0022-538X/78/0025-0129$02.00/0
JOURNAL OF VIROLOGY, Jan. 1978, p. 129-137

Vol. 25, No. 1
Printed in U.S.A.

Isolation and Characterization of an Endogenous Type C RNA Virus of Mink (Mv1Lu) Cells

MARIANO BARBACID, STEVEN R. TRONICK, AND STUART A. AARONSON*
Laboratory of RNA Tumor Viruses, National Cancer Institute, Bethesda, Maryland 20014

Received for publication 22 August 1977

Cells of the established Mv1Lu mink line spontaneously released a reverse transcriptase-containing virus after long-term passage in tissue culture. By molecular hybridization, DNA of normal mink cells was found to possess extensive nucleotide sequence homology with a reverse-transcription product of the viral genome, demonstrating that the new isolate was an endogenous virus of mink origin. The mink virus shared antigenic determinants with the major structural proteins of known mammalian type C viruses. Double-antibody competition radioimmunoassays were developed by utilizing the purified major structural protein, p30, of the mink endogenous virus. The virus was shown to possess antigenic determinants unique from those of other known mammalian type C viruses. It exhibited a higher degree of immunological cross-reactivity with endogenous rat type C and horizontally transmitted feline leukemia viruses than with other mammalian type C viruses tested. The finding that mink cells can remain nonvirus producing for many cell generations argues that there normally exists some cellular restriction to endogenous virus expression in this species.

It is well documented that the germ lines of many vertebrate species contain information for endogenous type C RNA viruses (for a review, see reference 2). The widespread existence of such viruses makes it worth considering their possible effects in cell biological investigations. This is particularly the case since many of the most commonly used cell lines are derived from murine species from which endogenous viruses have been isolated. In recent years, cell lines derived from several nonmurine species have become increasingly important in RNA tumor virus research because of their susceptibility to virus infection or because their morphological characteristics allow recognition of focus formation by transforming viruses. A culture derived from Aleutian mink lung, designated Mv1Lu, is an example of one such cell line (17, 20, 21, 23, 29, 37, 42, 44, 49). In the present report, a reverse transcriptase-containing virus, spontaneously released by Mv1Lu cells, has been characterized with respect to its origin and immunological relationship to other mammalian retroviruses.

MATERIALS AND METHODS

Cells and Viruses. Cells were grown in Dulbecco-modified Eagle medium supplemented with 10% calf serum (Colorado Serum Co., Denver, Colo.). The Mv1Lu mink lung cell line was obtained at passage 41 from the American Tissue Culture Collection (ATCC). Canine fetal thymus (ATCC), canine M132 (C. Bowles, Hazleton Laboratories, Vienna, Va.), mouse BALB/3T3 and NIH/3T3 (26), normal rat kidney (11), rhesus monkey FRhL1 (51), and human HOS (38) and A673 and A172 (16) cell lines have been described previously. Secondary cultures of donkey and goat testicle were also used. Normal mink liver tissues were obtained from Space Farm, Sussex, N.J., and United Animal Science Laboratory, Middleton, Wis.

Mammalian retroviruses (13) were either grown in our laboratory (mink type C virus and the W/Fu strain of rat leukemia virus [RaLV]) or obtained as sucrose gradient-purified preparations from Frederick Cancer Research Center, Frederick, Md. (Gross murine leukemia virus [MuLV]), Electro-Nucleonics Laboratories, Rockville, Md. (NIH-MuLV, Rickard strain of feline leukemia virus [FeLV], and the woolly monkey virus [SSAV]), or Pfizer, Inc., Maywood, N.J. (RD-114, baboon [*Papio cynocephalus*] endogenous virus, and Mason-Pfizer monkey virus [MPMV]) through the Office of Resources and Logistics, Virus Cancer Program, National Cancer Institute.

Antisera. Antisera, prepared by immunization of goats with purified Rauscher-MuLV p30 protein or Tween-ether-disrupted viruses, as well as swine anti-goat immunoglobulin G, were provided by R. Wilsnack (Huntingdon Laboratories, Baltimore, Md.) through a contract with the Virus Cancer Program, National Cancer Institute.

Reverse-transcriptase assays. Reverse-transcriptase assays were performed as previously described (39). Briefly, reaction mixtures (0.1 ml) contained 50 mM Tris-hydrochloride buffer (pH 7.8), 90 mM KCl, 2 mM dithiothreitol, 0.5% Triton X-100 (vol/vol), 2.5 μM [*methyl*-^{3}H]TTP (8×10^3 cpm/pmol)

or 5 μM [8-^{3}H]dGTP (3.1 × 10^3 cpm/pmol), divalent cations (0.1 mM $MnCl_2$ or 5 mM $MgCl_2$), and synthetic templates [500 ng of poly(A) and 375 ng of oligo(dT)$_{12-18}$ or 100 ng of poly(rC) · oligo(dG)$_{12-18}$]. After incubation for 60 min at 37°C, [^{3}H]TMP or [^{3}H]GMP incorporation into acid-insoluble material was determined.

Molecular hybridization. Viral [^{3}H]complementary DNA (cDNA) was prepared from mink type C virus grown in canine fetal thymus cells. Reaction mixtures contained 20 mM Tris-hydrochloride (pH 7.8); 60 mM KCl; 1 mM dithiothreitol; 5 mM $MgCl_2$; 0.2 mM dATP, dCTP, and dGTP; 0.01 mM [^{3}H]TTP (50 Ci/mmol, New England Nuclear); 50 μg of actinomycin D (Calbiochem) per ml; 0.014% (vol/vol) Triton X-100; and 50 μg of viral protein per ml. The reaction was primed by the addition of 0.8 mg of DNase I (Worthington Biochemicals Corp.)-digested calf thymus DNA (48) per ml, which resulted in an 18-fold increase in the amount of cDNA synthesized. The reaction was terminated after 4 h of incubation at 37°C by the addition of 0.2 M Tris-hydrochloride (pH 7.5), 0.2 M NaCl, 0.05 M EDTA, 0.8% sodium dodecyl sulfate, 25 μg of calf thymus DNA per ml, and 500 μg of proteinase K (Boheringen) per ml. Viral [^{3}H]cDNA was purified as previously described (41). The mean size of the cDNA was 160 to 300 nucleotides on alkaline sucrose gradients relative to a 120-base [^{32}P]DNA fragment (kindly supplied by W. Haseltine, Harvard University). More than 90% of the [^{3}H]cDNA was degraded to acid-soluble material upon incubation with S1 nuclease (7, 30). Eighty-seven percent of the [^{3}H]cDNA was hybridized by excess mink viral RNA, and half-maximum hybridization was achieved at a C_rt value of 3×10^{-2} mol · s/liter. The T_m of the hybrids was 92°C. The genetic complexity of the cDNA was estimated by determining its ability to protect virion [^{32}P]RNA from digestion by RNase A. At a ratio of cDNA to RNA of 2, 40% of the [^{32}P]RNA was hybridized. Maximum hybridization (80%) was achieved at a ratio of 10.

Virion RNA was prepared as described by Schincariol and Joklik (41). DNA, purified from mink, dog, cow, and human tissues by a modified Marmur procedure (35) as described by Britten et al. (10), was fragmented to an average size of 450 nucleotides with a Virtis model 60K homogenizer. DNA-DNA hybridization was performed by mixing unlabeled cell DNA (4 mg/ml) with [^{3}H]cDNA (2.5 ng/ml; specific activity, 2×10^7 cpm/μg) in 10 mM Tris-hydrochloride buffer (pH 7.5) containing 0.6 M NaCl and 0.1 mM EDTA. The mixture was boiled for 5 min, quick-cooled, and incubated at 68°C. Samples were taken at various times and assayed for hybrid formation by the S1 nuclease method. Data are expressed in terms of C_0t (moles of nucleotide × seconds per liter) and have been corrected to a monovalent cation concentration of 0.18 M (10).

Isolation of retrovirus structural proteins. The major internal antigen, p30, and the envelope glycoprotein, gp70, of mammalian retroviruses, including Rauscher-MuLV, AKR-MuLV, RaLV, FeLV, SSAV, RD-114, baboon (*P. cynocephalus*) endogenous virus, and MPMV, were purified as previously described (5, 24, 46). Each protein was radiolabeled with ^{125}I (14 Ci/mg) by the chloramine T method (18) at specific activities ranging from 10 to 30 μCi/μg.

Radioimmunoassays. Double-antibody precipitation radioimmunoassays were performed by incubating for 3 h at 37°C twofold serial dilutions of the appropriate antiserum with 10,000 cpm of the ^{125}I-labeled antigen in a 0.2-ml reaction mixture containing 10 mM Tris-hydrochloride (pH 7.8), 10 mM NaCl, 1 mM EDTA, 0.4% Triton X-100, and 0.6% bovine serum albumin (fraction V; Sigma Chemical Co.). After incubation at 4°C for 18 h, 0.025 ml of undiluted swine anti-goat immunoglobulin G was added and incubated for another 3 h at 4°C. Samples, diluted to 1 ml with the same buffer except for the omission of bovine serum albumin, were centrifuged at 2,500 rpm for 15 min. Supernatants were aspirated, and the radioactivity in the precipitate was measured in a Searle 1285 gamma counter.

Competition radioimmunoassays were performed similarly except that twofold serial dilutions of the competing antigen were preincubated for 1 h at 37°C with an antiserum dilution able to precipitate 25% of the input ^{125}I-labeled antigen (25). Protein concentrations were determined as described by Lowry et al. (32).

RESULTS

Isolation and characterization of a type C virus from a mink (Mv1Lu) cell line. The mink Mv1Lu cell line has proved very useful for the replication of a number of mammalian retroviruses. In the course of studies with the Mv1Lu line, it was observed that after around 100 generations in tissue culture, the reference stock obtained from the ATCC began producing sedimentable reverse-transcriptase activity. Similar results were observed in at least three separate experiments with Mv1Lu cells, transferred independently from the same early passage culture. To determine whether this enzyme activity was due to a retrovirus, high-speed pellets of culture fluids were suspended and isopycnically banded in a sucrose gradient. Reverse-transcriptase activity was detected at a density of 1.16 g/cm^3 and was examined with respect to its synthetic template and divalent cation specificity. Poly(C)$_n$ · oligo(dG)$_{12-18}$ supported threefold-greater activity than did poly(A)$_n$ · oligo(dT)$_{12-18}$, whereas much less incorporation was observed when nuclease-gapped DNA or poly(dAdT) was used as template. The enzyme was activated to a much greater extent by Mn^{2+} than by Mg^{2+} in the presence of poly(A)$_n$ · oligo(dT)$_{12-18}$. These data suggested that the Mv1Lu cell line was releasing type C virus particles.

This laboratory has developed competition radioimmunoassays that specifically detect the major structural proteins of all known mammalian type C viruses (6). Figure 1 shows that virus purified from Mv1Lu cultures completely dis-

placed the ^{125}I-labeled RD-114 p30 protein from binding limiting amounts of anti-Rauscher-MuLV p30 serum. As a control, when supernatant fluids from early passage Mv1Lu cell cultures with no reverse-transcriptase activity were processed instead, no competition was observed. The above enzymatic and immunological data established the presence of a type C virus in long-term-passage Mv1Lu cells.

To rule out the possibility that the virus was a laboratory contaminant, it was tested for its ability to compete in several radioimmunoassays specific for the major internal antigens of well-characterized mammalian type C viruses. The type C virus produced by mink cells did not detectably react in group-specific assays for mouse (anti-AKR-MuLV serum:^{125}I-labeled Rauscher-MuLV p30) or RD-114/baboon endogenous [anti-baboon (*P. cynocephalus*) virus serum:^{125}I-labeled RD-114 p30] type C viruses (Fig. 1). Similar results were obtained when homologous radioimmunoassays for RaLV p30, FeLV p30, or SSAV p30 proteins were used. These findings indicated that the type C virus isolated from Mv1Lu mink cell cultures differed immunologically from known mammalian type C retroviruses.

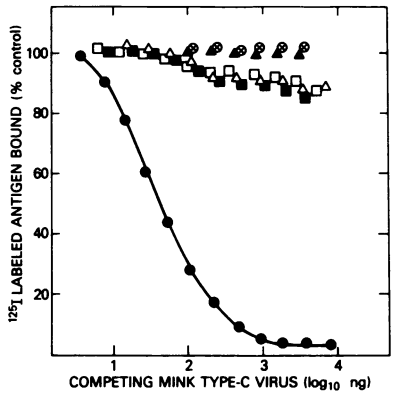

FIG. 1. *Immunological cross-reactivity of a retrovirus isolated from the mink Mv1Lu cell line with the p30 proteins of several mammalian type C viruses. Tissue culture fluids (250 ml) possessing reverse-transcriptase activity were obtained from long-term-passage Mv1Lu mink cells. After a low-speed centrifugation (3,000 × g for 10 min), the supernatants were centrifuged (100,000 × g for 90 min) through a 20% glycerol layer containing 10 mM Tris-hydrochloride (pH 7.8), 100 mM NaCl, and 1 mM EDTA. Viral pellets were resuspended in a final volume of 1 ml of the above buffer, but containing 1% Triton X-100 (vol/vol). After removal of insoluble material by a short centrifugation (3,000 × g for 10 min), detergent-disrupted viral pellets were tested at twofold serial dilutions as competing antigens in the homologous RaLV p30 (□), FeLV p30 (△), and SSAV p30 (⊗) assays, as well as in the group-specific anti-AKR-MuLV:^{125}I-labeled Rauscher-MuLV p30 (■), anti-baboon (P. cynocephalus) virus:^{125}I-labeled RD-114 p30 (▲), and the interspecies anti-Rauscher-MuLV p30:^{125}I-labeled RD-114 p30 (●) immunoassays. Results are expressed as the percentage of total ^{125}I counts per minute in the antigen-antibody precipitate normalized to 100% for maximal binding at infinite competing-antigen dilution.*

Host range of the mink type C virus. The host range of the mink virus was tested on assay cells of a variety of species. As shown in Table 1, the virus was infectious for early passage mink cells, although the level of virion-associated polymerase achieved was relatively low. The virus grew to highest levels in two canine cell lines and in a secondary culture derived from donkey testicle. No detectable polymerase activity was observed in infected primate or rodent cultures.

Nucleotide sequence homology between mink type C viral cDNA and mink cellular DNA. Endogenous mammalian RNA type C viruses have been shown to share nucleotide sequence homology with the DNA of their spe-

TABLE 1. *Host range of the mink endogenous type C virus*

Assay cell	Virion-associated reverse-transcriptase activity[a] after varying lengths of cultivation (weeks):			
	2	4	8	12
Carnivores				
Mink (Mv1Lu)	<0.2	0.8	6.1	10.2
Dog				
Canine fetal thymus	<0.2	4.2	20.6	100
M132	<0.2	1.0	5.3	18.6
Rodents				
Mouse				
BALB/3T3	<0.2	<0.2	<0.2	<0.2
NIH/3T3	<0.2	<0.2	<0.2	<0.2
Rat (normal rat kidney)	<0.2	<0.2	<0.2	<0.2
Ungulates				
Donkey (testicle)	1.5	4.2	15.7	24.8
Goat (testicle)	<0.2	<0.2	<0.2	<0.2
Primates				
Human				
HOS	<0.2	<0.2	0.3	4.7
A673	<0.2	<0.2	<0.2	<0.2
A172	<0.2	<0.2	<0.2	<0.2
Rhesus (FRhL1)	<0.2	<0.2	<0.2	<0.2

[a] Exponentially growing cultures were pretreated overnight with medium containing polybrene (2 μg/ml) and then infected as previously described (3). At the indicated weekly intervals, tissue culture fluids (10 ml) were harvested, clarified from cellular debris, and centrifuged at 100,000 × *g* for 1 h. Pellets were resuspended in 0.2 ml of 50 mM Tris-hydrochloride buffer (pH 7.8) containing 90 mM KCl and 0.5% Triton X-100 (vol/vol) and assayed for reverse-transcriptase activity as described in the text. Results are expressed as picomoles of [^{3}H]TMP incorporated per milliliter of tissue culture fluids. They represent mean values from two separate determinations.

cies of origin. To study the genetic relationship of the mink virus isolate to mink cellular DNA, a [^{3}H]cDNA transcript of the mink viral RNA was prepared. The purified viral DNA probe was then reacted with DNA isolated from normal mink liver tissues. Figure 2 shows that 80% of the probe was hybridized at the highest C_0t value (10^4 mol · s/liter). The C_0t value for half-maximum hybridization (80) indicated the presence of multiple copies of virus-related nucleotide sequences. The T_m of hybrids formed was 92°C (data not shown), indicating extensive base-pair homology. The specificity of the reaction was further demonstrated by the low extent (<10%) of hybridization of the mink viral cDNA with DNA of canine fetal thymus, the cells in which the virus was propagated (Fig. 2). These findings established the mink virus as an endogenous virus of mink cells.

Isolation of the p30 protein of mink endogenous type C virus. Sucrose gradient-purified mink type C virus (6 mg of protein) was disrupted with 1% Triton X-100 and fractionated by phosphocellulose chromatography at pH 6.5 as previously described (45) (Fig. 3A). Column fractions were assayed in the anti-Rauscher-MuLV p30:^{125}I-labeled RD-114 p30 interspecies assay. Peak reactivity, eluting between 0.25 and 0.3 M KCl, was further fractionated by gel filtration chromatography (Fig. 3B). Mink viral p30 protein was found to elute at a position corresponding to an apparent molecular weight of 30,000. Its purity, as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, was greater than 90% (Fig. 4).

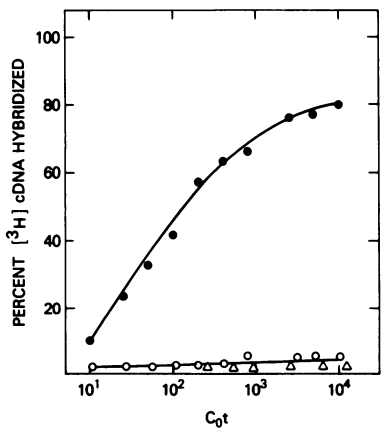

FIG. 2. *Hybridization of mink type C viral cDNA with mink cellular DNA. DNA, purified from fresh livers of normal minks, was tested for its ability to hybridize with 2.5 ng of mink viral [^{3}H]cDNA (●) per ml as described in the text. Other cellular DNAs tested included those isolated from dog liver and canine fetal thymus cells (○), as well as from calf thymus (△).*

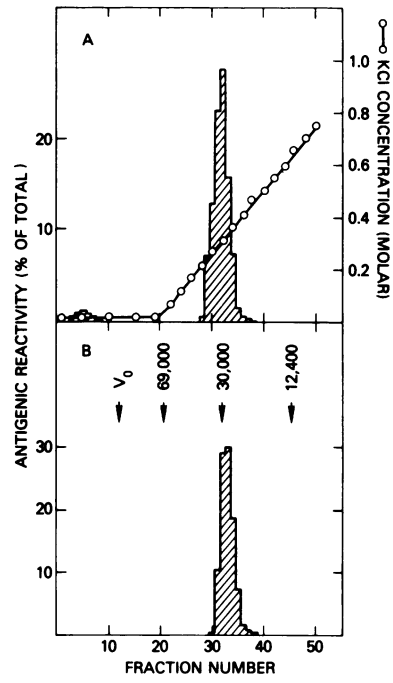

FIG. 3. *Purification of mink type C p30 viral protein. (A) Mink virus (6 mg of protein) was disrupted by sonic treatment for 1 min after the addition of 0.05 volume of 1 M Tris-hydrochloride buffer (pH 9.0) containing 20% Triton X-100 (vol/vol). Nonsolubilized material was removed by high-speed centrifugation (100,000 × g for 30 min), and the remaining supernatant was dialyzed against 500 volumes of BET buffer [10 mM N,N-bis-(2-hydroxyethyl)-2-aminoethanesulfonic acid (pH 6.0)-1 mM EDTA-0.2% Triton X-100]. The sample was then applied to a P-11 phosphocellulose (Whatman) column (1 by 3 cm) previously equilibrated with the same buffer and eluted with 80 ml of a 0 to 0.8 M KCl linear gradient. Portions (0.025 ml) from each fraction were tested as competing antigens in the anti-Rauscher-MuLV p30:^{125}I-labeled RD-114 p30 interspecies assay as described in the legend to Fig. 1. (B) Peak fractions were pooled, concentrated to 1 ml, and applied to a gel filtration column (1.5 by 90 cm) (Ultragel Aca 54, LKB Products) equilibrated with a 10 mM Tris-hydrochloride buffer (pH 7.8) containing 60 mM KCl, 1 mM EDTA, and 0.1% Triton X-100. Fractions containing mink type C viral p30, as determined by immunological criteria, were aliquoted and stored in liquid nitrogen. Molecular weight standards, including blue dextran (V_0), ^{125}I-labeled bovine serum albumin (69,000), ^{125}I-labeled Rauscher-MuLV p30 (30,000), and cytochrome C (12,400), were chromatographed in a previous experiment.*

For radioimmunological studies, purified mink viral p30 was labeled to high specific activity (8 to 12 μCi/μg) with ^{125}I by the chloramine T method. Antisera prepared against the mink vi-

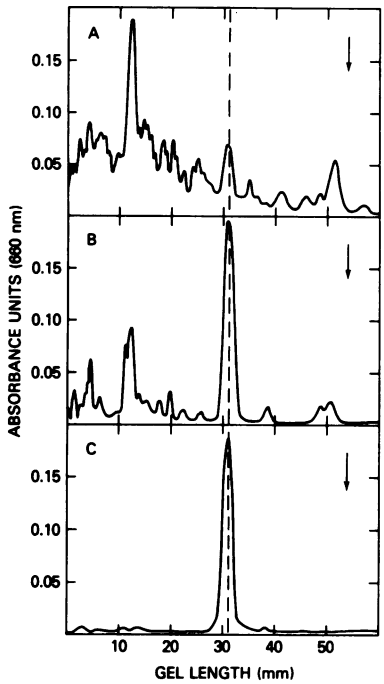

FIG. 4. *Purity of mink type C viral p30 protein. Portions (0.1 ml) from either (A) Triton X-100-disrupted mink virus, (B) phosphocellulose-partially purified mink viral p30 protein (Fig. 3A), or (C) phosphocellulose- and gel filtration-purified mink viral p30 protein (Fig. 3B) were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis by the method of Laemmli (28). Gels, after Coomassie brilliant blue staining, were scanned for absorbance at 660 nm in an ISCO gel scanner model 1310. The broken line represents the position of mink type C viral p30, and the arrow indicates the distance migrated by the tracking dye.*

rus, as well as against type C virus isolates of other mammalian species, were tested for their abilities to bind the ^{125}I-labeled mink viral p30. As shown in Table 2, the high level of binding (87%) achieved with anti-mink type C virus serum indicated the preservation of the immunological determinants of this viral protein during the two-step purification procedure. Immunoprecipitation of mink viral p30 was also observed with antisera prepared against several other mammalian type C viruses. The failure of anti-MPMV serum to precipitate this protein (Table 2) is in agreement with the concept that the major internal proteins of type C and D mammalian retroviruses lack immunological cross-reactivity (45).

An homologous competition radioimmunoassay for mink virus p30 was next developed. When several Triton X-100-disrupted mammalian type C viruses were tested as competing antigens, only the mink type C virus showed a complete displacement of the ^{125}I-labeled probe (Fig. 5). These results were independent of the cell line (canine or human) used to grow the mink virus, strengthening the conclusion that the p30 protein isolated here was virus coded. Among the other mammalian type C viruses tested, only RaLV showed a shallow, although significant, competition curve (Fig. 5).

TABLE 2. *Comparison of abilities of different antisera to bind ^{125}I-labeled mink type C viral p30 protein*

Antiserum to:	Mink p30		Homologous p30	
	Titer[a]	Maximum binding[b]	Titer[a]	Maximum binding[b]
Mink type C virus	80,000	87	80,000	87
AKR-MuLV	10,000	77	150,000	96
RaLV	160,000	83	500,000	100
FeLV	200,000	83	600,000	100
SSAV	320	48	250,000	100
RD-114	3,000	65	250,000	100
Baboon (*P. cynocephalus*) virus	3,000	60	150,000	68
MPMV	<40	5	600,000	90

[a] Titers are expressed as the reciprocal of the highest serum dilution capable of binding 20% of the appropriate ^{125}I-labeled p30 protein.

[b] Maximum binding values represent the percentage of ^{125}I-labeled p30 proteins bound at the lowest serum dilution tested (1:40).

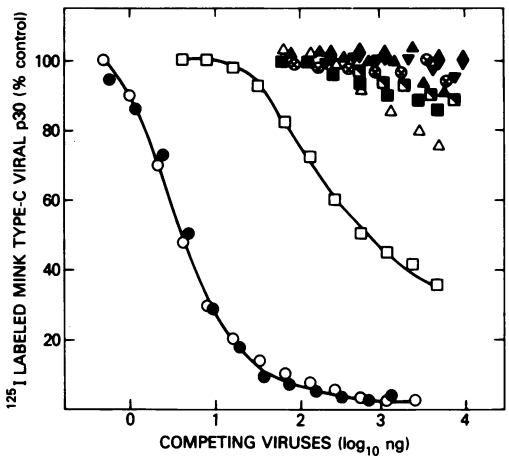

FIG. 5. *Homologous radioimmunoassay for the p30 protein of mink endogenous type C virus. Triton X-100-disrupted mammalian retroviruses were tested at twofold serial dilutions for their ability to compete with 0.5 ng of ^{125}I-labeled mink viral p30 (10,000 cpm) for binding limiting amounts of an antiserum elicited against mink type C virus. Competing viruses included mink type C virus grown in canine fetal thymus cells (●) or in human HOS cells (○), RaLV (□), FeLV (△), AKR-MuLV (■), NIH-MuLV (◪), SSAV (⊗), RD-114 (▲), baboon (P. cynocephalus) virus (▼), and MPMV (◆).*

Immunological relatedness between the mink endogenous type C virus and other mammalian retroviruses. To define the antigenic relationship of the mink type C virus to known mammalian virus isolates, heterologous immunoassays were established by use of limiting amounts of antisera directed against RaLV, FeLV, AKR-MuLV, and RD-114, respectively, to bind ^{125}I-labeled mink viral p30. It was reasoned that the more distantly related the mammalian type C viruses used in such interspecies assays, the more broadly shared antigenic determinants would be detected. As shown in Fig. 6A and B, only the mink virus and the corresponding homologous viruses, RaLV or FeLV, respectively, exhibited complete competition curves in the anti-RaLV or anti-FeLV:^{125}I-labeled mink type C virus p30 immunoassay. When anti-AKR-MuLV serum was used instead, not only mink and mouse type C viruses, but also FeLV and RaLV, demonstrated a high degree of cross-reactivity, whereas the baboon/RD-114 group of viruses showed minimal competition (Fig. 6C). Finally, all of the mammalian viruses tested demonstrated extensive cross-reactivity in an immunoassay in which anti-RD-114 serum was used to precipitate ^{125}I-labeled mink viral p30 (Fig. 6D). These results, taken together, indicate that the mink endogenous type C virus, although clearly distinct, is more closely related to RaLV and FeLV than to the other mammalian type C viruses tested.

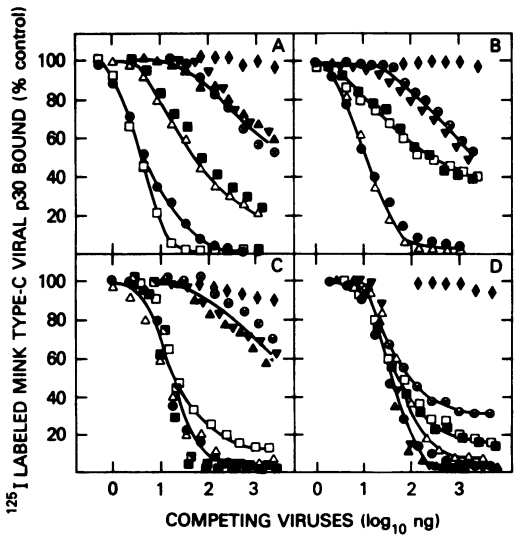

FIG. 6. *Interspecies radioimmunoassays for the p30 protein of mink endogenous type C virus. Limiting amounts of (A) anti-RaLV, (B) anti-FeLV, (C) anti-AKR-MuLV, and (D) anti-RD-114 sera were used to precipitate 0.5 ng of ^{125}I-labeled mink viral p30 protein (10,000 cpm). Detergent-disrupted mammalian retroviruses tested as competing antigens were those described in the legend to Fig. 5.*

These studies were extended to the analysis of a protein coded for by another type C viral gene. The *env* gene-coded glycoprotein gp70 has been shown to contain interpecies antigenic determinants somewhat less broadly reactive than those of the *gag* gene-coded p30 protein (24, 47). Mink type C virus failed to compete in the anti-FeLV:^{125}I-labeled Rauscher-MuLV gp70 interspecies assay, in which murine and FeLV type C viruses showed extensive competition. Similar findings were obtained in the anti-baboon (*P. cynocephalus*) virus:^{125}I-labeled RD-114 gp70 assay (data not shown). However, as shown in Table 3, caprine serum elicited against mink type C virus was able to precipitate the gp70 glycoprotein of FeLV, but not those of Rauscher-MuLV, AKR-MuLV, SSAV, RD-114 or baboon (*P. cynocephalus*) type C viruses. These results strengthen the conclusion that the mink endogenous virus is more closely related to RaLV and FeLV than to other known mammalian endogenous retroviruses.

DISCUSSION

The present report describes the isolation of a reverse transcriptase-containing virus from a continuous line of mink lung cells, Mv1Lu. The virus was shown to cross-react in a broad interspecies immunoassay that detects shared antigenic determinants of the major structural proteins of known mammalian type C viruses (6). Its lack of immunological identity with the p30 proteins of other mammalian type C viruses, as well as the unique antigenic determinants of its

TABLE 3. *Binding of mammalian type C viral gp70's by antiserum to the mink endogenous virus*

Type C viral gp70	Antisera elicited against:			
	Mink type C virus		Homologous type C virus	
	Titer[a]	Maximum binding[b]	Titer[a]	Maximum binding[b]
Rauscher-MuLV	<20	<5	4,000	95
Gross-MuLV	<20	<5	4,000	85
NIH-MuLV	<20	<5	1,200	80
FeLV	160	34	12,000	95
SSAV	<20	<5	4,000	90
RD-114	<20	<5	20,000	98
Baboon (*P. cynocephalus*) virus	<20	8	4,000	80

[a] Titers are expressed as the reciprocal of the highest serum dilution capable of binding 20% of the appropriate ^{125}I-labeled glycoprotein.

[b] Maximum binding values represent the percentage of ^{125}I-labeled glycoprotein bound at the lowest serum dilution tested (1:20).

major structural protein, p30, as defined in a homologous immunoassay developed for this protein, established the mink virus as a new type C virus isolate. By molecular hybridization, it was possible to show extensive nucleotide sequence homology between a reverse-transcription product of the mink viral genome and normal mink cellular DNA, thus demonstrating the virus to be an endogenous virus of mink cells.

Minks (*Mustela vison*) are carnivores, which along with skunks, ferrets, weasels, martens, badgers, and otters form the *Mustelidae* family (50). To date, few carnivores have been shown to contain endogenous retroviral information. Genetic sequences related to the endogenous cat virus RD-114 (4, 14, 31, 33, 40), which closely resembles endogenous viruses of Old World monkeys (9, 22, 43), are limited to a few species of the *Felis* genus (9). In addition, cellular DNAs of the same *Felis* species also possess homology with the genome of FeLV (8, 36), a horizontally transmitted virus, which causes a high incidence of leukemias and lymphosarcomas in cats (for a review, see reference 12). In the present studies, the virus isolated from Mv1Lu mink cells bore striking immunological similarities to rat type C viruses as well as to FeLV. Moreover, preliminary evidence indicates that the mink virus lacks significant homology with DNA of the ferret, another member of the genus *Mustela*. Thus, this endogenous virus appears to be an example of a relatively recent cross-species transfer of type C viral genetic information.

The Mv1Lu cell line has become increasingly useful in studies of the biology of mammalian retroviruses. These cells are permissive for replication of a variety of type C viruses, including mouse xenotropic and endogenous primate viruses (20, 21, 37, 49), as well as retroviruses that are difficult to propagate, such as mouse mammary tumor virus (29) and MPMV (17). Furthermore, the contact-inhibited nature of the Mv1Lu line makes it useful as an assay cell for transforming viruses (21, 23, 37, 44). Recently, type C viruses were isolated after cocultivation of Mv1Lu cells nonproductively transformed by the Kirsten strain of murine sarcoma virus with cells of primate origin (42). The viruses were postulated to be recombinants involving the genomes of the sarcoma virus and endogenous viruses of primate cells (42). The major internal antigens and reverse transcriptases of these viruses closely resembled those of FeLV and RaLV, respectively. As shown here, the endogenous mink virus, although clearly distinguishable, exhibited a high degree of immunological cross-reactivity with FeLV and RaLV. Thus, whether or not the virus isolates of Sherr et al. (42) actually differ from the endogenous mink virus, the fact that this virus can be spontaneously activated raises a note of caution for investigators utilizing the Mv1Lu line for propagation of retroviruses.

There is much evidence indicating that conditions favoring high levels of type C virus production are detrimental to the host. When type C viruses either exogenously infect an animal permissive for virus release and spread or are spontaneously activated during embryonic development, there is an associated high incidence of neoplasia (12, 15, 19, 27, 34). Thus, if there were any advantage to the host conferred by the evolutionary persistence of type C viral genes, there must be strong selective pressures for the development of regulatory mechanisms that control active virus replication. It has been observed that cells that normally restrict virus expression may demonstrate spontaneous virus activation and persistence after prolonged passage in tissue culture (1). The mink type C virus represents another example of an endogenous virus whose cellular restriction can be overcome, allowing it to propagate in cells of its species of origin.

ACKNOWLEDGMENTS

We thank L. L. Long and B. McLaughlin for excellent technical assistance and S. Hino and J. M. Krakower for helpful discussions.

This work was supported by Public Health Service contract NCI-3-73-3212 from the Virus Cancer Program of the National Cancer Institute. M.B. is a Visiting Fellow of the National Institutes of Health, on leave of absence from the Instituto de Bioquímica de Macromoléculas, Consejo Superior de Investigaciones Cientificas, Madrid, Spain.

LITERATURE CITED

1. **Aaronson, S. A., J. W. Hartley, and G. J. Todaro.** 1969. Mouse leukemia virus: "spontaneous" release by mouse embryo cells after long term *in vitro* cultivation. Proc. Natl. Acad. Sci. U.S.A. **65**:87-94.
2. **Aaronson, S. A., and J. R. Stephenson.** 1976. Endogenous type-C RNA viruses of mammalian cells. Biochim. Biophys. Acta Rev. Cancer **458**:323-354.
3. **Aaronson, S. A., G. J. Todaro, and E. M. Scolnick.** 1971. Induction of murine type-C viruses from clonal lines of virus-free BALB/3T3 cells. Science **174**:157-159.
4. **Baluda, M. A., and P. Roy-Burman.** 1973. Partial characterization of RD-114 virus by DNA-RNA hybridization studies. Nature (London) New Biol. **244**:59-62.
5. **Barbacid, M., J. R. Stephenson, and S. A. Aaronson.** 1976. Structural polypeptides of mammalian type-C RNA viruses: isolation and immunologic characterization of a low molecular weight polypeptide, p10. J. Biol Chem. **251**:4859-4866.
6. **Barbacid, M., J. R. Stephenson, and S. A. Aaronson.** 1977. Evolutionary relationships between *gag* gene-coded proteins of murine and primate endogenous type-C RNA viruses. Cell **10**:641-648.
7. **Benveniste, R. E., and E. M. Scolnick.** 1973. RNA in mammalian sarcoma virus transformed nonproducer cells homologous to murine leukemia virus RNA. Virology **51**:370-382.
8. **Benveniste, R. E., C. J. Sherr, and G. J. Todaro.** 1975. Evolution of type-C viral genes: origin of feline

leukemia virus. Science **190**:886–888.
9. **Benveniste, R. E., and G. J. Todaro.** 1974. Evolution of C-type genes: inheritance of exogenously acquired viral genes. Nature (London) **252**:456–459.
10. **Britten, R. J., D. E. Graham, and B. R. Neufeld.** 1974. Analysis of repeating DNA sequences by reassociation. Methods Enzymol. **29**:363–418.
11. **Duc-Nguyen, H., E. N. Rosenblum, and R. F. Zeigel.** 1966. Persistent infection of a rat kidney cell line with Rauscher murine leukemia virus. J. Bacteriol. **92**:1133–1140.
12. **Essex, M.** 1975. Horizontally and vertically transmitted oncornaviruses of cats. Adv. Cancer Res. **21**:175–248.
13. **Fenner, F.** 1976. The classification and nomenclature of viruses: summary of results of meetings of the international committee on taxonomy of viruses in Madrid, September 1975. Virology **71**:371–378.
14. **Fischinger, P. J., P. T. Peebles, S. Nomura, and D. K. Haapala.** 1973. Isolation of an RD-114-like oncornavirus from a cat cell line. J. Virol. **11**:978–985.
15. **Furth, J., H. R. Siebold, and R. R. Rathbone.** 1933. Experimental studies on lymphomatosis of mice. Am. J. Cancer **19**:521–604.
16. **Giard, D. J., S. A. Aaronson, G. J. Todaro, P. Arnstein, J. H. Kersey, H. Dosik, and W. P. Parks.** 1973. *In vitro* cultivation of human tumors: establishment of cell lines derived from a series of solid tumors. J. Natl. Cancer Inst. **51**:1417–1423.
17. **Goldberg, R. J., R. Levin, W. P. Parks, and E. M. Scolnick.** 1976. Quantitative analysis of the rescue of RNA sequences by mammalian type C viruses. J. Virol. **17**:43–50.
18. **Greenwood, F. C., W. M. Hunter, and J. S. Glover.** 1963. The preparation of ^{131}I-labeled human growth hormone of high specific radioactivity. Biochem. J. **89**:114–123.
19. **Gross, L.** 1951. Spontaneous leukemia developing in C3H mice following inoculation in infancy with AK-leukemic extracts or AK-embryos. Proc. Soc. Exp. Biol. Med. **76**:27–43.
20. **Hartley, J. W., and W. P. Rowe.** 1976. Naturally occurring murine leukemia viruses in wild mice: characterization of a new "amphotropic" class. J. Virol. **19**:19–25.
21. **Hartley, J. W., N. K. Wolford, L. J. Old, and W. P. Rowe.** 1977. A new class of murine leukemia virus associated with development of spontaneous lymphomas. Proc. Natl. Acad. Sci. U.S.A. **74**:789–792.
22. **Hellman, A., P. T. Peebles, J. E. Strickland, A. K. Fowler, S. S. Kalter, S. Oroszlan, and R. V. Gilden.** 1974. Baboon virus isolate M-7 with properties similar to feline virus RD-114. J. Virol. **14**:133–138.
23. **Henderson, J. C., M. M. Lieber, and G. J. Todaro.** 1974. Mink cell line Mv1Lu (CCL64): focus formation and the generation of "nonproducer" transformed cell lines with murine and feline sarcoma viruses. Virology **60**:282–287.
24. **Hino, S., J. R. Stephenson, and S. A. Aaronson.** 1975. Antigenic determinants of the 70,000 molecular weight glycoprotein of woolly monkey type-C RNA virus. J. Immunol. **115**:922–927.
25. **Hino, S., J. R. Stephenson, and S. A. Aaronson.** 1976. Radioimmunoassays for the 70,000-molecular-weight glycoproteins of endogenous mouse type C viruses: viral antigen expression in normal mouse tissues and sera. J. Virol. **18**:933–941.
26. **Jainchill, J. L., S. A. Aaronson, and G. J. Todaro.** 1969. Murine sarcoma and leukemia viruses: assay using clonal lines of contact-inhibited mouse cells. J. Virol. **4**:549–553.
27. **Kawakami, T. G., S. D. Huff, P. M. Buckley, D. L. Dungworth, S. P. Snyder, and R. V. Gilden.** 1972. Isolation and characterization of a C-type virus associated with gibbon lymphosarcoma. Nature (London) New Biol. **235**:170–171.
28. **Laemmli, U. K.** 1970. Cleavage of structural protein during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
29. **Lasfargues, E. Y., A. B. Vaidya, J. C. Lasfargues, and D. H. Moore.** 1977. *In vitro* susceptibility of mink lung cells to the mouse mammary tumor virus. J. Natl. Cancer Inst. **57**:447–449.
30. **Leong, J. A., A. C. Garapin, H. Jackson, L. Fanshier, W. Levinson, and J. M. Bishop.** 1972. Virus-specific ribonucleic acid in cells producing Rous sarcoma virus: detection and characterization. J. Virol. **9**:891–903.
31. **Livingston, D. M., and G. J. Todaro.** 1973. Endogenous type-C virus from a cat cell clone with properties distinct from previously described feline type-C virus. Virology **53**:142–151.
32. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
33. **McAllister, R. M., M. Nicolson, M. B. Gardner, R. W. Rongey, S. Rasheed, P. S. Sarma, R. J. Huebner, M. Hatanaka, S. Oroszlan, R. V. Gilden, A. Kabigting, and L. Vernon.** 1972. C-type virus released from cultured human rhabdomyosarcoma cells. Nature (London) New Biol. **235**:3–6.
34. **MacDowell, E. C., and M. N. Richter.** 1935. Mouse leukemia. IX. The role of heredity in experimental cases. Arch. Pathol. **20**:709–724.
35. **Marmur, J.** 1961. A procedure for the isolation of deoxyribonucleic acid from microorganisms. J. Mol. Biol. **3**:208–218.
36. **Okabe, H., E. Twoddy, R. V. Gilden, M. Hatanaka, E. A. Hoover, and R. G. Olsen.** 1976. FeLV-related sequences in DNA from a FeLV-free cat colony. Virology **69**:798–801.
37. **Peebles, P. T.** 1975. An *in vitro* focus-induction assay for xenotropic murine leukemia virus, feline leukemia virus C and the feline-primate viruses RD-114/CCC/M-7. Virology **67**:288–291.
38. **Rhim, J. S., H. Y. Cho, and R. J. Huebner.** 1975. Nonproducer human cells induced by murine sarcoma virus. Int. J. Cancer **15**:23–29.
39. **Ross, J., E. M. Scolnick, G. J. Todaro, and S. A. Aaronson.** 1971. Separation of murine cellular and murine leukemia virus DNA polymerases. Nature (London) New Biol. **231**:163–167.
40. **Sarma, P. S., J. Tseng, Y. K. Lee, and R. V. Gilden.** 1973. Virus similar to RD-114 virus in cat cells. Nature (London) New Biol. **244**:56–59.
41. **Schincariol, A. L., and W. K. Joklik.** 1973. Early synthesis of virus-specific RNA and DNA in cells rapidly transformed with Rous sarcoma virus. Virology **56**:532–548.
42. **Sherr, C. J., R. E. Benveniste, M. M. Lieber, and G. J. Todaro.** 1976. Type C viruses from Kirsten sarcoma-transformed mink cells co-cultivated with primate cells and expressing p30 antigens related to feline leukemia virus. J. Virol. **19**:346–358.
43. **Sherr, C. J., M. M. Lieber, R. E. Benveniste, and G. J. Todaro.** 1974. Endogenous baboon type-C virus (M7): biochemical and immunologic characterization. Virology **58**:492–503.
44. **Staal, S. P., J. W. Hartley, and W. P. Rowe.** 1977. Isolation of transforming murine leukemia viruses from mice with a high incidence of spontaneous lymphoma. Proc. Natl. Acad. Sci. U.S.A. **74**:3065–3067.
45. **Stephenson, J. R., S. Hino, E. W. Garrett, and S. A. Aaronson.** 1976. Immunological cross-reactivity of Mason-Pfizer monkey virus with type-C RNA viruses endogenous to primates. Nature (London) **216**:609–611.
46. **Strand, M., and J. T. August.** 1973. Structural proteins of oncogenic ribonucleic acid viruses: interspec II, a new interspecies antigen. J. Biol. Chem. **248**:5627–5633.
47. **Strand, M., and J. T. August.** 1974. Structural proteins of mammalian oncogenic RNA viruses: multiple anti-

genic determinants of the major internal protein and envelope glycoprotein. J. Virol. 13:171–180.
48. **Taylor, J. M., R. Illmensee, and J. Summers.** 1976. Efficient transcription of RNA into DNA by avian sarcoma virus polymerase. Biochim. Biophys. Acta 442:324–330.
49. **Todaro, G. J., C. J. Scherr, R. E. Benveniste, and M. M. Lieber.** 1974. Type-C viruses of baboons: isolation from normal cell cultures. Cell 2:55–61.
50. **Walker, E. P.** 1968. Mammals of the world, p. 1189–1223. The Johns Hopkins Press, Baltimore.
51. **Wallace, R. E., P. J. Vasington, J. E. Petricciani, H. E. Hoppe, D. E. Lorenz, and Z. Kadanka.** 1973. Development and characterization of cell lines from subhuman primates. In Vitro 8:333–341.