Two Distinct Endogenous Type C Viruses Isolated from the Asian Rodent *Mus cervicolor*: Conservation of Virogene Sequences in Related Rodent Species

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The cocultivation of a lung cell line from the Southeast Asian mouse *Mus cervicolor* with cells from heterologous species has resulted in the isolation of two new distinct type C viruses. Both viruses are endogenous to *M. cervicolor* and are present in multiple copies in the cellular DNA of these mice. One of the viruses, designated *M. cervicolor* type CI, replicates readily in the SIRC rabbit cell line and is antigenically related to the infectious primate type C viruses isolated from a woolly monkey (simian sarcoma-associated virus) and gibbon apes (gibbon ape leukemia virus). This virus is also closely related by both immunological and nucleic acid hybridization criteria to a type C virus previously isolated from a second Asian murine species, *Mus caroli*. The isolation of the *M. cervicolor* type C I virus thus provides further evidence that the infectious primate type C viruses originated by trans-species infection of primates by an endogenous virus of mice. The second virus, designated *M. cervicolor* type C II, replicates well in various cell lines derived from the laboratory mouse *Mus musculus*. While antigenically related to type C viruses derived from *M. musculus*, the *M. cervicolor* type C II virus isolate can be readily distinguished from standard murine leukemia viruses. Both new type C viruses from *M. cervicolor* are unrelated to the previously described retrovirus (M432) isolated from the same *Mus* species. The DNA of *M. cervicolor* therefore contains multiple copies of at least three distinct classes of endogenous viral genes. An examination of the cellular DNA of other rodent species for nucleic acid sequences related to the genomes of both *M. cervicolor* type C I and II reveals that both viruses have been highly conserved evolutionarily, and that other species of rodents, such as laboratory mice and rats, contain endogenous virogenes related to those in the DNA of *M. cervicolor*.

Infectious primate type C viruses have been isolated from several colonies of gibbon apes (21, 22) and from one woolly monkey (47). These viruses, GALV (gibbon ape leukemia virus) and SSV-SSAV (simian sarcoma virus-simian sarcoma-associated virus), spread from animal to animal under natural conditions and are able to induce tumors when inoculated into primates (53). Nucleic acid sequences homologous to those of the RNA genomes of SSAV and GALV have not been detected in the cellular DNA of normal primates, indicating that these are not endogenous primate viruses (4, 41).

While studying the relationships between various mammalian type C viruses using nucleic acid hybridization techniques, the infectious primate viruses were shown to share partial sequence homology with the endogenous

type C viruses of the laboratory mouse *Mus musculus* (4, 8). Such studies suggested that viruses of the SSAV/GALV group originated from endogenous viruses of mice. Since most infectious primate type C viruses have been obtained from gibbons whose natural habitat is Southeast Asia, we attempted to isolate type C viruses with properties similar to those of SSAV and GALV from Asian murine species. We found that cells from the Asian mouse *Mus caroli* can produce an endogenous, xenotropic type C virus, which, by immunological and interference criteria, is closely related to SSAV and GALV and differs from the previously described type C viruses of *M. musculus* (31). These results support the conclusion that the infectious primate type C viruses originated by trans-species infection of gibbons with an en-

dogenous, xenotropic type C virus from either *M. caroli* or another closely related mouse species.

In this report we describe the properties of two different type C viruses obtained from another Asian murine species, *Mus cervicolor*. One of the new viruses is closely related to the virus previously isolated from *M. caroli*, whereas the second isolate is more closely related to type C viruses isolated from *M. musculus*. An examination of the cellular DNA of other rodent species for nucleic acid sequences related to the genomes of type C viruses from *M. caroli* and *M. cervicolor* reveals that all three viruses are more evolutionarily conserved among rodents than are any isolates from the laboratory mouse, *M. musculus*. Whereas viruses related to SSAV and GALV have not been isolated from the laboratory mouse, cells of *M. musculus* would appear to have the genetic potential to code for viruses related to this type C viral group.

MATERIALS AND METHODS

Cell cultures. A cell line established in this laboratory from the lung of an *M. cervicolor* mouse has been continuously passaged for over 1 year. The cells initially grew slowly, but after 15 subcultures areas of more rapidly proliferating cells were observed. These cells showed a shorter doubling time, a greater saturation density, and a greater ability to form colonies from single cells as compared with the normal diploid fibroblastic cells seen in early subcultures. This pattern of spontaneous transformation has been frequently reported for *M. musculus* cells in culture (cf. 48). The other cell lines and viruses used in these studies have been previously described (5, 30, 43, 44). For viral isolations, indicator cell lines at 50% confluency were treated with 2 μ g of Polybrene per ml for 24 h prior to cocultivation with late-passage (40 to 60) *M. cervicolor* cells. The mixed cultures were transferred by trypsinization at approximately 1-week intervals; where the *M. cervicolor* cells were seen to be overgrowing the indicator cells, additional indicator cells were added. Supernatants were assayed for the presence of viral polymerase activity at 2-week intervals for 5 months.

Viral polymerase assays. Virus from culture supernatants was concentrated by centrifugation and assayed for viral RNA-directed DNA polymerase activity with synthetic template and primer as described (30). Results are expressed as counts per minute of [3 H]TMP incorporated into radioactive polydeoxythymidylic acid product in a 60-min incubation at 37°C. Polymerase antibody inhibition studies were performed as described (44).

Immunological assays. Radioimmunoassays for the major type C viral structural protein (p30) (42) were performed as described (45). The results are plotted as percentage of displacement of the radiolabeled test antigen (linear scale ordinate) versus micrograms of competing protein (log scale abscissa).

Preparation of 3 H-labeled DNA transcripts of the viral genome. A 2-h endogenous reverse transcriptase reaction with detergent-disrupted virus was used to synthesize [3 H]thymidine-labeled DNA in the presence of actinomycin D (30 μ g/ml) and 12 mM magnesium acetate (13). The labeled product was deproteinized and further purified as described previously (4). The 3 H-labeled DNA had a sedimentation coefficient of 7S-10S in alkaline sucrose and a specific activity of 1.8×10^7 cpm/ μ g. Protection experiments using 32 P-labeled 70S viral RNA (4) showed that the various 3 H-labeled DNA probes contained 55 to 84% of their respective 70S viral RNA sequences at a 3 H-labeled DNA- 32 P-labeled viral RNA molar ratio of 1.0, indicating that they contain most of the sequences present in viral RNA in proportions similar to their content in 70S RNA.

Extraction of nucleic acids. Viral and cellular RNA and cellular DNA were purified as described (4). Cellular DNA had a sedimentation coefficient of 7S-11S (the size of the 3 H-labeled DNA viral probes), as determined by centrifugation on alkaline sucrose gradients (4).

Preparation of radioactively labeled "nonrepetitive" cellular DNA. The DNA of various diploid cell strains labeled with [3 H]thymidine (120 μ Ci/ml of cell culture media) for 72 h was extracted (4). Nonrepetitive cellular DNA was isolated by removing the highly reiterated DNA sequences that anneal by a C_0t of 200 (35 to 43% of the total DNA) by fractionation on hydroxyapatite as described (9). The specific activity of this DNA ranged from 1.2×10^4 to 2.8×10^4 cpm/ μ g.

Molecular hybridization. DNA-DNA and DNA-RNA hybridizations were incubated for varying times at 65°C in reaction mixtures containing 0.01 M Tris-hydrochloride (pH 7.4), 0.75 M NaCl, 2×10^{-3} M EDTA, 0.05% sodium dodecyl sulfate, 30,000 to 40,000 cpm of 3 H-labeled viral DNA per ml, and 2 to 4 mg of cellular DNA or RNA per ml. Hybridizations were initiated by heating the reaction mixtures to 103°C for 10 min in an ethylene glycol water bath and then incubating them at 65°C. At varying times, 0.025-ml portions were removed and frozen at -80°C until digested with S_1 nuclease (3, 6). 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 (15) and corrected to a monovalent cation concentration of 0.18 M (16).

RESULTS

Isolation of viruses from *M. cervicolor*. A cell line established from the lung of a *M. cervicolor* mouse was treated with 5-bromodeoxyuridine (100 μ g/ml for 24 h) and cocultivated with various heterologous cell lines, and the culture supernatants were assayed at biweekly intervals for viral polymerase activity. Approximately 7 weeks after the initiation of cocultivation, reverse transcriptase activity was detected in cultures of a rabbit cell line (SIRC). When filtered supernatant fluids from these

cultures were used to infect other mammalian cell lines, reverse transcriptase activity was again detected in rabbit SIRC and feline FEC cells 3 weeks after infection and in rhesus DBS-FRHL-1 cells 15 weeks after infection. Single cell clones of the *M. cervicolor* lung culture were also cocultivated with various mammalian cell lines. Several weeks after cocultivation, reverse transcriptase activity was again detected in cultures containing either FEC or SIRC cells, but also in cultures containing cells derived from *M. musculus* (SC-1, NIH/3T3).

Table 1 lists the host ranges of the type C viruses isolated in both rabbit SIRC and *M. musculus* SC-1 cells and compares them with the host ranges of viruses previously isolated from *M. caroli* and *M. musculus*. The viruses replicating in *M. musculus* SC-1 cells and in rabbit SIRC cells have readily distinguishable host range properties. The virus isolated in the SIRC cell line, designated *M. cervicolor* type C I (CERV C I), also grows in feline FEC cells and to a lower titer in a rhesus cell line. By contrast, the virus isolated in *M. musculus* SC-1 cells, designated *M. cervicolor* type C II (CERV C II) grows only in cell lines derived from *M. musculus*, such as NIH/3T3 and BALB/3T3 clone A31. Neither the CERV C I nor CERV C II isolate replicates in cells derived from *M. cervicolor*, the species of origin of both viruses. The host range properties of the two new *M. cervicolor* isolates appear to differ from those of a virus previously isolated from the closely re-

lated mouse species *M. caroli* (31), as well as from a xenotropic type C virus isolated from *M. musculus* (BALB/c strain) (5).

Antigenic properties of the viral polymerases and p30 proteins. Figure 1 shows the results of studies performed with immune immunoglobulin G directed against the polymerases of several groups of mammalian type C viruses. Using an antiserum against the polymerase of the infectious primate virus SSAV (Fig. 1A), partial inhibition of reverse transcriptase activity was seen with the enzyme from the virus isolated from *M. caroli*, as well as with the polymerase of the CERV C I virus. By contrast, the enzymes of the CERV C II virus, the rat virus RT21c, and the Rauscher murine leukemia virus (R-MuLV), were not significantly inhibited by this serum. These results indicate that, like the type C virus isolated from *M. caroli*, the CERV C I isolate appears more closely related by these criteria to infectious primate viruses than to the viruses isolated from *M. musculus*.

Figure 1B shows the results obtained with an antiserum to the reverse transcriptase of the rat virus RT21c; this latter serum, which inhibits the polymerases of rat viruses, cross-reacts strongly with the reverse transcriptases of viruses isolated from *M. musculus* (43). The antiserum inhibited the enzymes from both R-MuLV and the CERV C II isolate but did not cross-react with reverse transcriptases from SSAV, CERV C I, or the type C virus from *M. caroli*. Thus, the reverse transcriptase of the

TABLE 1. Host range of type C viruses isolated from various *Mus* species

Host cell line	Species	Supernatant reverse transcriptase assay ^a ([³ H]dTMP incorporated, cpm × 10 ⁻³)			
		<i>M. cervicolor</i> C I virus growing in rabbit SIRC cells	<i>M. cervicolor</i> C II virus growing in mouse SC-1 cells	<i>M. caroli</i> virus growing in cat FEC cells	<i>M. musculus</i> vi- rus (BALB/c xenotropic) growing in dog FCf2Th cells
<i>M. caroli</i> heart	<i>M. caroli</i>	NT ^b	NT	2.1	NT
<i>M. cervicolor</i> lung	<i>M. cervicolor</i>	2.0	1.8	NT	125.8
NIH/3T3	<i>M. musculus</i>	0.9	750.4	1.2	1.5
SC-1	<i>M. musculus</i>	1.2	175.3	1.4	1.5
NRK	Rat	3.0	1.1	3.5	2.8
SIRC	Rabbit	198.0	1.0	0.7	158.6
FEC	Cat	155.3	1.4	226.3	850.3
Tb1Lu	Bat	0.9	1.3	1.2	221.3
FCf2Th	Dog	2.5	1.4	2.3	461.5
64J1	Mink ^c	2.7	2.3	201.2	331.6
DBS-FRHL-1	Rhesus	86.5	2.4	2.1	2.1
A204	Human	4.0	3.6	4.2	4.2

^a Cell culture medium from the four viruses propagated in the cell lines shown was filtered and used to infect the host cell lines listed. Medium from infected cultures was assayed for viral polymerase activity at 2-week intervals after infection. The results shown are the values obtained 15 weeks after infection. Italicized numbers indicate levels of viral polymerase activity significantly above background.

^b NT, Not tested.

^c 64J1 is a Kirsten sarcoma virus transformed nonproducer clone of the mink cell line Mv1Lu (31).

FIG. 1. Inhibition of type C viral polymerases by immune immunoglobulin G (IgG) prepared against the polymerases of (A) the woolly monkey type C virus, SSAV; (B) the endogenous rat virus, VNRK; and (C) the *M. musculus* virus, R-MuLV. The conditions of the assay were as described (44). Enzymes were obtained from: SSAV (●); VNRK (□); R-MuLV (○); the endogenous *M. caroli* type C virus (▲); the *M. cervicolor* type C I virus (■); and the *M. cervicolor* type C II virus (△).

CERV C II virus shares certain interspecies antigenic determinants with several other rodent type C viruses, but not with the CERV C I viral enzyme. An antiserum to the polymerase of R-MuLV that inhibits the reverse transcriptases of all *M. musculus* type C viruses to a comparable extent (44) also showed a small degree of cross-reaction with the enzyme isolated from the CERV C II virus (Fig. 1C), whereas the polymerase of the CERV C I virus was less inhibited. The CERV C II virus, then, although readily distinguishable from laboratory strain MuLV's by these criteria, appears more closely related to type C viruses isolated from *M. musculus* than to viruses isolated from *M. caroli* or from primates (SSAV/GALV group).

Figure 2 shows the results of competitive radioimmunoassays for the p30 proteins of these various rodent and infectious primate type C viruses. The amount of protein required to initiate competition reflects the titer of virus in infected cells, whereas the slopes of the competition curves provide a relative index of the degree of antigenic similarity between the competing and test antigens (44, 45). In an assay for the p30 protein of SSAV (Fig. 2A), the CERV C I virus competed efficiently for the labeled SSAV p30 protein and could not be distinguished from the infectious primate virus by these criteria; however, the p30 protein of the CERV C II virus was not detected in the same assay. Assays which detect either interspecies (Fig. 2B) or group-specific (Fig. 2C) antigenic determinants of the p30 protein of laboratory strain MuLV's, however, detected the p30 pro-

FIG. 2. Radioimmunoassays for p30 proteins. Extracts of cells infected with type C viruses were used as competing antigens. Assays were initiated with 50% of the radiolabeled test antigens bound in the absence of competing protein. The results are plotted as percentage of displacement of the labeled test antigen (ordinate) versus micrograms of competing protein (abscissa). (A) Assay for SSAV p30 protein using antiserum to SSAV p30 and ¹²⁵I-labeled SSAV p30. (B) Interspecies assay using antiserum to feline leukemia virus (FeLV) p30 and ¹²⁵I-labeled murine p30 (Rauscher strain). (C) Assay for murine p30 protein using antiserum to S2CL3 p30 and ¹²⁵I-labeled S2CL3 p30 (endogenous BALB/c, N-tropic type C virus). (D) Assay for endogenous rat (RT21c) p30 protein using antiserum to RT21c p30 and ¹²⁵I-labeled RT21c p30. Symbols: (●) infectious primate type C virus, SSAV; (○) endogenous *M. musculus* type C virus, S16CL2; (□) endogenous rat type C virus, RT21c; (▲) endogenous *M. cervicolor* type C I virus; (△) endogenous *M. cervicolor* type C II virus.

tein of the CERV C II virus but failed to detect the p30 protein of the CERV C I isolate. The reduced slope of the competition curve seen with CERV C II in the assay for the group-specific determinants of the MuLV p30 protein (Fig. 2C) indicates that, like CERV C II polymerase, the CERV C II p30 protein can be readily distinguished from the p30 proteins of type C viruses isolated from *M. musculus*. Figure 2D shows that an assay for the p30 proteins of rat type C viruses failed to detect the p30 proteins of either the CERV C I or CERV C II isolates. Taken together, the data indicate that the CERV C I virus is immunologically closely related to viruses of the SSAV/GALV group, whereas the CERV C II isolate is more closely related to, but distinguishable from, type C viruses isolated from various strains of laboratory mice.

CERV C I and II are endogenous viruses. It is a characteristic property of all endogenous mammalian type C viruses that their genes are present in multiple copies (5 to 25 per haploid

genome) in the cellular DNA of normal animals (10). To determine whether the new viral isolates were endogenous to *M. cervicolor*, we examined the reiteration frequency of the viral nucleic acid sequences of both the CERV C I and II viruses in the cellular DNA of normal *M. cervicolor* mice. Radioactively labeled DNA transcripts of the viral genome were prepared from the CERV C I and II isolates using the endogenous reverse transcriptase reaction. The reassociation kinetics shown in Fig. 3 can be used to estimate the relative frequencies of each set of viral genes by determination of the half- C_0t values (the midpoint of the renaturation curve). Sequences related to the viral genome of each of the two isolates were detected in the cellular DNA extracted from normal tissues of *M. cervicolor* mice. A comparison of the kinetics of hybridization for these reactions ($C_0t_{1/2}$, 2.4×10^2) with that seen for the self-annealing of *M. cervicolor* nonrepetitive DNA ($C_0t_{1/2}$, 1.5×10^3) is consistent with the presence of approximately six copies of each set of *M. cervicolor* type C viral genes per haploid genome in the cellular DNA of normal *M. cervicolor*. Identical results were obtained when the *M. cervicolor* lung cell line itself was used as a

FIG. 3. Hybridization of ^3H -labeled DNA probes prepared from each of the *M. cervicolor* type C viruses to *M. cervicolor* cellular DNA. The radioactivity shown represents ^3H -labeled DNA product, which is resistant to digestion by nuclease S_1 . The DNA-DNA hybridizations were performed as described in Materials and Methods. A ^3H -labeled DNA probe prepared from the *M. cervicolor* type C I virus grown in a rabbit cell line (SIRC) was annealed to DNA extracted from *M. cervicolor* (●), to the DNA of the rabbit SIRC cell line infected with this virus (□), and to human cellular DNA (■). A ^3H -labeled DNA probe prepared from the *M. cervicolor* type C II virus grown in a mouse cell line (SC-1) was annealed to DNA extracted from *M. cervicolor* tissue (Δ) and to human DNA (■). (○) ^3H nonrepetitive *M. cervicolor* cellular DNA reassociation.

source of cellular DNA. Thus, the two new virus isolates are endogenous to *M. cervicolor* and, like other endogenous viruses, can be detected in multiple copies in the cellular DNA of normal animals of the species (10). In contrast, the DNA of rabbit cells infected with the CERV C I virus contains only one proviral copy per haploid genome ($C_0t_{1/2}$, 1.5×10^3).

Evolutionary relationships among rodents as determined by cellular DNA hybridization. The extent of reassociation and the thermal stability of DNA strands derived from different species are measures of their evolutionary proximity (25, 34, 37, 38). Nonrepeated sequences comprise a large fraction of mammalian DNA; each sequence is believed to occur only once per haploid genome (15). This DNA is therefore suitable for determining the extent of nucleotide diversity that has accumulated since two species diverged from their most recent common ancestor.

Three different species of *Mus*, as well as rats, hamsters, and guinea pigs, were examined for the extent of homology of their cellular DNA to that of *M. cervicolor*. Radioactively labeled, nonrepetitive *M. cervicolor* cellular DNA was hybridized to the DNA of various rodents (Fig. 4A). Under the conditions used, *M. caroli* and *M. musculus* DNAs are, respectively, 78 and 60% related to *M. cervicolor*

FIG. 4. Hybridization of ^3H -labeled DNA probe from *M. cervicolor* type C I virus and *M. cervicolor* nonrepetitive cellular ^3H -labeled DNA to various rodent cellular DNAs. (A) Annealing of *M. cervicolor* nonrepetitive cellular ^3H -labeled DNA to rodent cellular DNA. The *M. cervicolor* lung cell line was labeled with [^3H]thymidine and extracted. Nonrepetitive cellular DNA was isolated by removing the highly reiterated DNA sequences that anneal by a C_0t of 200 (34% of the total DNA) by fractionation on hydroxyapatite. The [^3H]thymidine-labeled nonrepetitive DNA was then hybridized to DNA extracted from *M. cervicolor* (○), *M. caroli* (●), *M. musculus* (Δ), rats (▲), Chinese hamsters (□), and guinea pigs (■). (B) Annealing of ^3H -labeled DNA probe prepared from the *M. cervicolor* type C I virus grown in the SIRC rabbit cell line to DNA extracted from various rodents. Symbols are the same as in (A).

DNA, whereas rat and hamster nonrepetitive cellular DNAs are approximately 21 and 8% related, respectively. The relative final extents of hybridization agree well with data previously obtained using somewhat different conditions for determining the evolutionary relationships of rodents (20, 24, 36–38).

The thermal stability of nucleic acid hybrids was also used as an index of the degree of base pair mismatching between the various DNAs. Mismatching results in the formation of hybrids that melt at a lower temperature; the effect of mismatched base pairs on the thermal stability has been estimated at between 0.7 and 1.7°C per 1% altered pairs (33). The decrease in thermal stability between the homologous cellular DNA hybrids and the hybrids formed with the DNA of other rodent species is expressed as ΔT_m . Table 2 shows the thermal stability and final percentage of homology of various rodent nonrepetitive cellular DNA hybrids. Radioactively labeled cellular DNA from cell cultures of different *Mus* species was hybridized to the cellular DNA of various rodent species. It is evident that the cellular DNA of those rodents

that show progressively less homology also show a decrease in their thermal stability relative to the homologous DNA hybrid. Therefore, the decrease in the final extent of nucleic acid homology is most likely due to an accumulation of base pair mutations, reflecting an increasing phylogenetic distance. Among the three *Mus* species examined, *M. cervicolor* and *M. caroli* yield a $\Delta T_m R$ (13, 24) of approximately 4.3°C when their cellular DNAs were hybridized to each other. *M. musculus* DNA is slightly less related, yielding a $\Delta T_m R$ value of approximately 6.6°C.

The thermal stabilities of the various nonrepetitive cellular DNA hybrids were used to construct a phylogenetic tree of rodent evolution (Fig. 5). All the divergence times shown depend on the choice of 10 million years as the time when the *Mus* and *Rattus* lineages initially diverged (24, 36). Since this yields a $\Delta T_m R$ value of about 16.2°C, 1 million years of rodent evolution would appear to correspond to 1.6°C $\Delta T_m R$ under the assay conditions used. The data show *M. cervicolor* and *M. caroli* as having had a common ancestor somewhat more

TABLE 2. Nucleic acid homology and thermal stability between rodent nonrepetitive cellular DNAs^a

Species	³ H-labeled nonrepetitive cellular DNA					
	<i>M. cervicolor</i>		<i>M. caroli</i>		<i>M. musculus</i>	
	% Hybrid	$\Delta T_m R$ (°C)	% Hybrid	$\Delta T_m R$ (°C)	% Hybrid	$\Delta T_m R$ (°C)
Mouse						
<i>M. cervicolor</i>	100		79	4.7	64	6.6
<i>M. caroli</i>	78	3.9	100		65	7.0
<i>M. musculus</i>	68	6.2	67	6.5	100	
Rat						
<i>R. norvegicus</i>	21	17.5	25	15.7	23	16.1
<i>R. rattus</i>	NT ^b	NT	23	16.1	22	15.8
Chinese hamster						
<i>Cricetulus griseus</i>	9	>25	7	>25	9	>25
Syrian hamster						
<i>Mesocricetus auratus</i>	7	>25	7	>25	6	>25
Guinea pig						
<i>Cavia porcellus</i>	0	>25	1	>25	2	>25

^a The DNA of diploid cell lines derived from the three species of *Mus* shown was labeled with [³H]thymidine (120 μ Ci/ml of cell culture media) for 72 h and extracted. Nonrepetitive cellular DNA was isolated by fractionation on hydroxyapatite as described in Materials and Methods. Hybridizations were carried out to a C_0t value of 3×10^4 , and the extent of annealing was determined with the single-strand-specific nuclease S_1 (3, 6). The percent hybrid is the saturating normalized value obtained after hybridizing to the DNA of the species listed; the actual final extent of cellular DNA hybridization to the respective nonrepetitive cellular ³H-labeled DNA varied from 72 to 84%. The temperature at which 50% of the hybrids are dissociated (T_m) ranged from 85 to 91°C in various experiments. The ΔT_m is the difference in T_m between the other DNA-DNA hybrids and the T_m of the homologous hybrid. $\Delta T_m R$ is the thermal stability difference for all the nonrepeated DNA (24) and is the ΔT_m corrected for the final percentage of hybridization of the cellular DNA to the ³H-labeled cellular DNA probe, using S_1 nuclease to detect hybrids (13). This correction is based on the assumption that the difference between the various rodent DNAs is due to divergence from common ancestral nucleic acid sequences and not to the addition or deletion of large amounts of DNA unrelated to that in the species being tested. The T_m values were derived from at least three experiments in which hybrids were melted at 2°C intervals over a range of $\pm 8^\circ\text{C}$ from the T_m .

^b NT, Not tested.

FIG. 5. Phylogenetic tree depicting relationships among rodent cellular DNAs. The $\Delta T_m R$ ($^{\circ}\text{C}$) of the various DNA-DNA hybrids shown in Table 2 are plotted as a function of millions of years (Myr) of evolution. The points shown depend on the choice of 10 Myr for the time when rats and mice diverged (36, 37); this choice results in a value of $1^{\circ}\text{C } \Delta T_m R$ equal to 0.6 Myr of evolution. Among Old World monkeys, similar studies have yielded a value of $1^{\circ}\text{C } \Delta T_m R$ equal to 4 to 6 Myr of evolution. The different ratios obtained for primate and rodent evolution may reflect the different generation times of these two groups of mammals (24).

recently (about 2.5×10^6 years ago) than either species had with *M. musculus*. The rat and hamster species examined are believed to have diverged from the mouse about 10×10^6 and 20×10^6 years ago, respectively. The latter value is taken from previous estimates (20, 36, 37). The dates of divergence for two species of rat (*Rattus rattus* and *Rattus norvegicus*) approximately 2.3×10^6 years ago and Chinese and Syrian hamsters (about 7.5×10^6 years ago) were derived from experiments using labeled nonrepetitive rat and hamster DNA. If the choice of 10×10^6 years for the rat-mouse divergence and 20×10^6 years for the hamster-mouse divergence were shown to be incorrect, the relative relationships among the various rodent species would nevertheless remain the same.

M. cervicolor type C viral sequences in related rodent species. We examined the rodent species shown in Table 2 for the presence of nucleic acid sequences related to a ^3H -labeled DNA probe prepared from the CERV C I virus. As shown in Fig. 4B, related sequences were found in the DNA of other rodent species, approximately paralleling the relationship shown for the nonrepetitive cellular DNA in Fig. 4A.

Table 3 summarizes the data obtained for the CERV C I probe. Also listed are the hybridization results obtained for the CERV C II virus,

TABLE 3. Nucleic acid homology and thermal stability between retroviruses isolated from *M. cervicolor* and *M. caroli* and the DNA of various rodent species

Species ^a	Rodent viral ^3H -labeled DNA ^a							
	<i>M. cervicolor</i> type C I		<i>M. cervicolor</i> type C II		<i>M. cervicolor</i> M432		<i>M. caroli</i> type C I	
	% Hybrid	$\Delta T_m R$ ($^{\circ}\text{C}$)	% Hybrid	$\Delta T_m R$ ($^{\circ}\text{C}$)	% Hybrid	$\Delta T_m R$ ($^{\circ}\text{C}$)	% Hybrid	$\Delta T_m R$ ($^{\circ}\text{C}$)
Mouse								
<i>M. cervicolor</i>	100		100		100		51	9.8
<i>M. caroli</i>	66	8.4	35	13.9	31	15.3	100	
<i>M. musculus</i>	39	14.8	47	10.8	23	17.5	33	14.9
Rat								
<i>R. norvegicus</i>	25	16.1	14	19.2	11	20.8	22	17.2
Chinese hamster								
<i>C. griseus</i>	15	18.4	9	21.5	5	>25	12	19.8
Guinea pig								
<i>C. porcellus</i>	3	NT ^c	0	NT	1	NT	1	NT

^a ^3H -labeled DNA viral probes were prepared as described and hybridized to the DNA of the species listed. DNA-DNA hybridizations were incubated as described in Materials and Methods, and the extent of hybridization was determined with the single-strand-specific nuclease S_1 . The percentage of hybridization represents the average final saturation values to the ^3H -labeled DNA probes from several experiments; these values ranged from 76 to 88%. All hybridizations were carried out to a C_0t of at least 3×10^4 . $\Delta T_m R$ values were obtained as described in Table 2. The ΔT_m of the homologous hybrids varied from 88 to 91°C . The *M. cervicolor* type C I and M432 viruses were propagated in rabbit SIRC cells and mouse NIH/3T3 cells, respectively. The *M. cervicolor* type C II virus was spontaneously released from a clone of the *M. cervicolor* lung cell line. The *M. caroli* virus was grown in the feline FEC cell line.

^b DNA was extracted from various tissues of the species listed as described in Materials and Methods.

^c NT, Not tested.

for a third endogenous retrovirus previously isolated from *M. cervicolor* and designated M432 (17), and for the type C virus isolated from *M. caroli* (31). Nucleic acid sequences related to all four isolates can be detected in rodents as distantly related as the hamsters, although not in the guinea pig, which is believed to have diverged from the ancestors of the *Mus* species as long as 50×10^6 years ago (36). The decreased thermal stability that parallels the decrease in nucleic acid sequence homology indicates that the partial homologies detected are not the result of a strict preservation of discrete continuous segments of the viral genomes in these species, but rather are due to a more general base pair substitution throughout the genomes. Thus, sequences related to the three *M. cervicolor* isolates and the one *M. caroli* virus that have been isolated to date are very well conserved among rodent species, since they can be detected in animals that diverged from mice 20×10^6 years ago.

In sharp contrast to these data are the hybridization values obtained with the DNA of these same rodent species using ^3H -labeled

DNA probes prepared from viruses previously isolated from the laboratory mouse *M. musculus* (BALB/c or from *M. musculus* subsp. *molossinus* [feral subspecies; 29]), from rats, and from Chinese hamsters. Nucleic acid sequences related to the latter viruses cannot be detected to a significant extent in the DNA of most other rodent species (Table 4). For example, only a very low degree of nucleic acid sequence homology (<14%) to the BALB/c xenotropic virus isolated from *M. musculus* (5) was detected in the cellular DNA of either *M. caroli* or *M. cervicolor*. Similar results were also obtained with a probe prepared from an ecotropic *M. musculus* isolate (48) (data not shown). Although nucleic acid sequences related to the CERV C I virus can be detected in rat and hamster cellular DNA (as shown for comparison in Table 4), probes prepared from type C viruses isolated from the latter two species did not hybridize to the DNA of any *Mus* species. Moreover, whereas the Chinese and Syrian hamsters are evolutionarily separated by approximately 8×10^6 years (approximately the length of time separating the mouse and rat), there was essen-

TABLE 4. Nucleic acid homology between various rodent and primate type C viral DNA probes and the DNA from various species

Species ^b	% Hybridization to rodent cellular DNA ^a				
	<i>M. cervicolor</i> type C I	<i>M. musculus</i> (BALB/c xenotropic) ^c	Rat (VNRK)	Chinese hamster (CCL 14.1)	Gibbon (GBr-1)
Mouse					
<i>M. cervicolor</i>	100	14	NT ^d	3	16
<i>M. caroli</i>	66	9	3	NT	13
<i>M. musculus</i>	39	100	7	6	17
Rat					
<i>R. norvegicus</i>	25	1	100	2	4
<i>R. rattus</i>	NT	NT	71	NT	NT
Chinese hamster					
<i>C. griseus</i>	15	2	4	100	2
Syrian hamster					
<i>M. auratus</i>	NT	1	3	9	3
Guinea pig					
<i>C. porcellus</i>	3	0	0	0	0
Hippopotamus					
<i>Hippopotamus amphibius</i>	0	0	0	0	0
Gibbon					
<i>Hylobates agilis</i>	0	0	0	0	0

^a The normalized final percentage values obtained after hybridizing the various cellular DNAs to the ^3H -labeled DNA probes is shown; the actual final extents for the homologous hybridizations varied from 78 to 91%. ^3H -labeled DNA probes were prepared from the viruses as described in Materials and Methods. *M. cervicolor* type C I virus was grown in SIRC cells, *M. musculus* (BALB/c xenotropic) virus was grown in a canine thymus cell line, the rat and Chinese hamster viruses were spontaneously released from their own cell lines (28), and a virus isolated from normal gibbon brain tissue was propagated in a human rhabdomyosarcoma cell line, A204 (51).

^b DNA was extracted from various tissues of the species listed as described in Materials and Methods.

^c Similar data were also obtained with a ^3H -labeled DNA probe prepared from a xenotropic virus isolated from a feral subspecies of *M. musculus* (*M. musculus* subsp. *molossinus*; 29) and propagated in canine cells.

^d NT, Not tested.

tially no detectable sequence homology between the genomes of the type C viruses of these two species. Similarly, although sequences related to the rat type C viruses were found in both rat species (divergence time, 2×10^6 years), homologous sequences were not detected in the other rodents. Thus, the type C viruses isolated to date from the laboratory mouse, rat, and hamster are less highly conserved in rodents than are the three viruses isolated from *M. cervicolor* or the virus isolated from *M. caroli*.

Table 4 also shows data obtained with a probe prepared from a virus isolated from the brains of normal gibbons (51). Sequences homologous to those of the RNAs of SSAV and GALV have not been detected in the cellular DNA of normal primates (4, 41). However, we have previously reported a partial nucleic acid sequence homology between gibbon ape type C viral ^3H -labeled DNA and normal laboratory mouse cellular DNA (4). As is evident from the data in Table 4, all three species of *Mus* tested contain nucleic acid sequences partially homologous to those of the infectious primate type C viruses. These results are consistent with the concept that the infectious primate viruses arose by trans-species infection of certain primates with endogenous type C viruses of a *Mus* species.

Differences in the RNA genomes of CERV C I and II viruses. ^3H -labeled DNA probes prepared from the various murine viral isolates, as well as from the infectious primate

viruses (woolly-gibbon group), were also hybridized to the RNA of cells producing the various rodent and primate viruses to determine whether the detection of related sequences in the DNA of rodents reflected a homology with their respective endogenous type C viral genomes. As was predicated from the immunological and biological properties of the two *M. cervicolor* isolates, these two viruses appear virtually unrelated by nucleic acid hybridization (Table 5). The low level of homology between the genomes of CERV C I and II (about 10%) may reflect the fact that both viruses are derived from the same species and may contain common sequences of host cellular origin.

In reciprocal hybridization studies, the CERV C I and *M. caroli* isolates appear closely related (54 to 60% nucleic acid sequence homology). Both these viruses share similar immunological properties with the infectious primate type C viruses. Although the relationship of the CERV C I and *M. caroli* viruses to the woolly and gibbon type C viruses is not pronounced (5 to 8% homology) when assayed by molecular hybridization, these values are greater than those observed between the CERV C II isolate and the infectious primate viruses (0 to 1% homology). Paradoxically, the xenotropic and ecotropic viruses from *M. musculus* show a greater extent of homology (11 to 21%) to the genomes of SSAV and GALV than does either CERV C I or the *M. caroli* virus, even though the former viruses are less related to SSAV and

TABLE 5. Nucleic acid homology between various type C viruses

Species	Virus ^b	³ H-labeled transcripts ^a				
		<i>M. cervicolor</i> type C I	<i>M. cervicolor</i> type C II	<i>M. caroli</i> type C I	<i>M. musculus</i> xenotropic	Woolly monkey (SSAV)
<i>M. cervicolor</i>	<i>M. cervicolor</i> C I	100	9	60	3	7
	<i>M. cervicolor</i> C II	10	100	11	17	0
	M432	0	0	NT ^c	0	NT
<i>M. caroli</i>	<i>M. caroli</i> C I	54	14	100	1	6
<i>M. musculus</i>	BALB/c xenotropic	3	27	2	100	12
	BALB/c ecotropic	4	51	9	73	21
Rat	RT21c	3	6	4	2	3
Chinese hamster	CCL 14.1	0	4	3	1	1
Gibbon	GALV-1	6	1	6	14	65
Gibbon	GBr-1	5	0	4	11	72
Woolly monkey	SSAV	8	1	5	13	100
Baboon	M28	0	0	0	0	1
Cat	FeLV	0	0	1	2	0

^a ^3H -labeled DNA probes were prepared from the various viruses as described in Materials and Methods. The normalized final percentage values obtained after hybridizing the various DNA transcripts to the cellular RNAs are shown; the actual final extents for the homologous hybridizations varied from 92 to 97%.

^b Cellular RNA was extracted (see Materials and Methods) from the various viruses grown in the cell lines described in the footnotes to Tables 3 and 4. The baboon virus (M28) (52) was grown in a canine thymus cell line (FCf2Th), and feline leukemia virus (FeLV) was grown in the human cell line A204.

^c NT, Not tested.

GALV by immunological criteria.

The CERV C I isolate does not hybridize to the RNA of any cell line producing a rodent type C virus (except for *M. caroli*). The nucleic acid sequence homology detected with this viral probe to the cellular DNA of *M. musculus*, rats, or hamsters therefore appears to represent a homology with sequences coding for viruses that have not yet been isolated. Similarly, whereas ³H-labeled DNA transcripts of the *M. caroli* virus hybridize to a large extent to *M. musculus*, rat, and hamster DNA, they do not hybridize to the genomes of viruses that have heretofore been isolated from these species.

Table 5 shows that whereas the CERV C II viral probe shares little sequence homology with the genome of the CERV C I virus, a greater extent of hybridization was obtained with the RNA of cells infected with viruses derived from *M. musculus*. These data are consistent with the immunological studies, which indicated that both the reverse transcriptases and p30 protein of CERV C II were more closely related to those of viruses derived from laboratory mice than to the homologous proteins of CERV C I. The greater extent of hybridization to the BALB/c ecotropic virus (grown in *M. musculus* cells) as compared with the BALB/c xenotropic virus (grown in SIRC cells) could reflect the transcription of additional sequences in *M. musculus* cells homologous to the CERV C II virus, rather than to differences between the genomes of *M. musculus* ecotropic and xenotropic viruses.

DISCUSSION

The cellular DNA of the Asian mouse species *M. cervicolor* contains multiple copies of endogenous type C viral genes able to code for at least two distinct classes of infectious type C viruses. The two new isolates, designated CERV C I and CERV C II, have very different viral host ranges, antigenic properties, and RNA genomes and are almost completely unrelated to one another even though both viruses were isolated from the same lung cell line. The replication-defective type C viruses spontaneously released by certain rat cells also contain two totally distinct classes of viral RNA genomes (40). That these rat viruses have antigenically identical polymerases and p30 proteins suggests, however, that one of the RNA genomes is defective in specifying these antigens. Feline cells, as well, contain two completely unrelated sets of viral genes coding for the endogenous virus, RD-114, and the feline leukemia virus. In the latter case, viruses related to feline leukemia virus have never been induced from cat cells.

Moreover, the differences between the two cat viruses appear to reflect the fact that both were acquired by horizontal infection from viruses that are endogenous in other species (7, 11). Of the many mammalian cell lines from which type C viruses have been obtained, the *Mus cervicolor* cell line is the first from which two nondefective, endogenous type C viruses showing so little in common with one another have been isolated.

Based on immunological and nucleic acid hybridization criteria, the CERV C I virus is very closely related to a type C virus isolated from another Asian mouse species, *M. caroli* (31). Both of these murine viruses are closely related antigenically to type C viruses isolated from woolly monkeys (SSV/SSAV) and gibbon apes (GALV). These findings, considered together with previous molecular hybridization and immunological data (4, 8, 31, 44), support the view that the infectious primate viruses originated subsequent to a trans-species infection of primates with type C viruses of murine origin, derived from *M. caroli*, *M. cervicolor*, or a related mouse species. Since the viruses of the SSAV/GALV group are not endogenous to primates, there is no way at present to pinpoint the time at which such an infection may have occurred. However, viruses of the SSAV/GALV group now can readily cause leukemia and sarcomas in a variety of primate species (53); the oncogenic potential of the *M. cervicolor* endogenous viruses has not yet been established.

While the CERV C I and *M. caroli* type C viruses are much more related by immunological and interference criteria to SSAV and GALV than to standard laboratory strain MuLV's, neither virus shows a greater extent of genome homology to the RNAs of infectious primate viruses than do the viruses isolated from *M. musculus*. Moreover, ³H-labeled DNA probes prepared from SSAV and GALV do not hybridize to a significantly greater extent to the DNA of *M. cervicolor* and *M. caroli* than to that of *M. musculus*. The precise interpretation of these results is unclear at present. One possibility is that viruses of the SSAV/GALV group have been horizontally transmitted in primates for a sufficient length of time to have evolved rapidly. It is apparent that whereas endogenous virogenes evolve at rates approximating that of unique sequence cellular DNA (13), horizontally transmitted viruses, naturally selected for their ability to replicate, might acquire mutations or recombine with other type C viral genes at a much greater frequency than endogenous viral genes (7, 50). In the case of horizontally transmitted viruses, those genes that confer the greatest selective advantage for

replicative functions might be conserved, while other viral genes, less crucial for viral replication, might be more free to undergo variation. Thus, the homology observed between type C viruses of the SSAV/GALV group and those of mice might reflect similarities between only certain subsets of viral genes selected to maximize the potential for infectious virus spread, while homologies between the endogenous virogenes of related species might more closely reflect the evolutionary conservation of genes required for nonreplicative, cellular functions.

Unlike the CERV C I isolate, the CERV C II virus appears to be more closely related to MuLV's isolated from *M. musculus*. The antigenic determinants of the reverse transcriptase and p30 protein of the CERV C II virus show weak cross-reactions with the homologous proteins of laboratory strain MuLV's, and the RNA genome of CERV C II is partially homologous to endogenous type C viruses isolated from *M. musculus* cells. Of the numerous indicator cells tested to date, the CERV C II virus replicates well only in cells derived from *M. musculus*. Thus, although the CERV C II virus shares certain properties in common with standard MuLV's, *M. musculus* cells cannot restrict its replication.

Besides the two new type C viruses described here, another class of endogenous retrovirus (M432) with some properties similar to the *M. musculus* type B group has been previously isolated from spleen cells of *M. cervicolor* mice (17). Like the CERV C I and II viruses, multiple copies (about 20 per haploid genome) of endogenous viral genes related to the genome of M432 have also been detected in the DNA of normal mice from this species. Thus, there are at least 30 copies per haploid genome of retroviral genes in the cellular DNA of *M. cervicolor*. Assuming that there are 3×10^9 nucleotide

bases per haploid genome in mouse DNA (46), our results would suggest that at least 0.01% of the chromosomal DNA of *M. cervicolor* is composed of endogenous viral gene sequences.

Table 6 lists representative isolates of endogenous retroviruses that have been obtained to date from various *Mus* species. Since all of these viruses are genetically transmitted, it is possible to examine the cellular DNA of various rodent species for the presence of related viral genes. Radioactively labeled DNA probes prepared from various *M. musculus* type C viruses can barely detect related sequences in the cellular DNA of *Mus* species that are evolutionarily only 2×10^6 to 4×10^6 years distant. By contrast, probes prepared from the CERV C I and II viruses and from the *M. caroli* type C virus hybridize readily to the DNA of other rodent species in a manner that closely approximates the evolution of the nonrepetitive cellular DNA of these same animals (see Table 3). In fact, using the latter probes, partially related nucleic acid sequences can be detected in the DNA of the hamster, which is believed to have diverged from mice approximately 20×10^6 years ago. Thus, the endogenous viruses isolated from *M. cervicolor* appear to have been more highly conserved than the type C viruses previously isolated from *M. musculus* or from other rodent species (rat and hamster).

Previous studies characterizing the rate of evolution of endogenous primate type C viral genes revealed that a decrease of 1°C in the thermal stability of DNA duplexes corresponded to 4×10^6 to 6×10^6 years of evolution (13). Among rodents, a similar decrease corresponds to only 0.6×10^6 year of evolution (see Fig. 5). This 6- to 10-fold increase in the accumulation of base pair mutations in rodent cellular DNA as a function of time may reflect the approximately 6- to 10-fold faster generation time of rodents as compared to primates (24).

TABLE 6. Endogenous retroviruses isolated from various murine species

Species	Virus class	Representative isolates ^a	Reference
<i>M. musculus</i>	Ecotropic type C	AKR	18
		KiMuLV, S2CL3	23, 48
		BALB-1	1
		Rad-LV	32
	Xenotropic type C α	BALB-2, S16I/60	2, 5
	Xenotropic type C β	AT124, NZB	51, 27
	Amphotropic type C ^b	Lake Casitas	35, 19
<i>M. caroli</i> <i>M. cervicolor</i>	Type B	MMTV	14, 26
	Xenotropic type C I	CARO C I	31
	Xenotropic type C I	CERV C I	This paper
	Xenotropic type C II	CERV C II	This paper
	New retrovirus class	M432	17

^a KiMuLV, Kirsten MuLV; MMTV, mouse mammary tumor virus; Rad-LV, radiation leukemia virus.

^b These isolates have not satisfied all the criteria of an endogenous virus.

The detection of virogene sequences in hamster DNA using probes prepared to the *M. cervicolor* type C viruses would thus be analogous to the detection of primate virogene sequences in species 120×10^6 years distant from the baboon. The viruses isolated from *M. cervicolor* and *M. caroli*, then, are among the most highly conserved mammalian type C viruses.

Whereas the cellular DNA studies indicate that *M. cervicolor* and *M. caroli* are more closely related to one another than either is to *M. musculus*, the CERV C II probe hybridizes to the DNA of *M. musculus* to a somewhat greater extent than to the DNA of *M. caroli* (see Table 3). The same results were obtained with a CERV C II probe prepared from the virus grown in *M. musculus* cells or with virus spontaneously released from a subclone of the *M. cervicolor* lung cell line. The data can therefore not be attributed to the presence of *M. musculus* cellular sequences in the CERV C II probe. The unexpected results may reflect an environmental effect on the rate of virogene evolution (13) or a transfer of type C viral information between different *Mus* species (7, 12). Whether either phenomenon accounts for the data observed with the CERV C II viral probe is not yet clear. Nevertheless, the fact that nucleic acid sequences related to both the CERV C I and II viruses can be readily detected in the cellular DNA of rats and hamsters suggests that both sets of virogenes have been genetically transmitted in rodents for at least 20×10^6 years.

Table 7 summarizes results obtained with probes prepared to four different retroviruses. Neither of these four probes hybridizes to a significant extent to the RNA genomes of the other three isolates, indicating that each probe should detect a different subset of virogenes in the DNA of mice. Although nucleic acid sequences related to each of these probes can be detected in the DNA of all mice, representatives of each viral class have not been isolated from all the murine species. For example, the ability of the CERV C I probe to detect closely related virogene sequences in the DNA of *M. musculus* suggests that the DNA of the laboratory mouse has the potential to code for viruses more closely related to those of *M. cervicolor* and *M. caroli* than the viruses isolated to date from this species. The failure to isolate viruses of this class from *M. musculus* suggests that the expression of these virogenes is under tight cellular control or, alternatively, that these virogenes are defective and are no longer capable of coding for complete, infectious virions.

The ability of the *M. cervicolor* type C viruses to replicate in cells of heterologous species should facilitate an understanding of their potential to cause disease. Whereas the virus isolated from *M. caroli* has not been shown to replicate in primate cells, it is clear that the CERV C I virus will replicate in at least certain primate cell lines. Thus, it should be possible to experimentally determine whether a class of viruses that have putatively given rise to a group of infectious tumor viruses of primates

TABLE 7. Endogenous retroviruses in murine species^a

Species	<i>M. musculus</i> type C		<i>M. cervicolor</i> - <i>M. caroli</i> type C I		<i>M. musculus</i> MMTV		<i>M. cervicolor</i> M432	
	Sequences in cellular DNA	Isolate ob- tained	Sequences in cellular DNA	Isolate ob- tained	Sequences in cellular DNA	Isolate ob- tained	Sequences in cellular DNA	Isolate ob- tained
<i>M. musculus</i>	+	+	+	—	+	+	+	—
<i>M. caroli</i>	+	—	+	+	+	—	+	+ ^b
<i>M. cervicolor</i>	+	+ ^c	+	+	+	—	+	+

^a Four major classes of retroviruses have been isolated from the various *Mus* species. The *M. musculus* type C class prototype is the xenotropic virus isolated from BALB/c 3T3 (5). The prototype virus for the type C I class is the *M. caroli* isolate, CARO C I (31), or the CERV C I isolate described here. The "M432" class prototype is another isolate from *M. cervicolor* (17). This virus possesses a reverse transcriptase that requires magnesium as a cofactor but is unrelated by immunological and nucleic acid hybridization criteria to MMTV (mouse mammary tumor virus). The MMTV prototype is from *M. musculus*. Nucleic acid sequences related to the DNA of this virus have been detected in *M. cervicolor* and *M. caroli* DNA (39).

^b An isolate closely related to the M432 virus, isolated from the *M. cervicolor* lung line, has recently been obtained from a *M. caroli* heart cell line (our unpublished observations).

^c The CERV C II virus is considered to be related to the various *M. musculus* ecotropic and xenotropic isolates because of their partial nucleic acid sequence homology (Table 5). The *M. musculus* viruses of this class can be further subdivided into ecotropic, xenotropic, and amphotropic viruses based on their host range properties, but all isolates of this class share a partial nucleic acid sequence homology as well as immunological similarities between their polymerases and certain structural proteins.

can, in fact, cause disease in these latter species. Similarly, the ability of the CERV C II virus to replicate in cells of a second murine species should provide a convenient model system in which the oncogenic potential of murine xenotropic viruses can be studied.

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