10^4 mol·s/liter with total cell RNA. The calculated copy number of RNA per cell for each species is based on 10^4 mol·s/liter divided by the cDNA $C_rt_{1/2}$.

ruses have arisen in nature by a mechanism involving recombination of a type C RNA helper virus with some cellular genetic information. Efforts to investigate genetic information specific to the sarcoma virus have utilized molecular probes selected against helper virus nucleotide sequences contained within the sarcoma virus genome. To date, two classes of sarcoma virus-specific sequences have been characterized. One class, exemplified by Moloney MSV, is represented in only a small number of copies within the mouse cell genome, analogous to the representation expected of a cellular gene (11, 24). Moloney MSV-specific sequences are relatively well conserved within mammalian cellular DNAs, and are generally not expressed at high levels by normal mouse cells (12).

A second class of sarcoma virus-specific sequences is demonstrated by Kirsten and Harvey MSV strains. Genetic information specific to these viruses is represented as multiple copies within cellular DNA of their species of origin, the rat (3, 23). Moreover, RNA related to Kirsten MSV- and Harvey MSV-specific RNA sequences are expressed at relatively high levels by normal rat cells (3, 23), and can even be pseudotyped and transmitted to new cells by a type C helper virus (Scolnick et al., submitted for publication). Since many of these properties are analogous to those of type C viruses, it has been postulated that the sarcoma virus-specific sequences of Kirsten and Harvey MSV strains have originated from a new class of endogenous type C virus of rat origin (23).

In the present report, molecular approaches analogous to those used to analyze the genomes of murine transforming viruses have been applied to the investigation of three independent isolates of replication-defective transforming viruses of outbred cats. By a series of molecular hybridizations, cDNA's prepared from SM- and ST-FeSV strains were selected for sequences specific to the respective sarcoma virus genome and for nucleotide sequences shared with those of FeLV. The FeSV-specific cDNA's were then used as molecular probes to study the relatedness between independent FeSV isolates as well as the relationship of each virus to the genome of the species from which it originated.

We were able to demonstrate extensive homology between ST-FeSV-specific sequences and the genome of an independent feline transforming virus isolate, GA-FeSV. In contrast, ST- and SM-FeSV showed no detectable relatedness in their sarcoma virus-specific nucleotide sequences. By molecular hybridization, each set of FeSV-specific sequencess was demonstrated to be present within the cat cell genome, as evidenced by the extensive hybridization observed and the high T_m of the hybrids formed. By C_0t analysis, the copy number of each sarcoma-specific cDNA was similar to that of unique-sequence domestic cat cellular DNA. Moreover, the sequences were relatively well conserved within cellular DNAs of other mammalian species. Thus, the FeSV-specific sequences, like those of Moloney MSV, exhibit characteristics that might be expected of a cellular gene. If such sequences represent the tranforming regions of their respective sarcoma viral genomes, the present findings imply the existence of at least two distinct sets of cat cellular sequences, whose normal or altered expression may be sufficient for malignant transformation.

All three FeSV strains demonstrated partial relatedness to FeLV, findings consistent with the fact that each virus codes for some FeLV *gag* gene translational products (21). The FeLV-related sequences were represented within cat cellular DNA at higher copy number than that observed for the sarcoma virus-specific nucleotide sequences and with a distribution limited to Mediterranean cats. These later findings are consistent with the previously reported distribution of FeLV-related sequences in feline cellular DNAs (6). The present findings, thus, support the concept that each of three independent transforming isolates of outbred cats have arisen by a mechanism involving recombination of FeLV with some other cellular genetic information of feline origin.

Normal cat embryo cells in tissue culture did not express detectable levels of either SM- or ST-FeSV-specific RNA. Of interest, also, was the lack of expression of these sequences in FL74 cells, a feline lymphoma line that is the reference cell for detection of the feline oncornavirus membrane antigen (FOCMA). Recently, FOCMA has been reported to be an FeSV-coded protein, translated as part of a precursor containing FeLV *gag* gene-coded p15 and p12 (26, 28, 30). It is possible that the FeSV-specific cDNA probes utilized here may represent regions of the transforming viral genome, that differ from that coding for FOCMA. However, it is also known that viral genes that are not demonstrably related by molecular hybridization may code for antigenically related translational products (2). Thus, FOCMA could still be a translational product antigenically related to transforming gene products of SM- and/or ST-FeSV, but cellular sequences coding for FOCMA may have sufficiently diverged so as to be undetectable using FeSV-specific cDNA probes. Efforts are currently underway to localize sarcoma virus-specific sequences of FeSV to the restriction map of the FeSV genome. This information, along with the identification of restric-

tion fragments of the viral genome capable of causing transformation, should help to resolve these possibilities.

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