Isolation of an Endogenous Type C Virus Related to the Infectious Primate Type C Viruses from the Asian Rodent *Vandeleuria oleracea*

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A tissue culture cell line derived from the Asian rodent *Vandeleuria oleracea* has been shown to release an infectious, xenotropic type C virus. The virus-associated reverse transcriptase (RNA-dependent DNA nucleotidyltransferase) and the major internal protein p30 are immunologically related to the respective proteins of the woolly monkey-gibbon ape group of infectious primate viruses. By these criteria the *V. oleracea* viral isolate is similar to the murine type C-I class of endogenous retroviruses and has been designated Vand C-I. Nucleic acid homology studies show that *V. oleracea* cellular DNA shares similar levels of homology with DNA from members of the *Mus* and *Rattus* genera and lower levels of homology with other rodent genera. The Vand C-I viral genome is present in *V. oleracea* cellular DNA in multiple copies, and partially related sequences can be detected in other rodent genera. These results support the conclusion that the Vand C-I viral genome is genetically transmitted in *V. oleracea* and that the type C-I class of endogenous retroviral genes has been highly conserved during evolution.

Members of the genus *Mus* contain genetic information for at least four different identifiable classes of endogenous retroviruses (2, 12, 13, 21). The major internal protein (p30) and virus-associated reverse transcriptase of one of these classes, type C-I, share antigenic determinants with the respective proteins of the woolly monkey-gibbon ape group of infectious primate type C viruses (2, 13, 17). The virus-related nucleic acid sequences corresponding to the type C-I endogenous retroviruses are highly conserved in the cellular genome of members of the genus *Mus* and can be detected in other more distantly related genera (2, 13). Although the type C-I class has been readily isolated from a number of different Asian species of the genus *Mus*, other genera of rodents have not previously yielded this virus.

In this report, we describe the properties of a type C virus isolated from the Asian rodent *Vandeleuria oleracea* (long-tailed tree mouse) (14, 27). This rodent inhabits the region between India and Vietnam and is anatomically distinguishable from other rodent genera by structural features of the hands including adhesive pads on the tops of the digits and soles of the feet (14, 27). Other distinguishing features include a non-tufted tail and the structure of the teeth and skull. By karyological criteria, this genera has a diploid chromosome number of 26 and is distin-

guished by the occurrence of numerous Robertsonian fusions (14). As shown here, the non-repetitive cellular DNA of *V. oleracea* shares approximately equal homology with cellular DNA of the genera *Rattus* and *Mus*, and lower extents of homology with other genera of rodents. By these criteria it is considerably more distant from *Mus musculus*, for example, than are the most distantly related *Mus* species, e.g., *M. shorridgei* (13). The type C viral isolate obtained from *V. oleracea* is endogenous to this species as shown by nucleic acid hybridization to normal cellular DNA. By immunological criteria, the virus-associated reverse transcriptase and p30 proteins are related to the respective proteins of the woolly monkey and gibbon infectious primate type C viruses. These results support our previous observations, based on nucleic acid hybridization data, that the type C-I class of endogenous virogenes has been conserved in various members of the Muridae family (2, 13). Moreover, these results suggest that, in addition to various members of the genus *Mus*, other Asian rodent species may be a source, via horizontal transmission, of the infectious leukemia-producing, type C viruses found in certain populations of gibbons.

MATERIALS AND METHODS

Cell cultures. Animals of the species *V. oleracea*

were originally trapped in the Loei province of Thailand by J. Marshall (Fish and Wildlife Service). They subsequently were sent to our laboratory for the establishment of a breeding colony. The *V. oleracea* tissue culture cell line, designated M867, was established from the kidney tissue of one specimen and has been passaged continuously for over a year. The sources and methods of propagation of other cell lines and viruses used were as described (4, 13, 16, 23, 24).

Viral polymerase assays. Virus from culture supernatants was concentrated and assayed for viral reverse transcriptase with synthetic template and primer as described (16). Results are expressed as counts per minute of [³H]dTMP incorporated into acid-insoluble polydeoxythymidilic acid product in a 60-min incubation at 37°C. Polymerase antibody inhibition studies were performed as described (24).

Radioimmunoassays. Radioimmunoassays for the major type C viral structural protein (p30) (22) were performed as described (25). Extracts of cells infected with type C viruses were used as competing antigens. The results are plotted as percent displacement of radiolabeled test antigen (linear scale ordinate) versus micrograms of competing protein (log scale abscissa).

Preparation of ³H-labeled viral DNA transcripts. The Vand C-I virus, the designation for the *V. oleracea* viral isolate, was propagated on the cat cell line FEC. Virus from 12-h culture supernatant collections was purified and concentrated as previously described (9). The [³H]thymidine-labeled viral DNA probe was prepared from detergent-disrupted virus in the presence of actinomycin D as described (9). The specific activity of the ³H-labeled DNA probe was 2×10^7 cpm/ μ g.

Extraction of nucleic acids. Cellular DNA and RNA were extracted and purified as described (3). Cellular DNA was fragmented by sonic oscillation and had a sedimentation coefficient of 7 to 11S as determined by centrifugation on alkaline sucrose gradients (3). The DNA of various tissue culture cell lines was labeled with [³H]thymidine (120 μ Ci/ml of cell culture media) for 72 h and extracted (3). Nonrepetitive cellular DNA was then fractionated (7).

Molecular hybridization. DNA-DNA and DNA-RNA hybridizations were incubated for varying times in reaction mixtures containing 0.01 M Tris-hydrochloride (pH 7.5), 0.7 M NaCl, 2×10^{-4} M EDTA, 0.05% sodium dodecyl sulfate, 30,000 to 40,000 cpm of ³H-labeled viral DNA probe or nonrepetitive cellular DNA per ml, and 0.2 to 4 mg of cellular DNA or RNA per ml. Hybridizations were initiated by heating at 100°C for 10 min and then incubation at 65°C. At varying times, 0.025- or 0.05-ml portions were removed and frozen at -80°C until digested with S1 nuclease (1, 5) using high stringency conditions as described previously (5) (0.15 M monovalent cation concentration and incubation at 45°C for 0.75 h). Low stringency conditions of digestion included 0.6 M monovalent cation concentration and an incubation at 37°C for 1 h. C_0t values (C_0 is the concentration of cellular DNA in moles of nucleotide per liter and t is the time in seconds) were calculated as suggested by Britten and Kohne (10) and corrected to a monovalent cation concentration of 0.18 M (11).

RESULTS

A tissue culture cell line (M867) was established from the kidney of a male *V. oleracea*. After 24 weeks in culture and 12 passages, reverse transcriptase activity was detected in culture supernatants. Filtered supernatant fluids from these cultures were used to infect other mammalian tissue culture cell lines. Infected cultures were monitored at 2-week intervals for virus-associated reverse transcriptase activity in supernatant fluids. Table 1 lists the host range of the *V. oleracea* virus (Vand C-I). Four weeks after infection, reverse transcriptase activity was detected in culture supernatants of the human line A549 and the cat line FEC. After 8 weeks, virus replication could also be detected in mink, bat, and rabbit, but not murine, cell cultures.

The antigenic properties of the virus-associated reverse transcriptase were determined using immunoglobulin G prepared against the polymerases of Rauscher murine leukemia virus (R-MuLV) and the woolly monkey infectious

TABLE 1. Host range of the *Vandeleuria oleracea* type C virus (Vand C-I)

Species	Host cell line	Supernatant reverse transcriptase assay ^a ([³ H]-dTMP incorporated, cpm $\times 10^{-3}$)
<i>Mus caroli</i>	<i>Mus caroli</i> lung	4.5
<i>M. cervicolor</i>	<i>M. cervicolor</i> lung	1.7
<i>M. dunni</i>	<i>M. dunni</i> kidney	7.0
<i>M. musculus</i>	Feral embryo (SC-1)	5.4
<i>M. shortridgei</i>	<i>M. shortridgei</i> lung	2.9
Human	Lung carcinoma (A459)	938.2
Chimpanzee	Glial cells (Cha-15)	3.5
Rabbit	Cornea (SIRC)	52.2
Cat	Embryo (FEC)	129.9
Bat	Lung (Tb-1-Lu)	162.6
Mink	Lung (Mv-1-Lu)	108.8
Dog	Thymus (FCf2Th)	2.9

^a Cell culture medium from the *V. oleracea* kidney tissue culture cell line was filtered and used to infect the host cell lines listed. Media from infected cultures were assayed at 2-week intervals for virus-associated reverse transcriptase activity. The results shown are values obtained 8 weeks after infection. Numbers in italics indicate levels of viral polymerase activity significantly above background.

FIG. 1. The inhibition of type C virus-associated reverse transcriptase by antisera (immunoglobulin G) prepared against: (A) the woolly monkey type C virus, SSAV; (B) the *M. musculus* type C virus, R-MuLV. The conditions for the assay have been described (24). The enzymes were obtained from SSAV (○), Vand C-I (●), and R-MuLV (▲).

primate type C virus (SSAV). As shown in Fig. 1A, the antiserum to the SSAV enzyme almost completely inhibits the Vand C-I enzymatic activity, whereas the R-MuLV enzyme is only partially inhibited (24). In contrast, the antiserum to the R-MuLV enzyme only partially inhibits the Vand C-I enzymatic activity and does not inhibit the SSAV enzyme (24). By this criteria, the Vand C-I polymerase appears antigenically to be closely related to the SSAV enzyme. A similar pattern of inhibition by these sera has been previously reported for the type C-I class of endogenous retroviruses isolated from Asian species of the genus *Mus* (2, 17).

Figure 2 depicts the results of competitive radioimmunoassays for the major internal viral protein (p30) of SSAV and *M. musculus* type C-II virus S2C13 (BALB/c ecotropic type C virus) using antisera prepared against these proteins. In this assay, the slope of the competition curve can be used as a relative measure of the antigenic relatedness between the radiolabeled protein and the homologous antigens in the competing viral preparation (24, 25). Figure 2A shows an assay for the SSAV p30 protein in which Vand C-I competes, but with a reduced slope. Vand C-I does not compete in the S2C13 radioimmunoassay (Fig. 2B). These results show that, like the virus-associated polymerase, the major internal viral protein of Vand C-I is antigenically partially related to the respective SSAV protein. We have previously shown that the murine type C-I viruses compete in an interspecies assay which detects antigenic determinants common

to endogenous porcine and infectious primate type C viruses (13, 17). Rat and hamster type C viruses do not compete in this assay. The Vand C-I virus competes with radiolabeled SSAV p30 protein when anti-pig virus p30 antiserum is used (Fig. 2C). Thus, on the basis of immunological criteria, the Vand C-I virus-associated proteins studied here exhibit antigenic reactivities similar to the various viruses of the murine type C-I class.

FIG. 2. Radioimmunoassays for viral p30 proteins. (A) Assay for SSAV p30 protein using antisera to SSAV p30 and ¹²⁵I-labeled SSAV p30. (B) Assay for murine p30 protein using antisera to S2C13 (BALB/c ecotropic type C virus) p30 and ¹²⁵I-labeled S2C13 p30. (C) Interspecies assay using antisera to the p30 protein of endogenous porcine virus, PK(15), and ¹²⁵I-labeled SSAV p30 (41). Symbols: ●, human cells infected with SSAV; ○, cat cells infected with Vand C-I; ▲, mouse cells infected with S2C13 virus; △, rat cells spontaneously releasing VNRK virus.

Vandeleuria is a taxonomically distinct genus containing one species, *V. oleracea* (14, 27). We have compared the evolutionary relationship of *V. oleracea* to other rodent genera by measuring the conservation of nucleic acid sequence homology between their cellular genomes. *V. oleracea* nonrepetitive cellular DNA was purified from [³H]thymidine-labeled tissue culture cells and used as a probe to measure the extent of sequence homology and the thermal stability (ΔT_m) of the hybrids formed with cellular DNA from various other rodent species. ΔT_m is an index of base-pair mismatching between DNAs of different species (20). The results are shown in Table 2. Using standard S1 nuclease digestion conditions (high stringency) to detect hybridization, the labeled *V. oleracea* nonrepetitive cellular DNA hybridizes to similar final extents with *Rattus* and *Mus* cellular DNAs (20 to 30%) and to lesser extents with hamster (12%) and guinea pig (1%) cellular DNAs. When the final extents of hybridization are measured under low stringency conditions, less stable hybrids are detected (see Materials and Methods). The *V. oleracea* cellular DNA probe hybridized 38 to 45% with *Rattus* and *Mus* cellular DNAs and again to lesser extents with hamster DNAs.

Moreover, the ΔT_m of the *Rattus* and *Mus* DNA hybrids are similar (10.5 to 11.7°C), whereas the hamster DNA hybrids have a ΔT_m ranging from 18.7 to 19.3°C. In reciprocal experiments, *R. norvegicus*- and *M. cervicolor*-labeled nonrepetitive cellular DNAs hybridized to similar extents (22 and 18%, respectively) with *V. oleracea* cellular DNA. Similarly, members of the genera *Rattus* and *Mus* share 16 to 22% nucleic acid sequence homology with each other under stringent conditions for S1 nuclease digestion. These results are consistent with the divergence of the genera *Rattus*, *Mus*, and *Vandeleuria* from a common ancestor subsequent to the divergence of other rodent genera including *Cricetulus griseus* (Chinese hamster), *Mesocricetus auratus* (Syrian hamster), and *Cavia porcellus* (guinea pig).

Endogenous retroviruses are characterized by the presence of multiple copies of virus-related nucleic acid sequences in the cellular DNA of the species of origin, and related sequences in other closely related species (6, 13, 17). To determine whether Vand C-I is endogenous to *V. oleracea*, we prepared a ³H-labeled DNA transcript of the viral genome using the endogenous reverse transcriptase reaction. The single-strand

TABLE 2. Nucleic acid homology between rodent nonrepetitive DNAs

Species ^a	³ H-labeled nonrepetitive cellular DNA ^b			
	<i>Vandeleuria</i> (% hybrid)	ΔT_m (°C) ^c	Rat (% hybrid)	Mouse (% hybrid)
<i>Vandeleuria oleracea</i>	100		22	18
Rat				
<i>Rattus norvegicus</i>	21 (40) ^d	11.7	100	16
<i>R. rattus</i>	28 (44)	10.7	70	14
Mouse				
<i>Mus cervicolor</i>	20 (45)	10.5	24	100
<i>M. pahari</i>	29 (44)	11.2	25	44
<i>M. shortridgei</i>	22 (38)	NT	19	36
Chinese hamster				
<i>Cricetulus griseus</i>	13 (18)	19.3	9	6
Syrian hamster				
<i>Mesocricetus auratus</i>	10 (14)	18.7	2	2
Guinea pig				
<i>Cavia porcellus</i>	1.0 (1.0)	28	0	0

^a Cellular DNA was extracted from various tissues or cultured cell lines of the species listed, as described (3), and fragmented to a mean size of 7 to 11S (see text).

^b The preparation of [³H]thymidine-labeled nonrepetitive cellular DNA and conditions for hybridization are described in the text. Hybridization reactions were incubated at 65°C for 72 h and then digested with single-strand-specific nuclease, S1, as described (5). The values given are percent hybridization at a C_0t of 2×10^4 and were normalized to the final extent of hybridization with the homologous DNA. These values varied from 60 to 70%.

^c The temperature at which 50% of the hybrids are dissociated (T_m) was determined as described (2), except that low stringency conditions were employed (as above). The ΔT_m is the difference in T_m between the homologous hybrid (91°C) and the other DNA-DNA hybrids.

^d Values in parenthesis were obtained using low stringency conditions of S1 nuclease digestion. The monovalent cation concentration was 0.6 M, and incubation was at 37°C for 1 h; other components of the reaction were as described (5).

^3H -labeled DNA probe had a sedimentation value of 21S on an alkaline sucrose gradient, which corresponds to the approximate size expected for a full-length DNA copy of the viral genome. We have compared the kinetics of hybridization of this probe to cellular DNA extracted from the *V. oleracea* tissue culture cell line ($C_{0t_{1/2}} = 2 \times 10^1$) and the self-annealing of *V. oleracea* nonrepetitive cellular DNA ($C_{0t_{1/2}} = 1.5 \times 10^3$), used here as a standard for nucleic acid sequences which are present once per haploid genome (Fig. 3). By this criterion, the virus-related sequences are repeated approximately 75 times per haploid genome in *Vandeleuria*. This represents a 15-fold increase in the copy number of type C-I endogenous viral sequences than was previously observed in *Mus cervicolor* (2). To determine whether these repeated viral sequences in *Vandeleuria* are identical to the viral genome, we have compared the thermal stabilities of the hybrids formed between the Vand C-I viral DNA probe and, respectively, *V. oleracea* and Vand C-I-infected cat cellular DNA. The latter cell line contains one or perhaps two copies of virus-related sequences per haploid genome (Fig. 3). The virus-related sequences in *Vandeleuria* DNA melt over a broad temperature range with a T_m of 88.5°C, whereas the viral sequences in the DNA of the infected cat cell line have a T_m of 92°C (Fig. 4). The observed ΔT_m of 3.5°C suggests that there is extensive base-pair mismatching between the viral genome and the multiple copies of viral sequences in the *Vandeleuria* cellular DNA.

Cellular DNAs of other species were tested with the viral ^3H -labeled DNA probe for the presence of nucleic acid sequences related to the Vand C-I viral genome (Table 3). Since *Vandeleuria* is only distantly related to other rodent genera, low stringency conditions for S1 nuclease digestion were used to measure hybrid formation. Under these conditions, virus-related sequences could be detected in the cellular genome of all rodents tested, whereas DNA from nonrodent species lacked these sequences. The final extents of hybridization of the Vand C-I viral ^3H -labeled DNA with the rodent cellular DNAs are similar to those obtained with the *Vandeleuria*-labeled nonrepetitive cellular DNA probe (Table 2). Thus, *Rattus* and *Mus* cellular DNAs shared more sequence homology (45 to 48% and 28 to 32%, respectively) with the viral DNA probe than did hamster or guinea pig DNAs. These results are consistent with the evolutionary conservation of the type C-I class of endogenous retroviral sequences in the cellular genome of members of the Muridae family. The fact that the final extent of hybridization of the

FIG. 3. Hybridization of ^3H -labeled Vand C-I viral DNA and *V. oleracea* nonrepetitive cellular DNA probes with cellular DNA from *V. oleracea* and a cat cell line infected with Vand C-I. The DNA-DNA hybridizations were performed as described in the text. Hybridization was detected using stringent conditions of S₁ nuclease digestion. A Vand C-I viral ^3H -labeled DNA probe was hybridized with cellular DNA from *V. oleracea* (●), Vand C-I-infected FEC tissue culture cells (○), and domestic cat liver (Δ). A ^3H -labeled nonrepetitive *V. oleracea* cellular DNA probe was hybridized with *V. oleracea* cellular DNA (▲).

viral DNA probe to mouse cellular DNA was lower than observed with rat DNA may suggest that the viral sequences are evolving faster in the genus *Mus* than they are in the genus *Rattus*, relative to their respective nonrepetitive cellular DNAs.

We have previously shown nucleic acid sequence homology between the genomes of the murine type C-I endogenous retroviruses (2; and unpublished observations). The extent of homology depends on the evolutionary distance between the species from which the viruses were isolated. Moreover, low levels of sequence homology could be demonstrated between the *M. cervicolor* type C-I viral genome and the genomes of SSAV and the gibbon ape leukemia virus (GaLV) (2). We have used the Vand C-I viral DNA probe to measure nucleic acid sequence homology between the Vand C-I viral genome and the genomes of other retroviridae. No sequence homology could be demonstrated, under low stringency conditions of S1 nuclease digestion, with RNA from murine, rat, and hamster endogenous type C viruses nor sarcoma viruses (Table 4). Other nonrodent type C viral

FIG. 4. Thermal stability of hybrids formed between Vand C-I viral [^3H]DNA and cellular DNA from *V. oleracea* (●), and Vand C-I-infected FET tissue culture cells (○). Hybridization was carried out to a C_{ot} of 2×10^4 at 65°C . Duplicate samples (0.025-ml) were diluted 10-fold in 0.7 M NaCl and incubated for 10 min at the indicated temperature. Hybrids were detected using low stringency conditions for S1 nuclease digestion (see text).

RNAs including SSAV, simian sarcoma virus, and GaLV were also negative.

DISCUSSION

We have previously reported the isolation of a class of endogenous type C viruses (designated type C-I) from a variety of Asian species of the genus *Mus* (2, 13, 17). The type C-I class of viruses was initially distinguished by virus-associated antigenic determinants which are related to those of the infectious primate type C viruses isolated from certain populations of gibbon apes in Southeast Asia and a woolly monkey fibrosarcoma (2, 17). More recently, it was found that the type C-I class of endogenous murine retroviruses could be further distinguished from other murine type C viruses (designated type C-II) (2), associated with neoplastic disease in mice, by at least three additional criteria. (i) Type C-I viruses are restricted from replicating on murine tissue culture cells, but can be propagated on a wide variety of nonrodent tissue culture cells, including some derived from primates (2, 13). (ii) By hybridization criteria, the type C-I and type C-II viral genomes share little nucleic acid homology (2). (iii) The type C-I

endogenous virogenes are highly conserved in the genus *Mus* and have evolved at rates comparable to the nonrepetitive cellular DNA (2, 13). Moreover, related viral sequences could be demonstrated in the cellular DNA of other rodent genera, including *Vandeleuria* (13; and unpublished observations).

The cellular DNA of members of the genus *Vandeleuria*, as shown here by nucleic acid hybridization, share similar levels of nucleic acid homology with cellular DNA from members of the genera *Mus* and *Rattus*, suggesting that the three genera diverged from a common ancestor. We have described here the isolation of a type C virus, designated Vand C-I, from *V. oleracea*, which exhibits properties characteristic of the endogenous type C-I class of retroviruses. Vand C-I grows only on nonrodent tissue culture cell lines and, thus, like other members of this class, is xenotropic (15). The virus-associated proteins, similar to the *Mus* type C-I viruses, share antigenic determinants in common with the infectious primate type C viruses (SSAV and GaLV).

On the basis of antigenic determinants shared with the murine type C-I viruses and the absence of, respectively, SSAV- and GaLV-related nucleic acid sequences in the cellular DNA of un-

TABLE 3. Nucleic acid homology between *Vandeleuria* type C viral DNA and the cellular DNA of various species

Species ^a	³ H-labeled viral DNA ^b (% hybrid formed)
Rodent	
<i>Vandeleuria oleracea</i>	100
Rat	
<i>Rattus norvegicus</i>	45
<i>R. rattus</i>	48
Mouse	
<i>Mus cervicolor</i>	32
<i>M. pahari</i>	30
<i>M. shortridgei</i>	28
Chinese hamster	19
Syrian hamster	20
Guinea pig	6
Nonrodent	
Cow	0
Cat	0.2
Pig	0.5
Human	0.1

^a The cellular DNA of tissues or cell cultures of the species listed was extracted as described in the text.

^b The preparation of [^3H]thymidine-labeled viral DNA and conditions for the hybridization reactions are described in the text. The extent of hybridization was measured at a C_{ot} of 2×10^4 by S1 nuclease digestion under conditions of low stringency (see text). The values given are the percent hybridization normalized to the value obtained with *V. oleracea* cellular DNA (80 to 100%).

TABLE 4. Nucleic acid homology between the *Vandeleuria* type C viral genome and other retroviral genomes

Species of origin	Virus designation ^a	% Hybrid ^b
Rodent		
<i>Vandeleuria oleracea</i>	Vand C-I	100.0
Rat		
<i>Rattus norvegicus</i>	VNRK	4.0
	RT21C	3.0
	64J1 (KSV)	2.0
Mouse		
<i>Mus cervicolor</i>	Cervicolor C-I	6.0
	M432	3.0
<i>M. musculus</i>	Ecotropic (Musculus C-II)	6.0
	Xenotropic (Musculus C-II)	6.0
	SC-1 (RAS)	4.0
	Mo-A31 (MSV)	2.0
<i>M. shortridgei</i>	Shortridgei C-I	5.0
Syrian hamster	Y22	5.0
Chinese hamster	CCL-14.1	6.0
Nonrodent		
Pig	PK (15)	4.0
Cat	FeLV	6.0
Woolly monkey	SSAV	1.0
Woolly monkey	SSAV/SSV	
Gibbon ape	GaLV	6.0
Baboon	BaLV (M28)	4.0

^a Cellular RNA was extracted (see text) from various tissue culture cell lines releasing the listed viruses (2, 3). Retroviral-associated sequences (RAS) were from the feral mouse cell line, SC-1 (26).

^b The ³H-labeled DNA probe was prepared as described in the text. The final extents of hybridization were obtained for the various cellular RNAs using low stringency S1 nuclease digestion conditions and were normalized to the homologous reaction (80 to 100%).

infected woolly monkeys and gibbon apes, it was proposed that infectious primate type C viruses arose via a recent horizontal transmission of type C-I virus from Asian species of *Mus* to certain populations of gibbon apes and a woolly monkey (2, 17). The infectious primate type D Mason Pfizer monkey virus represents a similar example of horizontal transmission of an endogenous retrovirus between species, in this case two separate subfamilies of primates (9). Although Mason Pfizer monkey virus is infectious in rhesus monkey populations, the viral genome shows the greatest sequence homology with the endogenous langur type D viral genome. These examples of natural transmission of currently infectious retroviruses contrast with the origin of RD114 endogenous type C virus of domestic cats (18, 19). In this case it has been postulated that an ancestor of domestic cats acquired the RD114 type C virus via a horizontal transmission from

baboons (8). Subsequent to the infection, the RD114 virogenes became incorporated into the germ line of domestic cats as well as some of its close relatives and is now genetically transmitted in these species.

Using a ³H-labeled DNA transcript of the Vand C-I viral genome, it was possible to detect multiple copies of partially related viral sequences in *V. oleracea* cellular DNA. Related viral sequences were also detected in the cellular DNA of other rodent genera. In general, the level of sequence homology with the viral DNA transcript paralleled that observed with *V. oleracea* nonrepetitive cellular DNA. Thus, although there are many examples of horizontal transmission of retroviral genes between species, these data are most consistent with the genetic transmission of Vand type C-I-related virogenes in *V. oleracea* and other rodent genera.

Previously we have shown that the *M. cervicolor* type C-I viral genome shared nucleic acid sequences in common with the *Vandeleuria* cellular DNA (13). Paradoxically, as shown here, the labeled Vand C-I viral DNA does not hybridize significantly above background with *M. cervicolor* or *M. shortridgei* type C-I viral RNA. We interpret the apparent lack of nucleic acid homology as reflecting the evolutionary distance between the *Mus* and *Vandeleuria* genera as well as the lower stability, under the hybridization conditions employed, of DNA-RNA hybrids. In addition, these results suggest that the infectious primate type C viruses, GaLV or SSAV, are not the result of a recent horizontal transmission from *Vandeleuria*.

The data presented here on the Vand C-I endogenous retrovirus, in addition to our previous work on the *Mus* endogenous type C-I viruses, are consistent with the conclusion that this class of retroviruses is the most evolutionarily conserved, as well as the most easily activated, and suggests that related type C viruses will be isolated from other rodent genera. In this respect, although the rodent species of origin of SSAV and GaLV has not as yet been identified, it is possible that this class of infectious viruses may have arisen via a horizontal transmission from any of a number of distantly related rodents. Moreover, the broad host range of the type C-I viruses suggests the possibility that other examples of horizontal transmission of this class of endogenous rodent type C virus to other mammalian species may be encountered.

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