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Genetics

Bvr-1, a restriction locus of a type C RNA virus in the feline cellular genome: Identification, location, and phenotypic characterization in cat × mouse somatic cell hybrids

(endogenous leukemia virus/murine leukemia virus/feline leukemia virus/isozyme mapping)

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ABSTRACT Somatic cell hybrids were constructed between BALB/c-RAG mouse cells and feline lymphoma cells by the hypoxanthine-aminopterin-thymidine selection scheme. RAG cells spontaneously produce an endogenous B-tropic type C virus. Cat–mouse hybrids preferentially segregate feline chromosomes and retain murine chromosomes demonstrable by karyotypic and isozyme analyses. Despite the presence of the complete mouse genome, including the viral genome, virus production was diminished to 1–5% of the levels observed in RAG parents based upon particle-associated RNA-dependent DNA polymerase (reverse transcriptase) activity in the culture fluid. Thirty-seven hybrids made on four different occasions had suppressed virus levels, and no hybrids expressed parental virus levels. Reverse selection experiments on 6-thioguanine demonstrated that a restriction gene, tentatively named *Bvr-1*, was linked to the feline structural genes for hypoxanthine phosphoribosyltransferase (IMP:pyrophosphate phosphoribosyltransferase; EC 2.4.2.8) and glucose-6-phosphate dehydrogenase (D-glucose-6-phosphate:NADP$^+$ 1-oxidoreductase; EC 1.1.1.49) in cats, probably on the X-chromosome. The genetic mode of action of *Bvr-1* is *trans* dominant in restriction of murine leukemia virus. The restriction locus results in a block late in virus maturation but prior to release, since expression of antigens for viral structural proteins and mature budding particles is apparent on surfaces of restricted hybrid cells but not in high-speed pellets from culture fluid of restricted cells.

The distribution of multiple copies of endogenous oncornavirus-related sequences in normal cellular DNA is widespread within mammalian species (1–4). The regulation of expression of these usually silent sequences presents an important problem not only in eukaryote gene control, but also in the further understanding of the participation of the viruses and their virogenes in neoplastic processes. In the mouse, the availability of inbred strains has provided the foundation for the description of virus structural genes (*Akv-1, Akv-2*), partially active viral genes whose alleles control expression of viral structural proteins in the absence of complete virus (*Gv-1, Mlv-1*), and genes whose pattern of expression suggests that they play a regulatory role in unscheduled production of endogenous viruses (*Fv-1, Fv-2, Gv-2,* and *Rgv*) (5–12). Additionally, at least four phenotypically distinct endogenous viruses are harbored by the BALB/c mouse and each is under a different mode of control of induction and/or expression (13–17).

Various isolates of type C viruses from mammalian species exhibit characteristic host ranges with respect to which species of tissue culture cell will permit their infection and reproduction. Host range phenotype can be attributed to the evolution and fixation of viral restriction genes in various animal species concordant with the evolutionary divergence of viral target genes on whose products the restriction genes act. Somatic cell hybridization permits the placement of such restriction genes and endogenous viral genes in the same nucleus of cultured cells. The preferential segregation of the chromosomes of one of the parents provides an experimental system for the detection and genetic localization of viral structural genes, as well as the restriction genes. The study of the mechanism of interaction of restriction loci and virogenes has the potential of elucidation of the developmental sequence of genetic events involved in the induction of leukosis viruses *in vivo*. This report describes the detection of a feline restriction gene (*Bvr-1*) that acts dominantly in the block of an endogenous B-tropic murine leukemia virus in cat × mouse hybrid cells.

Abbreviations: HAT medium, hypoxanthine-aminopterin-thymidine medium; HPRT, hypoxanthine phosphoribosyltransferase; MuLV and FeLV, murine and feline leukemia virus, respectively; G6P dehydrogenase, glucose-6-phosphate dehydrogenase.

MATERIALS AND METHODS

Cells and Cell Culture Conditions. The parent cells used in the production of somatic cell hybrid colonies were a continuous feline lymphoma suspension cell (FLA or FL-74) productively infected with FL-74 strain of feline leukemia virus (18–21) and the murine renal adenocarcinoma line (RAG), adapted to tissue culture and resistant to 6-thioguanine, which produces an endogenous B-tropic murine leukemia virus (17, 21).

Virus-negative cell lines included an embryonic Crandell feline kidney derivative, FCO-121 or CRFK, obtained from the Naval Biological Research Laboratory, Oakland, and a BALB/c adult skin cell, Basc-2, established in this laboratory. SC-1 cells are wild mouse embryonic fibroblasts that are permissive for both N- and B-tropic murine viruses. Monolayer cultures were carried in Dulbecco-Vogt minimal essential tissue culture medium supplemented with 10% fetal bovine serum in the absence of antibiotics. Cultures were checked for mycoplasma and were consistently negative.

Cell Hybridization. FLA cells (1.2×10^7) were mixed with 2×10^6 RAG cells (grown on 0.1 mM 6-thioguanine) in a sterile tube with an empirically tested optimum fusion titer of β-propiolactone-inactivated Sendai virus as described (21). Hybrids were plated on Dulbecco-Vogt medium supplemented with 0.1 mM hypoxanthine, 0.4 μM aminopterin (Nutritional Biochemical Co.), and 16 μM thymidine (HAT medium). The aminopterin kills the RAG parents and any RAG homokaryons due to the absence of a functional hypoxanthine phosphoribosyltransferase (IMP:pyrophosphate phosphoribosyltransferase; EC 2.4.2.8) (HPRT) (22). Surviving hybrid colonies were picked with cloning cylinders 3–4 weeks after fusion.

RNA-Dependent DNA Polymerase Assay. Virion production was detected by the recovery of particles containing RNA-dependent DNA polymerase from high-speed pellets of filtered (0.45 μm Millipore) tissue culture fluid. The polymerase was assayed with a synthetic template and primer with ^{3}H-

Table 1. Particulate RNA-dependent DNA polymerase activity in primary hybrid clones of RAG × FLA

Cell lines and hybrids	Polymerase (pmol/hr·10^6 cells)
FLA	151–294
RAG	17.7–63
FCO-121	≤0.4
Basc-2	≤0.4
FLA(HAT)	237
SC1(RAG-V+)	475
SC1(RAG-V+)(HAT)	432
Fusion I	
R × F 1B	2.8
R × F 2C	1.2
R × F 3B	5.4
R × F 5B	2.1
R × F 5E	1.1
R × F 5F	1.9
R × F 5G	1.1
R × F 6A	2.4
R × F 6D	0.8
R × F 8A	2.9
R × F 8E	6.8
R × F 8F	3.0
R × F 8G	8.7
R × F 9B	1.2
R × F 9C	0.8
R × F 9D	0.7
R × F 9E	4.1
R × F 13E	4.9
Fusion II	
F × R 2A	1.1
F × R 5C	1.1
F × R 6A	6.8
F × R 7A	1.3
F × R 21C	1.3
F × R 27B	2.1
Fusion III	
c1 20	0.4
Fusion IV	
RcF A12	1.3
RcF C8	2.1
RcF D1	0.5
RcF D2	1.0
RcF D3	1.8
RcF D4	1.2
RcF D5	2.1
RcF D6	4.6
RcF D7	2.2

labeled substrates from Schwarz/Mann as described (23). Results are expressed as pmol of [^{3}H]dTMP incorporated per hr per 10^6 cells using culture fluid from a 48-hr culture in logarithmic phase.

Electron Microscopy. This was performed in collaboration with Mr. Donald Stuart. Packed cells and high-speed pellets of filtered (0.45 μm) culture fluid were fixed at 3% glutaraldehyde, 1% osmium tetroxide and embedded in Epon. Sections were stained with lead citrate and uranyl acetate.

Antisera. Autologous bovine antiserum against FeLV was prepared by infecting a portion of thymic tissue from a young steer with ST-FeLV *in vitro* and inoculating these cells back into the donor (24). The serum was a gift to Dr. T. Kawakam. Monospecific goat antiserum against Rauscher murine leukemia virus-p30 was prepared by Dr. R. Wilsnack through the Office of Program Resources and Logistics, NCI.

FIG. 1. Karyotype of FLA, RAG, and F × R 2A hybrid between RAG × FLA.

Radioimmune Assay for Viral Associated Cell Surface Antigens. Viral antigens were measured quantitatively with ^{131}I-labeled immune serum and ^{125}I-labeled normal serum as described (24–26).

Gene-Enzyme Markers Detected by Isoenzyme Procedures. Standard isozyme procedures were used for electrophoresis and histochemical enzyme development (27–29). HPRT and adenine phosphoribosyltransferase (AMP:pyrophosphate phosphoribosyltransferase; EC 2.4.2.7) were assayed on opposite halves of an acrylamide gel in an EC apparatus and developed autoradiographically using ^{14}C-labeled substrates. Glucose 6-phosphate-dehydrogenase (D-glucose-6-phosphate:NADP$^+$ 1-oxidoreductase; EC 1.1.1.49) (G6*P* dehydrogenase) was routinely analyzed on cellulose acetate gels in a Beckman microzone cell. All other enzymes were separated in 11% hydrolyzed starch (Electrostarch) using a vertical Buchler electrophoresis system.

Karyology. Logarithmic phase cells were treated with colcemid for 3 hr, harvested, swollen in hypotonic sodium citrate (0.7%) for 10 min, and fixed in a 3:1 (vol/vol) methanol:acetic acid mixture. Samples were air dried and stained with acetoorcein. Chromosomes were photographed with Kodak high contrast film with a Zeiss photomicroscope-II.

RESULTS

Virus Production in Cat × Mouse Hybrid Cells. Both FLA and RAG cells produce considerable levels of type C RNA virus detectable by electron microscopy (17, 40) or by particle-associated RNA-dependent DNA polymerase in the culture fluid (Table 1). The host range of the RAG virus is restricted to murine cell lines homozygous for the *Fv-1*^{B} allele, thus classifying it as a B-tropic murine type C virus (17). The FL-74 strain of FeLV grows in all feline cells but fails to grow in cell lines of heterologous species, including mouse (ref 20; S.J.O., unpublished observations). Similarly, the RAG virus fails to grow in heterologous species' cell lines, including cat (17).

Over 80 somatic cell hybrids between RAG and FLA were selected in four independent experiments. RNA-dependent DNA polymerase activities of hybrid virus were invariably 2–3 logarithmic units lower than the FLA production and 1–2 logarithmic units below the RAG parent. The reduction in virus activity was not a reflection of inhibition in HAT medium since there was no restriction of virus production in nonmutant cells infected with either parent virus cultured on HAT medium.

Genetic Analysis of Hybrid Clones: Karyology. The distinction between feline and murine chromosomes is facilitated by the preponderance of telocentric mouse chromosomes compared to feline chromosomes, which are generally metacentric or acrocentric (Fig. 1). Four hybrids were examined; each had a large number of telocentric chromosomes and only

Table 2. Chromosome analysis of FLA × RAG hybrids

Cell	Modal chromosome no.*	Modal no. acrocentric plus metacentric	Modal no. telocentric
Parents			
FLA	38	36	2
RAG	59–65	5	51–57
Hybrids			
cl 20	110	9	101
RcF 1A	101	12	87
RcF 2A	95	12	83
RcF 1C	56	7	49

* Stem number determined for RAG cells was 60–65 chromosomes. Three hybrids had a 2*s* stem number. Polyploid chromosome spreads (2*s*, 3*s*, etc.) are approximate multiples of the RAG modal chromosome number and represent either homologous cell fusion of two or more RAG cells with an FLA cell or chromosome doubling in the absence of cell division.

a few biarmed chromosomes, indicating that the hybrids had retained the murine genome and segregated feline chromosomes preferentially. This conclusion is supported by isozyme data (see next section). When one considers the stem number of the hybrid colony (Table 2) and the modal number of metacentric chromosomes observed in the RAG parent, the number of feline chromosomes retained can be roughly estimated. In those hybrids examined thus far, the estimates range from one to five feline chromosomes.

Genetic Analysis of Hybrid Clones: Enzyme Gene Segregation. Twenty-five gene-enzyme differences between FLA and RAG cells were observed after gel electrophoresis and histochemical enzyme development (Table 3). These enzyme genes are distributed among at least 10 of the 20 mouse chromosomes (30). Since the feline biochemical genetic map is virtually nonexistent, it is impossible to know exactly how many feline chromosomes are represented, but by analogy to the distribution of the same gene enzyme markers in mouse and man, the number is probably close to 12 out of 19 feline chromosomes (30–32).

Table 3 lists the enzyme phenotype of 27 hybrid colonies examined at the same passage as the initial assays for RNA-dependent DNA polymerase. Every mouse enzyme was expressed in each of the hybrids but only a few (2 to 8) feline enzymes were observed. Twelve of the feline enzymes were never observed. Clearly, the hybrids retained the entire murine genome and only a few feline chromosomes.

The feline and murine HPRT have identical electrophoretic mobilities. The presence of the feline HPRT was deduced indirectly from the following observations. The RAG parent had no detectable HPRT in a gel assay or in a test tube quantitative assay, while all hybrid extracts were positive for HPRT. Furthermore, the specific activity of feline HPRT is approximately 10% of the murine enzyme under identical enzyme assay conditions. Each of the hybrids examined expressed the low feline-like HPRT levels, while two independently isolated $HPRT^+$ revertants of RAG cells (RAG-R1 and RAG-R2) expressed the higher murine level specific activities.

Each of the 26 hybrids examined expressed feline HPRT and G6*P* dehydrogenase, suggesting that their enzyme genes are syntenic. A secondary subcolony of one hybrid, RcF D1, failed to express the feline G6*P* dehydrogenase in later passages. This result would be explained by a chromosomal aberration with a breakpoint between the *Hprt* and *Gpd* loci.

Table 3. Enzyme phenotype of RAG × FLA hybrids

Cells	Feline enzymes expressed*	No. of feline enzymes tested
Parents		
FLA	1–25	25
RAG	0	25
Hybrids		
F × R 2A	5,10,11,14	13
F × R 6B	5,9,10,11,17	22
F × R 7A	5,10	21
F × R 21C	5,10	17
F × R 27B	1,5,10,11	16
RcF E6	5,10,20	6
RcF D1	5,10,11	6
R × F 1B	5,8,10,14,20	14
R × F 2C	5,8,10,17,20	23
R × F 3B	5,10,20	20
R × F 5B	5,10,11,14,17	22
R × F 5E	5,10,11,20	21
R × F 5F	5,10,11,20,23	21
R × F 5G	5,10,11,17,20,23	21
R × F 6A	1,5,8,10,11,17,23,24	23
R × F 6D	5,8,10	12
R × F 8A	5,10,11,17,23	20
R × F 8B	5,8,10,11,14,17,23,24	21
R × F 8E	5,10,11,13,14,24	15
R × F 8F	5,10,14	9
R × F 8G	5,10,11,23,24	22
R × F 9B	5,10,14,20	15
R × F 9C	5,10,14,20	16
R × F 9D	5,10	15
R × F 9E	5,10,11,20	22
R × F 13E	5,6,8,10,11,20	20

* Enzyme tests refer to the following enzymes resolved by gel electrophoresis: (*1*) acid phosphatase (EC 3.1.3.2) (*Ap-1*); (*2*) adenosine phosphoribosyltransferase (*Aprt*); (*3*) adenosine deaminase (EC 3.5.4.4) (*Ada*); (*4*) esterase (EC 3.1.1.1) (*Est*); (*5*) glucose-6-phosphate dehydrogenase (*Gpd*); (*6*) glucosephosphate isomerase-1 (EC 5.3.1.9) (*Gpi-1*); (*7*) glucosephosphate isomerase-2 (*Gpi-2*); (*8*) glutamate oxaloacetate transaminase-2 (EC 2.6.1.1) (*Got-2*); (*9*) hexokinase-1 (EC 2.7.1.1) (*Hk*); (*10*) hypoxanthine phosphoribosyltransferase (*Hprt*); (*11*) c-isocitrate dehydrogenase (EC 1.1.1.42) (*Id-1*); (*12*) m-isocitrate dehydrogenase (EC 1.1.1.42) (*Id-2*); (*13*) lactate dehydrogenase-A (EC 1.1.1.27) (*Ldh-A*); (*14*) lactate dehydrogenase-B (*Ldh-B*); (*15*) c-malate dehydrogenase (EC 1.1.1.37) (*Mor*); (*16*) c-malic enzyme (EC 1.1.1.40) (*Mod-1*); (*17*) mannose phosphate isomerase (EC 5.3.1.7) (*Mpi-1*); (*18*) nucleoside phosphorylase (EC 2.4.2.1) (*Np*); (*19*) peptidase A (EC 3.4.11.-) (*Dip-2*); (*20*) peptidase B (EC 3.4.11.-) (*Trip-1*); (*21*) phosphogluconate dehydrogenase (EC 1.1.1.44) (*Pgd*); (*22*) phosphoglucomutase-1 (EC 2.7.5.1) (*Pgm-1*); (*23*) phosphoglucomutase-2 (EC 2.7.5.1) (*Pgm-2*); (*24*) pyruvate kinase (EC 2.7.1.40) (*Pk*); (*25*) superoxide dismutase-2 (EC 1.15.1.1) (*Sod-2*). Symbols in parentheses refer to murine enzyme gene nomenclature. All mouse enzymes tested (up to 32) were expressed in every hybrid.

Experimental Demonstration of Chromosomal Synteny of *Hprt*, *Gpd*, and *Bvr-1*. The production of both feline leukemia virus (FeLV) and murine leukemia virus (MuLV) in each of the cat–mouse hybrids examined was severely restricted. The presence of the entire murine genome is consistent with the presence of integrated MuLV genome(s) whose expression is restricted by a dominant feline gene. Based upon the concordance of feline *Hprt*, *Gpd*, and *Bvr-1* expression, we hypothesized that *Bvr-1* was located on the same chromosome as the enzyme genes. Since both of these enzyme genes are X-linked

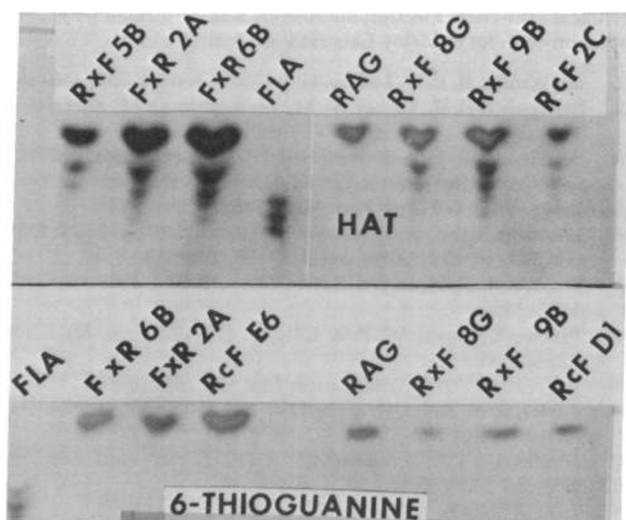

FIG. 2. Cellulose acetate gel electrophoretic patterns of G6*P* dehydrogenase in extracts of FLA, RAG, and six hybrids selected on HAT medium (top panel). The three-band feline pattern probably represents isozyme heterogeneity as opposed to a heterozygote between genetic variants, since of three feline cell lines examined all express the same pattern. The hybrid cells express hybrid enzyme bands in addition to the parental forms indicative of heterodimeric enzyme composed of subunits from each parent. The six hybrid colonies that were selected on 6-thioguanine are presented in the lower panel, expressing only the mouse enzyme.

in both human (31, 32) and mouse (33), the locus is likely to reside on the feline X-chromosome as well, especially in light of the evolutionary tendency to conserve homologous linkage relationships of X-linked genes in vertebrates (34).

A back-selection experiment designed to examine the hybrid cells in the presence and the absence of the feline X-chromosome, and presumably the three syntenic loci, was constructed. Hybrids grown on HAT medium compulsively retain the feline X-chromosomes since those few that spontaneously lose it by mitotic disjunction are killed by aminopterin. Six hybrids were taken off HAT medium in three steps: first a passage on HT(HAT medium minus aminopterin) for 2 weeks and then on complete medium with no supplements. After 2 weeks on complete medium, cells were plated on 0.1 mM 6-thioguanine to select positively for those isolated colonies derived from cells that spontaneously had lost the feline X-chromosome. These back-selected hybrids, like their RAG ancestors, are resistant to 6-thioguanine since their murine X-chromosome has a mutant HPRT which fails to incorporate the lethal analogue. The back-selected hybrid should be coordinately deficient in feline HPRT, G6*P* dehydrogenase, and *Bvr-1* virus restriction.

The results of this experiment are presented in Fig. 2 and Table 4. The six hybrids' secondary colonies adapted to 6-thioguanine were deficient in HPRT activity and in feline G6*P* dehydrogenase. Virus pellets were prepared from plates of the six hybrids on HAT, nonselective, and 6-thioguanine medium. The virus levels on HAT were restricted, but on nonselective and 6-thioguanine the virus levels in the culture fluid were increased 10 to 100-fold to levels comparable to the RAG parent. Virus collected from four of the back-selected hybrids was tested for viral host range and for inhibition with antisera for RNA-dependent DNA polymerase from MuLV and FeLV. In each case, the virus was distinct from FeLV but indistinguishable from the B-tropic MuLV of the RAG parent (S. O'Brien and J. M. Simonson, manuscript in preparation).

Point in Viral Assembly of *Bvr-1* Restriction. Thirty-one restricted hybrids were tested for cell surface associated viral antigens by a radioimmune assay for cell surface antigens. Typical results are presented in Table 5. The hybrids expressed MuLV p30 antigens at high levels, comparable to their RAG parents. The expression of FeLV cell surface antigens was also comparable to RAG levels, reflecting interspecies antigenicity of cell surface antigens between the two species (S. J. O'Brien, J. M. Simonson, and S. Davis, submitted for publication).

Six restricted hybrid cells, the parents, and controls were examined by transmission electron microscopy for cell membrane budding virions and for virus particles in high-speed pellets of filtered tissue culture fluid (Table 5). Each of the parents was positive, while each hybrid was negative for particles in the fluid pellets. Restricted hybrid cells (on HAT) did, however, possess numerous type C particles budding from the plasma membrane. These observations place the time of expression of *Bvr-1* late in viral maturation at the point of release from the plasma membrane. The time of action of *Bvr-1* could conceivably be earlier, e.g., during the synthesis of viral protein or nucleic acid components that are required for release. It is not yet clear whether the virus particles are rapidly disrupted after release or rather are prevented from release entirely.

Table 4. Virus production in RAG × FLA hybrids*

	Selective medium†		
	HAT X^+	DV(none)‡ $X^{\pm}$	6-Thioguanine X^-
Parent			
FLA	407	529	Dies
RAG	Dies	63	58
Basc-2	≤0.3	≤0.1	Dies
Hybrid			
F × R 2A	0.3	4.2	62.1
F × R 6B	2.2	45.5	31.5
R × F 8G	5.3	15.2	78.7
R × F 9B	1.7	14.9	24.6
RcF D1	1.6	8.6	12.0
RcF E6	1.3	38.5	53.4

* Expressed as pmol of dTMP/hr per ml of tissue culture fluid.
† X^+, X^-, $X^{\pm}$ refer to presence or absence of feline X-chromosomes.
‡ Dulbecco-Vogt medium.

Table 5. Viral phenotype of *Bvr-1* restricted RAG × FLA hybrid

Control cells	Surface antigens*		Virus particles†	
	α MuLV p30	α FeLV	Membrane budding	In fluid pellets
FLA	4.8	155	+	+
RAG	211	48	+	+
Basc-2	≤0.4	≤0.4	–	–
FCO-121	≤0.4	≤0.4	–	–
R × F 8G	201	23	+	–
R × F 9B	105	64	+	–
F × R 2A	106	65	+	–
F × R 6A	60	62	+	–
RcF D1	—	45	+	–
RcF E6	—	74	+	–

* ng of antibody bound/5 × 10^6 cells per ml.
† Virus particles were detected with transmission electron microscopy on cell membranes and in tissue culture fluid pellets.

DISCUSSION

The studies presented here indicate that a single genetic locus of the feline genome is a major determinant in the restriction of endogenous MuLV in somatic cell hybrids. The gene, tentatively designated *Bvr-1* (BALB/c virus restriction), is *trans* dominant in its mode of expression and is syntenic with the generally X-linked structural genes for HPRT and G6*P* dehydrogenase.

The presence of cellular restriction genes for type C RNA viruses has been suggested in human cells by Tennant and Richter (35), who observed restriction of infection of mouse × human heterokaryons with exogenously added Moloney leukemia virus, and by Minna *et al.* (J. D. Minna, T. H. Marshall, R. D. Burk, R. S. Lemons, S. H. Brown, and S. H. Wilson, in preparation), who observed diminution of particle-associated RNA-dependent DNA polymerase in culture fluid below parental levels in hybrids between virus-producing rodent cells and fresh human leukocytes. The action of the human restriction of murine virus is still unclear, however, since both exogenously applied murine ecotropic viruses and endogenous induced murine viruses replicated at high levels in different human × mouse hybrids containing a full human genome (36, 37).

The point of action of *Bvr-1* is apparently late in the assembly process of type C viruses. Virus production is blocked at the release of particles budding from the plasma membrane. This is exactly the point of action of interferon on type C virus production (38). If *Bvr-1* is producing an interferon, it must be able to distinguish between FeLV and MuLV since the FLA parent cell is a potent virus producer. It may also be important that both the human interferon producer locus and the susceptibility genes have been mapped to chromosomes 2, 5, and 21, and not on the X chromosome (31).

The diminution of FeLV production might be explained by the loss of certain critical gene functions normally provided by the segregant feline chromosomes, not the least of which would be the integration site of FeLV in feline chromosomal DNA. Since FeLV fails to infect murine cells, a murine restriction system must also be considered. In collaboration with G. Todaro, C. Sherr, and R. Benveniste, 12 of the RAG × FLA hybrids were examined for FeLV-associated group specific antigens and for FeLV production. Two hybrids were positive for FeLV antigens, and one of these produced quite low amounts of whole FeLV virus. The murine genome may exert a positive *trans* dominant restriction of FeLV in a post-infection period of the FeLV replication cycle. The results, however, do not exclude the participation of a second feline chromosome which is required for vigorous FeLV production, but which was lost in these two hybrids.

The approach outlined in this report promises to be an important protocol for the detection and characterization of various genes that participate in endogenous type C virus induction, assembly, and production. By analysis of the variety of restriction genes that prevent cross species infection of ecotropic, but not xeno- or amphotropic, endogenous virus (39), it should be possible to resolve the sequences of cellular events that control the developmental expression of these eukaryote episomes. The genetic events that participate in this sequence may possibly relate to the cascade of gene switches that result in a neoplastic cell.

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