1-1-1978

Bvr-1, a Restriction Locus of a Type C RNA Virus in the Feline Cellular Genome: Pleiotropic Restriction of Endogenous BALB Virus in Cat x Mouse Somatic Cell Hybrids

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Bvr-1, A RESTRICTION LOCUS OF A TYPE C RNA VIRUS IN THE FELINE CELLULAR GENOME: PLEIOTROPIC RESTRICTION OF ENDOGENOUS BALB VIRUS IN CAT × MOUSE SOMATIC CELL HYBRIDS

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Embryonic and neoplastic cells of different mouse strains contain endogenous type C viral information which can be transmitted vertically and whose complete or partial expression is under strict genetic control (1–5). BALB/c mice harbor at least five different endogenous viruses in their genome which differ in several biological properties including host range, mode and frequency of induction, and antigenic determinants on their structural proteins (2, 4, 6). These viruses include: (*a*) an N-tropic virus (3); (*b*) a B-tropic virus (7–9); (*c*) a xenotropic virus which grows in rat or rabbit cells (3, 4); (*d*) a defective (or restricted) viral genome which encodes a p12 structural protein immunologically distinct from the BALB/c xenotropic or N-tropic viruses (2); and (*e*) an endogenous virogene sequence in normal BALB/c cellular DNA which is homologous to cDNA probes prepared from *Mus cervicolor* type CI xenotropic virus but from which neither intact virus nor viral proteins have been detected (10). Although the biological function of endogenous oncornaviruses is not fully understood, there are clearly empirical connections of these viruses to neoplastic processes. The ecotropic viruses (N- and B-tropic) have been implicated as both leukemogenic vectors and as post-transformation symptoms of murine leukemia. Xenotropic viruses may play a key role in stimulating immune surveillance against spontaneous neoplasms since (*a*) circulating antibody to xenotropic virus proteins are found in various mouse strains (11) and (*b*) certain neoplastic transformation systems are characterized by unscheduled expression of xenotropic viral proteins (12).

The study of the host cellular regulation of expression of oncornaviruses provides a unique opportunity to study both an extremely well described system in eucaryote gene control as well as a genetic sequence related to leukemogenesis. We have recently described a feline chromosomal locus which effectively restricts the replication of murine leukemia virus in somatic cell hybrids between cat and mouse cells (13). The gene is apparently X-linked in cats and acts *trans*-dominantly to suppress murine leukemia virus (MuLV)[1]

[1] *Abbreviations used in this paper:* BudR, bromodeoxyuridine; DV, Dulbecco-Vogt MEM; FeLV, feline leukemia virus; G-6-PD, glucose-6-phosphate dehydrogenase; HAT, hypoxanthine aminopterin thymidine; HPRT, phosphoribosyl transferase; IdU, iododeoxyuridine; MuLV, murine leukemia virus; RDDP, RNA-dependent DNA polymerase; TMP, thymidine monophosphate; TTP, thymidine triphosphase.

production late in the viral life cycle. We describe in this report the pleiotropic action of *Bvr-1* on three recoverable endogenous BALB/c mouse viruses: N-, B-, and X-tropic retroviruses in cat × mouse somatic cell hybrids.

Materials and Methods

Cells and Culture Conditions. The cell lines utilized in this study are listed in Table I. FL-74 cells (also called FLA cells) were grown in RPMI-1640 (Grand Island Biological Co., Grand Island, N. Y.) medium supplemented with 10% heat inactivated fetal bovine serum. Monolayer cultures and hybrids were grown on either Dulbecco-Vogt MEM (DV) or Ham's F12M medium supplemented with 10% fetal bovine serum. Hybrids were selected and maintained on hypoxanthine aminopterin thymidine (HAT) medium, and back selected on Ham's F12M medium supplemented with 0.1 mM 6-thioguanine as described (13).

Type C Viral RNA-Dependent DNA Polymerase (RDDP) Assay. Type C virus activity was assayed by measuring particle associated RDDP or reverse transcriptase activity in tissue culture fluid of infected cells (14). Routine assays were performed with an $rA \cdot dT_{12-18}$ template (Collaborative Research, Waltham, Mass.), [^{3}H]thymidine triphosphate, [^{3}H]TTP, (Schwartz/Mann Div., Becton, Dickinson & Co., Orangeburg, N. Y.) and 1 mM Mn^{++}.

Antibody Inhibition of Viral RDDP. Tissue culture fluid associated virus was collected by ultracentrifugation and concentrated 36 times in a resuspension buffer (0.01 M Tris pH 7.9; 20 mM KCl, 1 mM EDTA, 1 mM dithiothreitol, 50% glycerol). Three antisera were tested for specific inhibition of feline leukemia virus (FeLV) vs. MuLV RDDP: goat anti-Rauscher MuLV reverse transcriptase, goat anti-Tween ether disrupted FeLV (provided by Dr. R. Wilsnack, Huntingdon Research Center, Baltimore, Md.) and normal goat serum (Grand Island Biological Co.). 10 μl of concentrated virus and 10 μl of various antiserum dilutions (in 0.1 M Tris HCl pH 8.0, 1 mM dithiothreitol) were incubated for 4 h at 4°C. At the end of the incubation period, the reaction cocktail plus [^{3}H]TTP was added to the incubation mixture and assayed for RDDP activity. Units are picomoles [^{3}H]thymidine monophosphate, [^{3}H]dTMP, incorporated per hour of reaction per milliliter of culture fluid.

Somatic Cell Hybridization. The construction of RAG × FL-74 hybrid clones by Sendai virus cell fusion has been described (13). Hybrid colonies were analyzed genetically by karyological and isozyme procedures, and generally retained the full murine genome, but had segregated the majority of feline chromosomes.

Iododeoxyuridine (IdU) Induction of N-Tropic and Xenotropic Virus from RAG and Hybrid Cells. Log phase hybrid clones and RAG parents were treated in 100-mm plates for 24 h with 30 μg IdU/ml culture fluid. The IdU medium was decanted and replaced with medium supplemented with 25 μg/ml mitomycin C for 1 h to irreversibly inhibit cell division, but not cell metabolism and virus production. A control plate was refed with complete medium and monitored for effective mitomycin action. To the remaining induced plates were added 2×10^6 target cells (NRK, SIRC, VA-2, N-3T3 cl 10) plus Ham's F12M medium. After 3 days the plates were subcultured with a 1:10 split ratio and monitored for virus production by culture fluid RDDP. The cocultivated plates were tested every 5 days for RDDP with a fluid sample 24 h after fresh feeding and subsequently subcultured again.

Results

Definition of Bvr-1 Restriction of B-MuLV in Hybrid Cells. Over 80 somatic cell hybrid colonies between murine RAG and feline FL-74 cells (Table I) were constructed and selected in four independent experiments with the HAT selection scheme (13). The parent RAG cells spontaneously produced a murine ecotropic oncornavirus with a host range of a B-tropic murine type C virus (Table II). There was no evidence of spontaneous production of BALB/c N-tropic or xenotropic viruses by RAG cells. The FL-74 parent is productively infected with FL-74 strain of FeLV. Neither of the parental species will support replication of the alternative parent's virus (Table II and reference 15).

TABLE I
Cell Lines and Their Viruses Used In This Study

Cells	Species	Histological origin	Spontaneous type C viruses*	Karyotype	Reference
FL-74‡	Feline	Lymphoma from kidney	FeLV(FL-74)	Pseudodiploid	27
RAG(BALB)§	Murine	Renal adenocarcinoma	B-tropic MuLV	Heteroploid	12, 28
CRFK	Feline	Kidney	Negative	Hypoploid	29
Basc-2 (BALB)	Murine (*FV-1^{BB}*)	Subcutaneous adult fibroblast	Negative	Heteroploid	13
BALB-3T3	Murine (*Fv-1^{BB}*)	Embryo	Negative	Heteroploid	30
NIH-3T3	Murine (*Fv-1^{NN}*)	Embryo	Negative	Heteroploid	31
Ncl1A cl 10‖ NIH-3T3	Murine (*Fv-1^{NN}*)	Embryo	Negative	Heteroploid	32
SC-1¶	Murine (*Fv-1^{-}*)	Embryo	Negative	–	33
MS-1	Chinese hamster	Ovary	Negative	–	
NRK	Rat	Kidney	Negative	–	34
SIRC	Rabbit	Cornea	Negative	–	4
VA-2(W1-18)§	Human	Embryonic lung	Negative	–	35

* Criteria of type C virus production include: particulate RDDP activity in cell supernates, budding particles on examination in transmission electron microscopy, and viral antigens (p30, gp70) on infected cell surfaces (14, 36).

‡ FL-74 is a lymphoid suspension cell which was a selective parameter used in hybrid production (13, 27, 36).

§ RAG and VA-2 are 6-thioguanine resistant and die on HAT medium.

‖ Ncl1A cl 10 is a bromodeoxyuridine-resistant subclone of 3T3 cells which dies on HAT medium (R. Goldberg, personal communication).

¶ SC-1 cells lack restriction of N and B tropic viruses. This result may be due to alternative *Fv-1* allele or a modifying gene which overrides the *Fv-1* genotype. Direct resolution of the question awaits genetic analysis of the SC-1 phenotype (33).

Karyological analysis and tests of 25 gene enzyme systems which are distinguishable between cat and mouse cells indicated that the majority of feline chromosomes were lost in the hybrids while the murine genome remained intact (13).

FeLV and FeLV group-specific p27 antigen detected by a competition radioimmunoassay have been found in only 2 of 18 hybrids tested thus far (C. Sherr and S. O'Brien, unpublished observations). Presumably the feline chromosomal virus integration site (or some other critical function provided by feline chromosomes) has been segregated in the FeLV-negative hybrids. On the other hand, the B-tropic MuLV integration site was certainly present since the entire murine chromosomal complement was retained in the hybrids. Nevertheless, the production of MuLV in 34 hybrids tested was invariably 1–2 logs lower than in the RAG parent. This diminution is due to a feline restriction gene, *Bvr-1* (for BALB virus restriction) which is syntenic (on the same chromosome) with the feline structural gene for two normally X-linked enzymes: hypoxan-

TABLE II
Host Range of RAG Endogenous Virus

Infected cell line	Species	Cell fluid reverse transcriptase*‡	
		Uninfected control	RAG Virus infected cells
NIH Swiss 3T3	Mouse	0.28	1.6
BALB-3T3	Mouse	1.1	*152.7*
Basc-2	Mouse	0.3	*58.0*
SC-1	Mouse	1.2	*115*
CRFK	Cat	0.15	0.9
SIRC	Rabbit	0.64	0.38
VA-2	Human	0.61	0.3
MS-1	Chinese hamster	0.0	0.0

* Units = picomoles [^{3}H]dTMP incorporated/hour/10^6 cells.

‡ Infecting virus was filtered (0.45 μm Millipore) tissue culture fluid collected 48 h after refeeding of a log phase culture of RAG cells. The virus containing fluid was supplemented with 2.0 μg polybrene/ml and added to test cell for 6 h. Cells were grown to confluence, split to three plates, and tested for reverse transcriptase as described (37). Assays were performed 10–14 days after infection.

thine guanine phosphoribosyl transferase (HPRT) and glucose-6-phosphate dehydrogenase (G-6-PD) (13).

The physiology of the HAT selective system lends itself to a back selection experiment whereby individual hybrid colonies can be selectively maintained in the presence or absence of *Bvr-1* (Table III). The original hybrids were selected on HAT medium which is toxic to any hybrids which spontaneously lose the feline *Hprt* and other X-linked loci. These hybrids were subcultured on HT medium (HAT minus aminopterin) for 1 wk, on complete DV medium for 1 wk, and then on medium supplemented with 10^{-4} M 6-thioguanine. The rationale for the intermediate stage of selection on HT and complete medium stems from the persistence of HPRT in cells for several days after the loss of the enzyme's structural gene (16). Only those hybrids which had spontaneously lost the feline X-chromosome by mitotic nondisjunction survived on 6-thioguanine since hybrids with a functional feline HPRT incorporated the toxic purine analogue into nucleic acids and were killed. The back selected hybrids, like their RAG parents, survive on 6-thioguanine because their mutant HPRT fails to incorporate the analogue. Thus, hybrids maintained on HAT medium are *Bvr-1*$^+$, while their back selected descendents maintained on 6-thioguanine are *Bvr-1*$^-$.

Bvr-1 Action is Reversible on B-MuLV Production. The reversibility of *Bvr-1* restriction is indicated by the increase in RDDP production in back selected (*Bvr-1*$^-$) hybrids (Table III). To establish the character of the virus recovered from *Bvr-1*$^-$ back selected hybrids, RDDP inhibition experiments with anti-FeLV and anti-Rauscher MuLV sera were performed (Fig. 1). Virus from five back selected hybrids were inhibited by anti-Rauscher MuLV, but not by anti-FeLV or normal goat serum in a manner indistinguishable from

TABLE III

*Back Selection Experiment to Segregate Feline Restriction Gene, Bvr-1**

	Selective pressure	Phenotype of feline genes		RDDP Production‡	Murine virus phenotype
		HPRT	G-6-PD		
FL-74 × RAG*					
↓ HAT					
Hybrids	Retain feline × (*Bvr-1*$^+$)	+	+	0.3–5.3	Restricted
↓ HT					
↓ DV					
Hybrids	None	+ or −	+ or −	4.2–45.5	Restricted or unrestricted
↓ 6-Thioguanine					
Hybrids	Lose feline X (*Bvr-1*$^-$)	−	−	12–79	Unrestricted

* Results summarized from reference 13. Six hybrids were back selected: FXR2A, FXR6B, RXF8G, RXF9B, RCFD1, and RCFE6.

‡ Range of values for six representative hybrids. Units are picomoles dTMP incorporated/hour/milliliter culture fluid. RAG parents produce 20–60 pmol/h/ml of culture fluid associated RDDP.

the RAG virus. RDDP obtained from FL-74 virus was inhibited by anti-FeLV-RDDP, but not by anti-MuLV-RDDP serum.

The biological host range of the virus recovered from the *Bvr-1*$^-$ back selected hybrids was also examined (Table IV). The hybrid virus failed to infect CRFK, SIRC, or NIH-3T3, thus excluding the presence of FeLV, xenotropic MuLV, or N-tropic MuLV. With the viral isolates tested, there was successful infection of SC-1, and B-3T3, confirming the identity of the virus as B-tropic MuLV similar to the RAG parental virus (Table II).

Bvr-1 Restricts IdU Induction of BALB Xenotropic MuLV but not BALB N-Tropic MuLV. BALB/c mouse cells contain two inducible oncornaviruses: an N-tropic virus (also called BALB:virus-1) and a xenotropic virus (also called BALB: virus 2) encoded in their cellular genome (3). To assess the effect of *Bvr-1* on induction of these two viruses, hybrid cells (4 *Bvr-1*$^+$ and 4 *Bvr-1*$^-$ clones) were treated for 24 h with IdU. The *Bvr-1*$^+$ hybrids were maintained on HAT medium to permit survival of only those hybrids which retain the feline X-chromosome, while the *Bvr-1*$^-$ hybrids were maintained on medium supplemented with 6-thioguanine. After induction with IdU, the test hybrids were cocultivated with heterologous virus-negative cell lines previously demonstrated to be susceptible to infection by these inducible BALB viruses (3, 4). The cocultivated lines were sampled every 5 days over a 6 wk period for virus

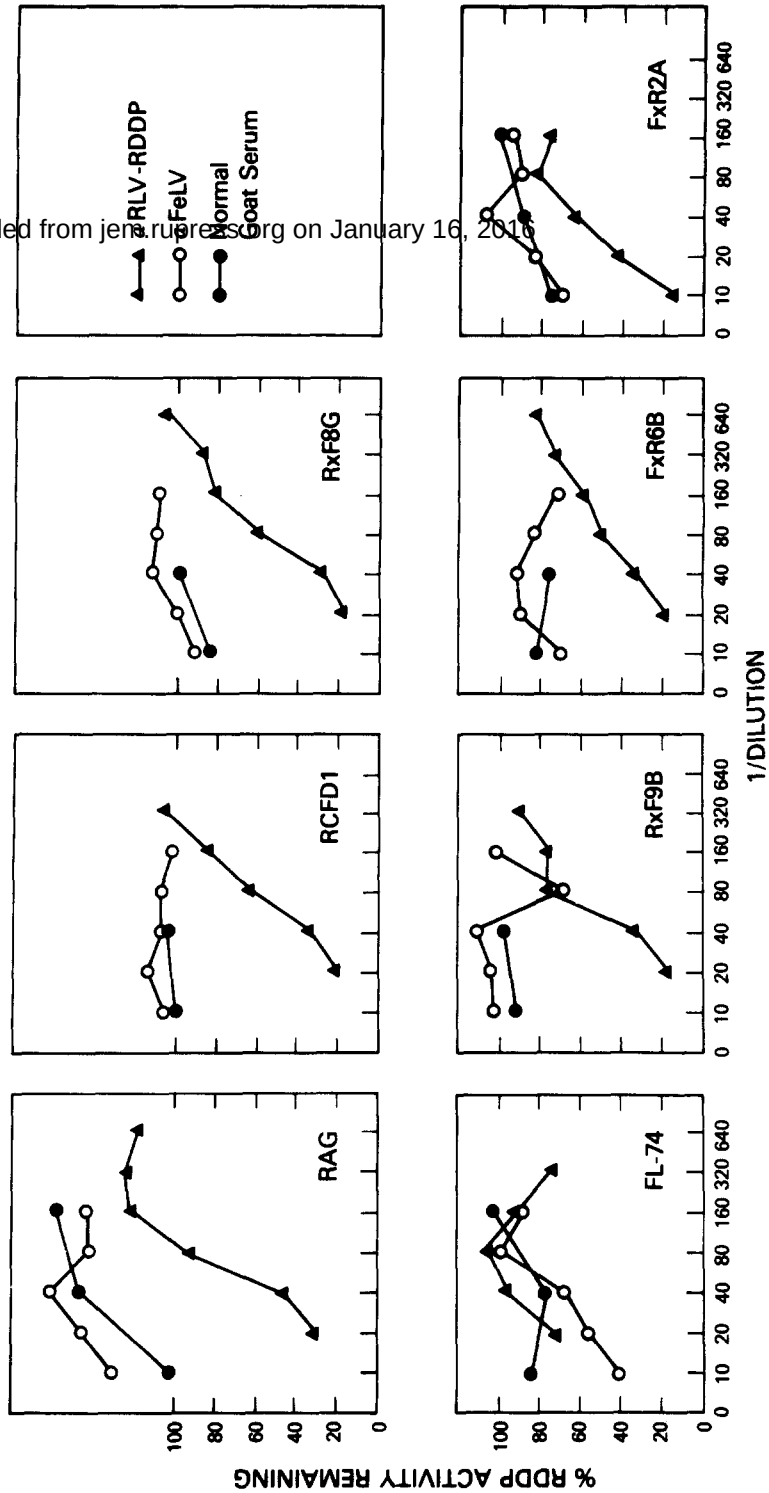

FIG. 1. Group-specific antiserum inhibition of viral RNA-dependent DNA polymerase obtained from *Bvr-1* hybrid colonies. Each culture was maintained on 6-thioguanine selective medium. Virus was harvested from log phase culture fluid and incubated with indicated antiserum as described in Materials and Methods. RDDP activity was determined after a 4 h incubation at 4°C.

TABLE IV
Host Range of Virus Obtained from Back Selected (Bvr-1⁻) RAG × FL-74 Hybrids

Virus source	RDDP Activity of infecting supernate*	Target cells‡				
		N-3T3	B-3T3	SC-1	SIRC	CRFK
FL-74	30	NT§	NT	NT	NT	*275*
RCFD1	3.2	≤4	*30.2*	*7.4*	0.48	≤2.2
RXF8G	35.3	0.8	*155*	*389*	0.0	0.2
FXR6B	6.4	0.5	*269*	*188*	0.1	0.6
RXF9B	17.8	≤1.7	*55*	*184*	0.4	0.43
Uninfected control	0.0	0.39	0.0	0.29	0.4	0

* Units are picomoles dTMP/hour/milliliter culture fluid. Infection and assays as in Table II.
‡ Units are picomoles dTMP/hour/10^6 cells.
§ NT = Not tested.

production. The results of induction with one hybrid pair (RXF9B *Bvr-1⁺* on HAT and RXF9B *Bvr-1⁻* on 6-thioguanine) are presented in Fig. 2.

Two *Bvr-1⁻* control cells were used in these experiments: RAG cells which were induced with IdU and carried on medium supplemented with 10^{-4} M 6-thioguanine, and RCFD1^x, an exceptional hybrid cell line which was induced with IdU and maintained on HAT medium. RCFD1^x was a subclone of RCFD1 which spontaneously began to produce parental levels of B-tropic MuLV despite maintenance on HAT medium. Examination of these colonies with isozyme techniques revealed that the feline G-6-PD was also no longer expressed. However, thermolability decay curves which distinguish between electrophoretically identical mouse and feline HPRT demonstrated that the feline HPRT was present (17). Apparently a portion of this hybrid's X-chromosome had been lost due to chromosome breakage rendering the feline genotype of this line as follows: *Bvr-1⁻, Gpd⁻, Hprt⁺*. This hybrid cell line provides an effective control for aminopterin effects on viral induction. Induction of the RAG cell provides a control for 6-thioguanine effects of viral induction.

IdU-mediated induction of both RAG and RCFD1^x cells resulted in release of a xenotropic virus detectable in 2–3 wk on NRK, SIRC, and VA-2 cells and an N-tropic virus detectable within 10 days on N-3T3 cells (Fig. 2). Virus obtained from SIRC cells which had been cocultivated with back selected FXR6B and RXF9B hybrids exhibited a murine xenotropic host range identical to BALB:virus 2 (data not shown).

The *Bvr-1⁻* subclone of RXF9B (grown on 6-thioguanine) was inducible for the xenotropic endogenous virus as well as the N-tropic endogenous virus (Fig. 2). The kinetics of induction were very similar if not identical to that of RAG or RCFD1^x control cells. The *Bvr-1⁺* hybrid (grown on HAT) was restricted, however, in production of the xenotropic virus on each of the three test cells. The restriction of induction of the xenotropic virus was not absolute since several weeks after the appearance of xenotropic virus from unrestricted hybrids or parent cells, low levels of xenotropic virus could be detected in each of the three target cells cocultivated with restricted (*Bvr-1⁺*) hybrids (Fig. 2b and 2c).

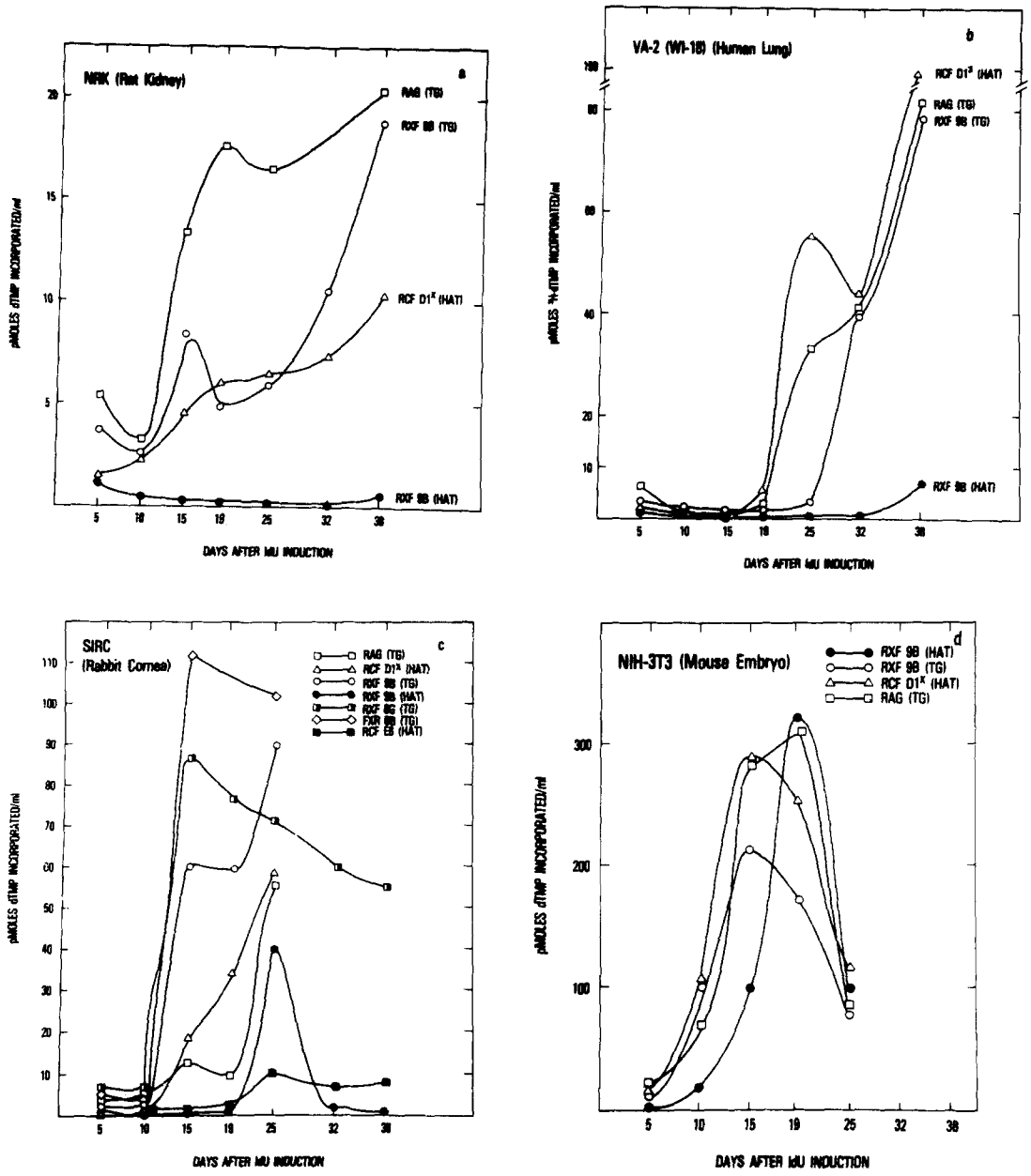

FIG. 2. Kinetics of IdU-mediated induction of X-MuLV (BALB:virus-2) and N-MuLV (BALB:virus-1) from RAG parent cells and a RAG × FL-74 hybrid (RXF9B) pair selectively retaining (HAT) or segregating (6-thioguanine) feline *Bvr-1*. Panels a, b, and c represent cocultivation of induced cells with cell lines from three heterologous species which are permissive for X-MuLV replication. Panel d represents induction of N-MuLV detected by murine *Fv-1* compatibility of NIH-3T3 mouse cells. RCFD1ˣ is an exceptional *Bvr-1*⁻ hybrid which is a positive control for aminopterin effects on IdU-mediated virus induction. RCFD1ˣ is phenotypically *Bvr-1*⁻, *G6PD*⁻, *Hprt*⁺, with respect to feline genes and probably represents a broken chromosome (see text).

TABLE V

Production of Xenotropic and N-Tropic Virus after IdU Induction of Hybrid Cells

Induced cell	Medium	Bvr-1 genotype	Supernatant RDDP picomoles dTMP/10^6 cells	
			VA-2*	NIH-3T3‡
RAG	TG	–	163	144
RCFD1×	HAT	–	204	188
RCFD1	TG	–	173	181
FXR6B	TG	–	191	NT
RXF8G	TG	–	137	120
RXF9B	TG	–	153	138
FXR6B	HAT	+	NT§	248
RXF9B	HAT	+	14.2	141
FXR2A	HAT	+	43	254
RCFE6	HAT	+	8.2	177

* Day 38.

‡ Day 25.

§ NT = Not tested.

The *Bvr-1*$^+$ subclone of RXF9B (grown on HAT) was not restricted in the production of N-MuLV (BALB: virus-1) (Fig. 2 d). N-MuLV was evident within 10 days after induction on NIH-3T3 cells and rises to optimum levels within 2 wk.

IdU-mediated inductions of endogenous MuLV were also performed with the following *Bvr-1*$^+$ hybrids on HAT medium: FXR6B, RCFE6, and FXR2A and with *Bvr-1*$^-$ hybrids on 6-thioguanine: RCF-D1, FXR6B, RXF8G (13). The kinetics of induction for each of these hybrids were essentially identical with the RXF9B curves, viz. that *Bvr-1*$^+$ restricts xenotropic MuLV induction but is leaky in this restriction, and that *Bvr-1*$^+$ has no effect on N-tropic virus induction. The residual xenotropic virus produced by restricted hybrids is apparently completely functional since RDDP levels produced in late stages approached that of xenotropic virus from *Bvr-1*$^-$ cells. A tabulation of intrinsic RDDP activities (picomoles dTMP/hour/10^6 cells) for each of these hybrids at the termination of two cocultivation experiments is presented in Table V.

Characteristics of Residual Virus Produced by Bvr-1$^+$ *Restricted Hybrid Cells.* *Bvr-1*$^+$ restricted cells do not produce an absolute block in oncornavirus production since low levels of RDDP (1–8 pmol/h/10^6 cells) are evident in culture fluid of restricted cells (Table III). We attempted to determine whether this activity represents a "leaky" escape of B-tropic MuLV or rather the simultaneous production of a second endogenous virus in the parent and/or hybrid cells which is not subject to *Bvr-1* restriction. The residual virus from six restricted hybrids (the same six which were back selected) was collected and tested in infectivity assays of murine and feline cells (Table VI). None of the residual virus preparations were infectious in NIH-3T3 or in CRFK cells, thus excluding the presence of FeLV, or murine N- or xenotropic virus. Three of the hybrid virus preparations infected SC-1 cells and replicated to high levels while the other three exhibited a weak infection at best in the same

TABLE VI

*Host Range Character of Residual Virus Obtained from Bvr-1$^+$ (HAT) RAG × FL-74 Hybrids**

Virus source	RDDP Activity of infecting supernate	Target cells				RDDP Activity of infecting supernate from SC-1 cells	Target cells			
		CRFK	N-3T3	B-3T3	SC1		Basc-2	B-3T3	N-3T3	SC1
RAG	7.0	0.0	0.0	*153*	*115*					
RCFD1	2.0	NT	0.64	NT	*145*	— 16.5 →	*429*	*70.3*	4.9	*36.6*
RCFE6	2.5	0.66	0.58	0.29	*248*	— 38.4 →	*495*	*97.1*	0.3	*147*
RXF8G	9.0	0.32	0.9	0.37	*112*	— 35.2 →	*800*	*164.5*	0.1	*133*
RXF9B	0.6	0.0	0.84	1.5	1.9					
FXR2A	2.1	0.07	0.39	0.94	1.47					
FXR6B	2.6	0.41	0.46	0.7	2.85					
Uninfected control	0.0	0.11	0.93	0.28	0.19	0.0	1.0	0.15	0.0	1.9

* Units are picomoles TMP/hour/10^6 cells. Infection and assays are as in Table II.

cells. An unexpected observation was the failure of hybrid virus from all six hybrids to infect B-3T3 even though the RAG B-MuLV replicated readily in B-3T3 cells at approximately the same input virus concentration (Table VI).

Virus recovered from the infected SC-1 cells was further tested for host range on four murine cell lines which differed in their *Fv-1* genotype: Basc-2 and B-3T3 (*Fv-1^{BB}*), NIH-3T3 (*Fv-1^{NN}*), and SC-1 (*Fv-1$^-$*). The residual virus recovered from SC-1 cells exhibited the host range of a B-tropic murine type C virus since they successfully infected Basc-2, B-3T3, and SC-1, but not N-3T3 (Table VI).

Discussion

The present studies demonstrate that a feline chromosomal gene, *Bvr-1*, exerts a *trans*-dominant restriction on the endogenous B-tropic virus produced by BALB/c tumor cells and also described in aged BALB/c mice (7–9, 14). The time of action of *Bvr-1* has been previously established as late in MuLV assembly since budding virions and viral structure proteins are detected in high amounts on restricted cell surfaces, but not in culture fluid pellets (13). The restriction is reversible since reverse selection experiments on 6-thioguanine showed that hybrids which have lost *Bvr-1* again express parental levels of B-tropic MuLV.

Bvr-1 is a pleiotropic gene which is restrictive of IdU-mediated induction of the BALB xenotropic virus in addition to the production of B-tropic virus. Alternatively, *Bvr-1* does not have an appreciable effect on the IdU-mediated induction of the N-tropic BALB/c endogenous virus. Apparently the object of *Bvr-1* restriction in the virus or in its assembly sequence (the viral target of *Bvr-1*) is similar in the B-tropic and xenotropic viruses but distinct from the same in the N-tropic virus.

The production of residual virus by *Bvr-1$^+$* restricted hybrids could be the result of at least three possible situations. (*a*) *Bvr-1* encodes a leaky function which interferes with MuLV production to a large extent but not completely. (*b*) *Bvr-1* is not leaky and the residual virus represents release from restriction in the small percentage of cells which have undergone spontaneous nondisjunction, resulting in loss of the feline X-chromosome. In RAG × FL-74 hybrid cells, the frequency of X-non disjunction is between 10^{-2} and 10^{-3} (S. J.

O'Brien, unpublished observations). These cells survive for several days on HAT medium due to persistence of residual HPRT enzyme and may produce MuLV. This situation would predict a longer half-life for feline HPRT than for the product of *Bvr-1*. (*c*) *Bvr-1* is not leaky and the residual virus represents either an additional B-tropic virus which is insensitive to *Bvr-1* or a precursor of infectious B-MuLV. This interpretation is suggested by the infectivity character of the residual virus.

Virus from *Bvr-1* restricted cells was not infectious in *Fv-1* compatible BALB-3T3 cells, but readily infected the SC-1 cell. The virus recovered from SC-1 cells was fully infectious B-tropic MuLV which was capable of infecting BALB-3T3 and BALB adult fibroblasts. Similar conversions of poorly infectious N-tropic viruses by passage through SC-1 cells have been observed with viruses obtained from C3H-L cells (18), from BALB/c spleen cells, and from C3H 10T1/2 cells (19–21). Rapp et al. (20) have suggested that SC-1 cells provide a microenvironment which results in conversion of a poorly infectious virus to a highly infectious population. The conversion is apparently a heritable viral modification since converted virus retain their high infectivities even after passage in cell lines incapable themselves of effecting the conversion, such as NIH-3T3 and C3H 10T1/2 (U. Rapp, personal communication). *Bvr-1* may block this conversion in the normal sequence of virus assembly which occurs in RAG cells.

It may also be important that recent genetic evidence coupled with DNA sequence similarities suggests that the B-MuLV and N-MuLV of BALB mice are post-transcriptional modifications of the same cellular virogene (22). Thus, the possibility of heritable modification of the endogenous viral genome during virus assembly is a phenomenon to be considered as a possible target of late acting restriction genes like *Bvr-1*. The study of *Bvr-1* restriction may serve to elucidate the sequences of virion modification which are operating in oncornavirus maturation in mouse cells.

The designation of *Bvr-1* as a restriction gene is not intended to connote that it resembles bacterial restriction genes in its mode of action. Bacterial restriction genes encode restriction enzymes which recognize and cleave specific viral DNA sequences as a defense mechanism against lytic infection (23). Eucaryote restriction genes like *Bvr-1* have the same net result, viz. to delimit virus (type C in this case) replication in various species, but their mode of action may be very different. Both murine *Fv-1* and feline *Bvr-1* are restriction gene candidates which act at very different points of viral replication (24–26, 13).

Summary

Bvr-1 is a dominant X-linked feline gene which restricts the replication of B-tropic murine leukemia virus (B-MuLV) in somatic cell hybrids between murine BALB/c-RAG cells and FL-74 feline cells. Since the hybrids were originally derived by the hypoxanthine aminopterin thymidine selection scheme, counter selection experiments on 6-thioguanine result in preferential survival of hybrid cells which have spontaneously lost the feline X-chromosome on which is located the structural gene for hypoxanthine guanine phosphoribosyl transferase (IMP: pyrophosphate phosphoribosyl transferase, E.C. 2.4.2.8) and *Bvr-1*. Back selected *Bvr-1*$^-$ cells express high parental levels of B-MuLV. *Bvr-1* effec-

tively restricts the IdU-mediated induction of the endogenous xenotropic BALB virus (BALB: virus 2) but not the endogenous N-tropic virus (BALB: virus 1). Pleiotropic restriction of B-MuLV and X-MuLV, but not N-MuLV suggests that the viral targets of *Bvr-1* (either viral components or functions in viral assembly) of the B-tropic and X-tropic endogenous BALB viruses are similar to each other but distinct from the target in the N-tropic virus. Very low levels of B-MuLV are detected in restricted cells, but this residual virus is not infectious in either NIH-3T3 or BALB-3T3 mouse cells which are genotypically $Fv\text{-}1^N/Fv\text{-}1^N$ and $Fv\text{-}1^B/Fv\text{-}1^B$, respectively. Passage of residual virus through host cells without *Fv-1* related restriction (SC-1) results in production of infectious B-MuLV indistinguishable from that produced by RAG parent cells.

We are grateful to Doctors R. Goldberg and R. Lemons for discussions and criticisms throughout the progress of this work.

Received for publication 6 May 1977.

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