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Detection of Baboon Type C Viral Sequences in Various Primate Tissues by Molecular Hybridization

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Nucleic acid sequences homologous to a single-stranded [^{3}H]DNA transcript prepared from a baboon type C virus replicating in dog thymus cells can be readily detected in the cellular DNA of several Old World monkeys (baboon, patas, African green, and two species of macaques—rhesus and stumptail). These results demonstrate that primates other than the baboon also contain endogenous type C viral genes. With the hybridization conditions employed (S_1 nuclease, 65 C), no homologous sequences were detected in DNA from human or New World monkey tissues. Of various nonprimate tissues examined, only domestic cat cellular DNA was partially homologous to the baboon virus [^{3}H]DNA transcript. In reciprocal experiments, [^{3}H]DNA transcripts of RNAs from endogenous cat viruses (RD-114/CCC group) show a significant partial homology with cellular DNA from Old World primates (baboon, patas, and rhesus monkey). The partial homology between type-C-related information in the DNA of domestic cats and various Old World monkeys suggests the possibility of horizontal transmission between the progenitors of these animals at some point in evolution. No nucleic acid sequences homologous to [^{3}H]DNA transcripts prepared from type C viruses isolated from tumor tissue of a woolly monkey and a gibbon ape could be detected in any primate tissue DNA examined; however, a partial nucleic acid homology was found between woolly monkey and gibbon ape type C viral [^{3}H]DNA and normal mouse cellular DNA.

Baboon cells in culture have a high probability of releasing infectious type C virus. The viruses of this group can be recognized by their ability to grow in cell lines from such diverse species as dog, bat, and man, but do not infect other baboon cell cultures (37). The baboon type C virus (M7) that has so far been most extensively characterized was derived from normal placental tissue (4). Nucleic acid sequences homologous to this baboon type C virus were found in the DNA of normal baboon liver and in the DNA of the placenta from which the virus was isolated (4). Other baboon type C isolates, however, have now been obtained from testicular, kidney, and lung cell cultures. By immunologic criteria, viral host range, and nucleic acid hybridization studies, these new viral isolates are closely related to the M7 type C virus and appear to be members of a distinct new type C virus group (37).

Type C viruses have previously been isolated from a woolly monkey fibrosarcoma (35) and a gibbon ape lymphosarcoma (19). In this report, we examine a variety of primate and nonprimate tissue DNAs for the presence of nucleic acid sequences homologous to single-stranded [^{3}H]DNA transcripts prepared from the various different putative primate type C viruses. In agreement with the data of Scolnick et al. (33), nucleic acid sequences related to either the woolly monkey or the gibbon ape type C viruses cannot be detected in primate cellular DNA. However, nucleic acid sequences related to the baboon type C virus can readily be detected in the cellular DNA of various primates, and, in the baboon, several tissues from normal animals are found to be expressing baboon type C viral specific RNA.

MATERIALS AND METHODS

Cells. The FCf2Th (canine thymus) cell strain was obtained from the Naval Biomedical Research Laboratory (Oakland, Calif.). A204 is a cultured human rhabdomyosarcoma line developed in this laboratory (17). RD is a human rhabdomyosarcoma cell line originally described by McAllister et al. (24). SV clone 80 is a simian virus (SV40)-transformed human diploid skin fibroblast cell line (38). BAB8-Lg are baboon lung cells obtained from J. Melnick. Cultures

of chimpanzee liver cells were obtained from Flow Laboratories (Rockville, Md.). All cells were grown in Dulbecco's modification of Eagle minimal essential medium containing 10% calf serum (Colorado Serum Co.), and were serially transferred by using 0.1% trypsin in phosphate-buffered saline.

Tissues. The baboon placenta (animal no. 1) (*Papio cynocephalus*) from which M7 was originally isolated and a liver from another baboon (animal no. 2) were obtained from the colony maintained at the Southwest Foundation for Research and Education (San Antonio, Tex.). Lung, spleen, uterus, and testis tissue was obtained from baboons housed at the Baylor College of Medicine, Houston, Tex. (animals no. 3 and no. 4).

Various tissues from patas (*Erythrocebus patas*), owl (*Aotus trivirgatus*), spider (*Ateles paniscus*), rhesus (*Macaca mulatta*), and stumptail (*Macaca arctoides*) monkeys were obtained from S. Rangan and P. Gerone of the Delta Regional Primate Research Center (Covington, Louisiana) and from Harvey Rabin (Litton Bionetics, Kensington, Md.). African green monkey (*Cercopithecus aethiops*) tissue was obtained from Flow Laboratories (Rockville, Md.). Capuchin monkey (*Cebus* sp.), dog (*Canis familiaris*), and cat (*Felis catus*) tissues were obtained from Pel-Freez Biologicals Inc. (Rogers, Ark.). Lion (*Panthera leo*) and snow leopard (*Uncia uncia*) tissues were obtained from E. Dolensek of the Bronx Zoo, N.Y.

Virus. The baboon virus, M7, was isolated and propagated on either the FCf2Th or the A204 cell line (4). The type C virus derived from a spontaneous fibrosarcoma of the woolly monkey (35) was grown in A204 cells. The type C virus derived from a gibbon lymphosarcoma (19) was grown in SV clone 80 cells. RD-114 virus was grown in RD cells at Pfizer Laboratories (Maywood, N.J.). The endogenous cat virus, CCC (14, 23), was grown in the FCf2Th canine thymus cell line. All viruses were concentrated from culture supernatants by continuous flow centrifugation and were banded on sucrose gradients.

Preparation of [^{3}H]DNA. A 3- to 16-h endogenous reverse transcriptase reaction from detergent-disrupted type C virus was used to synthesize [^{3}H]thymidine-labeled DNA in the presence of actinomycin D (30 μg/ml) as previously described (6). The [^{3}H]DNA product was deproteinized, double-stranded DNA was removed by fractionation on hydroxylapatite, the product was chromatographed on a Sephadex G-50 column, RNA was hydrolyzed, and the product was dialyzed as previously described (6). The specific activity of the [^{3}H]DNA was 1.5×10^7 counts per min per μg.

Some of the [^{3}H]DNA product prepared from an endogenous reaction of M7 virus in the presence of actinomycin D was annealed with a saturating amount of 35*S* RNA isolated from M7 virions; at a C_rt of 0.1, 75% of the [^{3}H]DNA probe was hybridized to the viral RNA. The resulting RNA:DNA hybrid was digested with the single-strand specific nuclease (S_1) isolated from *A. oryzae* (1, 6). This treatment removes any DNA sequences which do not hybridize with viral RNA. The RNA:DNA hybrid remaining after treatment with nuclease S_1 was deproteinized and treated with 0.5 N KOH at 37 C for 16 h to hydrolyze RNA. The remaining DNA product was neutralized, dialyzed, and concentrated by lyophilization.

Extraction of viral RNA. Purified virions were disrupted with 1.0% sodium dodecyl sulfate (SDS) and deproteinized by three extractions with a 0.5 volume of chloroform-isoamyl alcohol (24:1 vol/vol), and a 0.5 volume of aqueous-saturated and neutralized phenol containing 10% m-cresol. The deproteinized solution was layered on a linear density gradient consisting of 15 to 30% (wt/wt) sucrose in 0.01 M Tris, pH 7.4, 0.1 M NaCl, 10^{-3} M EDTA, 0.1% SDS, and centrifuged at 30,000 rpm for 4 h at 19 C in an SW41 rotor. Approximately 20 0.6-ml fractions were collected dropwise by gravity and the refractive index and adsorption at 260 nm (A_{260}) were determined. RNA banding in the 60 to 70*S* region was pooled and precipitated with two volumes of ethanol. To isolate 35*S* viral RNA, 60 to 70*S* RNA (obtained after velocity sedimentation) was heated at 65 C for 3 min in 0.01 M Tris, pH 7.4, 0.1 M NaCl, 0.001 M EDTA, layered on the same linear sucrose density gradient as described above, and centrifuged at 38,000 rpm for 7 h at 19 C in an SW41 rotor.

Extraction of cytoplasmic RNA. Cytoplasmic RNA was extracted as previously described (8).

Extraction of cellular DNA. Washed cells or tissue were suspended in three volumes of 0.05 M Tris, pH 8.3, 5 mM magnesium acetate, and 0.04 M NaCl (buffer A), homogenized in a Potter-Elvehjem homogenizer, and centrifuged at $4,000 \times g$ twice to pellet nuclei and unbroken whole cells. The pellet was resuspended in 20 volumes of buffer A, adjusted to 1.0% SDS and 0.1 M NaCl, and extracted several times at room temperature with chloroform-isoamyl alcohol (24:1 vol/vol) and with neutralized water-saturated phenol containing 10% m-cresol. After phenol extraction, the solution was extracted four times with ether and treated with 0.5 N KOH at 37 C for 12 to 16 h to hydrolyze RNA. The remaining DNA was neutralized, lyophilized, and dialyzed against four changes of 400 volumes of 0.01 M Tris, pH 7.4, 0.1 M NaCl, 10^{-4} M EDTA. The DNA was then sonically treated essentially as described by Parks and Scolnick (29): The microtip attachment for the Branson sonicator (model W185) was placed 1 cm from the bottom of a scintillation vial containing 15 ml of water which was placed on a top-pan balance. The power setting was adjusted so as to displace 6.0 g on the balance. A 15-ml amount of dialyzed DNA solution (0.5 to 3 mg/ml) was sonically treated at that setting for a total of 9 min at 5 C. This treatment consistently reduced cellular DNA to 6 to 8*S* (the size of the [^{3}H] DNA probes) as determined by centrifugation on alkaline sucrose gradients. After sonic treatment, the DNA solutions were centrifuged at $10,000 \times g$ to remove a small amount of metal particles that were present as a result of sonic treatment, lyophilized, and stored at −20 C at a concentration of 6 to 10 mg/ml.

Hybridization. Cellular or viral RNA and [^{3}H]DNA were incubated for varying times at 50 C in reaction mixtures containing 0.01 M Tris, pH 7.4, 0.40 M NaCl, 2×10^{-3} M EDTA, 0.05% SDS, 30%

formamide, and about 20,000 counts/min (1.3 ng) of [^{3}H]DNA per ml. The ratio of the concentration of cellular RNA to [^{3}H]DNA varied from 1.5×10^6 to 4×10^6; the ratio of viral RNA to [^{3}H]DNA was 3×10^3. Hybridizations were started by heating the mixtures to 90 C for 10 min, cooling on ice, and incubating at 50 C.

DNA:DNA hybridizations were incubated for varying times at 65 C in reaction mixtures containing 0.01 M Tris, pH 7.4, 0.75 M NaCl, 2×10^{-3} M EDTA, 0.05% SDS, 10,000 to 20,000 counts per min per ml of [^{3}H]DNA and 1 to 3 mg of cellular DNA per ml. The ratio of cellular DNA to [^{3}H]DNA varied from 1.5 to 4 $\times 10^6$. Hybridizations were started by heating the mixtures to 98 C for 10 min, cooling on ice to 4 C, and incubating at 65 C. At varying times (ranging from 6 min to 72 h), 0.05-ml portions were removed and frozen at −80 C until digested by S_1 nuclease. Duplicate zero-time samples were also withdrawn to monitor any effect of cellular DNA or RNA on the digestion of the [^{3}H]DNA product by S_1 nuclease. The amount of [^{3}H]DNA that was S_1 resistant in the presence of heterologous mammalian DNA from various sources (e.g., bovine and murine) varied from 1 to 7%. 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) as A_{260} per ml per $2 \times$ h, and corrected to a monovalent cation concentration of 0.18 M (11).

Annealing of unique sequence baboon and dog DNA. FCf2Th (dog) and BAB8-Lg (baboon) cells were labeled with 50 μCi of [^{3}H]thymidine per ml for 48 h and the DNA was extracted as described above. A 3-mg amount of dog liver DNA and 40,000 acid-precipitable counts/min of [^{3}H]thymidine-labeled FCf2Th DNA (2.6 μg) were heated at 98 C for 10 min in a 1.0-ml solution containing 0.01 M Tris, pH 7.4, 0.75 M NaCl, 2×10^{-3} M EDTA, and 0.05% SDS. The solution was cooled in ice and then incubated at 65 C. Portions of 0.05 ml were removed at various times, and the extent of hybridization was determined with nuclease S_1. The annealing of baboon DNA was performed in the same fashion: various concentrations of baboon spleen DNA and [^{3}H]thymidine-labeled BAB8-Lg DNA were hybridized to the indicated C_0t values.

Analysis of hybrids. At the end of the hybridization, to each 0.05-ml portion was added 2.3 ml containing 0.033 M sodium acetate, pH 4.5, 2.5×10^{-4} M zinc sulfate, 0.13 M NaCl, 30 μg of denatured calf thymus DNA, and sufficient purified S_1 nuclease (6) to render the single-stranded [^{3}H]DNA product trichloroacetic acid-soluble in the presence of 500 μg of cellular RNA or DNA after a 30-min incubation at 45 C. After incubation, 0.6 ml of 50% trichloroacetic acid was added, the reactions were chilled at 2 C for 15 min, the [^{3}H]DNA that was cold acid-precipitable was collected on Millipore filters, and the radioactivity was counted.

Preparation of radioactively labeled viral RNA. Three 32-oz prescription bottles containing 70% confluent cells were labeled for 3 h in phosphate-free MEM containing 10% dialyzed fetal calf serum (phosphate-free media) and 100 μCi of ^{32}P (carrier free) per ml from New England Nuclear Corp. This medium was replaced with phosphate-free medium; 24 h later, the cells were labeled with ^{32}P for an additional 3 h. The ^{32}P-containing medium was again replaced with phosphate-free medium, and fluid was collected at two 18-h intervals from a bat lung cell line (TblLu) infected with the baboon virus M7, from RD cells (24) infected with RD-114, and from a rat kidney line (NRK) (12) infected with woolly monkey type C virus. The fluid was clarified by centrifugation at $12,000 \times g$ for 10 min, and virus was pelleted through 20% glycerol in 0.05 M Tris-hydrochloride, pH 7.8, 0.10 M KCl at $105,000 \times g$ for 90 min at 4 C. The virus pellet was suspended in 0.2 ml of 0.1 M NaCl. 0.01 M Tris-hydrochloride, 0.001 M EDTA, pH 7.4 (buffer B), layered on a 5-ml gradient consisting of a 10 to 60% (wt/wt) sucrose gradient in buffer B, and centrifuged at 50,000 rpm for 3 h at 4 C in an SW50.1 rotor. The fractions containing the radioactive virus peak at a density of 1.16 g/ml were pooled and diluted fourfold with cold buffer B, and the virus was recovered by centrifugation at $105,000 \times g$. Radioactively labeled 70S viral RNA was isolated on a linear sucrose gradient as described above.

RESULTS

Characterization of single-stranded [^{3}H]-DNA viral probes. The [^{3}H]DNA probes prepared from baboon, cat (RD-114/CCC), woolly monkey, and gibbon ape type C viral RNAs were characterized as to the extent of complementarity to their 70S viral RNA templates. Seventy to 90% of each probe hybridized in the presence of saturating amounts of their respective purified viral RNA. In addition, the proportion of 70S viral RNA that was represented in the [^{3}H]DNA probes was also examined. As shown in Table 1, hybridization of 70S [^{32}P]RD-114 viral RNA with a 1.5-fold excess of the [^{3}H]RD-114 DNA probe resulted in the protection of 70% of the viral RNA from pancreatic ribonuclease digestion; with a 16-fold excess of DNA probe, 80% of the viral RNA was hybridized. Similarly, the baboon viral [^{3}H]DNA probe (M7/FCf2Th) contained 76% of the baboon 70S viral RNA sequences at a DNA:RNA molar ratio of 0.9. A molar ratio of 5.0 of DNA to RNA did not result in any further protection. Thus, although some of the viral RNA-specific sequences cannot be readily detected in the DNA product, over two-thirds of the 70S RD-114 and of the 70S M7 viral RNA sequences are well represented in the [^{3}H]DNA probes used in these studies. The woolly monkey viral [^{3}H]DNA product hybridizes to 50% of the [^{32}P]woolly 70S viral RNA when annealed to it in a twofold molar excess.

Hybridization of baboon viral [^{3}H]DNA probes to cellular DNA extracted from vari-

TABLE 1. *Hybridization of [^{32}P]70S viral RNA to the in vitro synthesized [^{3}H]DNA probes*[a]

DNA[b]	μg of DNA (× 10^3)	70S[^{32}P]RNA	μg of RNA (× 10^3)	DNA:RNA ratio	%[c]
[^{3}H]RD-114	1.1	RD-114[d]	0.75	1.5	70.0
[^{3}H]RD-114	2.2	RD-114	0.14	15.7	80.0
Calf thymus	50.0	RD-114	0.59	85.0	0.8
Human spleen	60.0	RD-114	0.80	75.0	1.0
[^{3}H]M7/FCf2Th	2.7	M7[e]	2.88	0.9	76.0
[^{3}H]M7/FCf2Th	2.5	M7	0.50	5.0	75.0
Calf thymus	50.0	M7	2.60	19.0	0.0
[^{3}H]Woolly/A204	2.7	Woolly monkey[f]	4.30	0.6	30.0
[^{3}H]Woolly/A204	9.7	Woolly monkey	5.10	1.9	50.0
Calf thymus	50.0	Woolly monkey	4.20	12.0	0.7

[a] Hybridizations were performed as described for viral RNA and [^{3}H]DNA in Materials and Methods, and were carried out to a C_0t of 1.0. At the end of the hybridization, to each 0.01-ml sample was added 1.0 ml containing 0.01 M Tris-hydrochloride, pH 7.4, 0.12 M NaCl, and 20 μg of pancreatic ribonuclease that had been previously heated to 80 C for 15 min to inactivate any contaminating deoxyribonuclease. After incubation at 37 C for 30 min, reactions were made 10% in cold trichloroacetic acid, filtered, and counted.

[b] The specific activity of the [^{3}H]DNA viral probes is 1.5×10^7 counts per min per μg.

[c] The percentage of ^{32}P viral RNA radioactivity that is resistant to digestion with pancreatic ribonuclease is listed.

[d] 70S viral RNA was isolated from RD-114 virus grown in a human rhabdomyosarcoma cell line (RD) (24) as described in Materials and Methods; the specific activity is 0.9×10^6 counts per min per μg.

[e] M7 virus was grown in a bat lung cell line (TblLu); the specific activity of the M7 70S viral RNA is 0.3×10^6 counts per min per μg.

[f] Woolly monkey virus was grown in a rat kidney cell line (NRK) (12); the specific activity of this 70S viral RNA is 0.1×10^6 counts per min per μg.

ous primate and nonprimate tissues. The baboon type C virus (M7) originally isolated from a baboon placenta (4) was grown in either canine thymus (FCf2Th), human rhabdomyosarcoma (A204), or a bat lung (TblLu) cell line. Nucleic acid sequences that are fully homologous to a single-stranded [^{3}H]DNA copy of baboon viral RNA have previously been shown to be present in the DNA of normal baboon liver and in the DNA of the placenta from which the virus was isolated.

To determine if sequences homologous to M7 could also be detected in the DNAs extracted from tissues of other baboons and several other species, a [^{3}H]DNA probe prepared from M7 virus grown in canine thymus cells was hybridized to DNAs obtained from various primate tissues. Figure 1 shows the reassociation kinetics obtained in this experiment, plotted as a function of C_0t. The data were obtained by varying either the concentration of nucleic acids or the time of incubation; similar results were obtained in both cases. The extent of DNA:DNA hybridization was monitored with the single-strand specific nuclease S_1. As shown in Fig. 1, the [^{3}H]M7 DNA probe hybridizes readily to the DNA from the original placenta from which the virus was isolated, and with DNA from a normal spleen and liver from two other baboons obtained from separate colonies. At the maximal extent of the reaction, 70% of the [^{3}H]DNA is resistant to digestion by S_1 nuclease. The probe also hybridizes to the same final extent with the DNA extracted from a canine thymus cell culture that is productively infected with M7.

DNA extracted from other Old World monkeys (patas, African green, rhesus) was also examined for homology to M7. DNA from patas monkey liver saturated about 35% of the M7 probe; this is one-half of the final extent of homology seen with baboon DNA. African green monkey liver DNA hybridizes to 18% of the DNA probe. In contrast, a sample of New World monkey DNA (woolly monkey liver) and of human spleen DNA hybridizes to about 4.0% of the M7 [^{3}H]DNA probe at C_0t values of about 8×10^3. This level of hybridization is close to the background levels (i.e., hybridization to various mammalian DNAs) obtained with this probe.

Table 2 lists the various primate and nonprimate tissues that have been examined for nucleic acid sequences related to M7. The data show the final saturating percent hybridization values. The first column represents experiments performed with an endogenous [^{3}H]DNA product prepared from M7 virus grown in canine thymus cells. The data indicate that there is information that is fully homologous to the

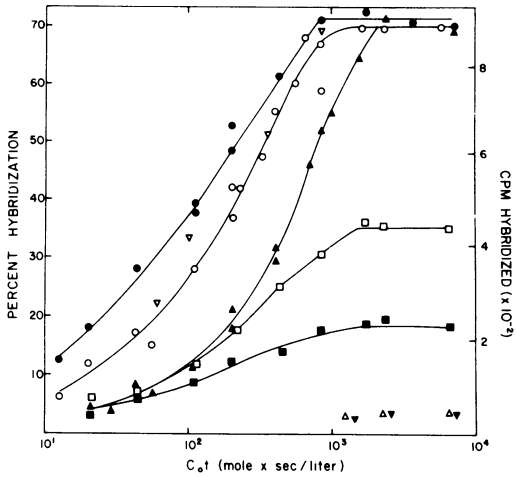

FIG. 1. *Hybridization of baboon type C viral [³H]DNA product to various primate cellular DNAs. Approximately 1,240 counts/min of M7/FCf2Th [³H]DNA were added per 0.05 ml of the reaction mixture. The radioactivity shown represents [³H]DNA product which is resistant to digestion by nuclease S_1; 18 counts/min of the [³H]DNA probe were resistant to S_1 nuclease at zero time; this has been subtracted from all values. The DNA:DNA hybridization was performed as described in Materials and Methods. Symbols: ●, cellular DNA extracted from baboon spleen; ○, DNA from baboon liver; ▽, DNA from baboon placenta; ▲, DNA from FCf2Th canine cell culture infected with baboon virus, M7; □, DNA from patas monkey liver; ■, DNA from African green monkey liver; △, DNA from a normal human spleen; ▼, DNA from a woolly monkey liver.*

probe in the DNA extracted from three normal baboons. DNA extracted from tissues of two patas, an African green, a rhesus, and a stumptail monkey also hybridize to 14 to 34% of the M7 probe. In contrast, DNA extracted from tissues of four New World monkeys (spider, woolly, marmoset, and capuchin) hybridize to less than 5% of the probe. Ape DNA extracted from gibbon liver and a chimpanzee liver cell culture, as well as DNA from human spleen and placenta, also hybridize to less than 5% of the baboon (M7) [³H]DNA probe. Thus, among the primates, other Old World monkeys contain nucleic acid sequences that are partially homologous to the baboon viral DNA probe but under the conditions used, nucleic acid sequences related to the baboon viral RNA cannot be detected in more distantly related primates.

The DNA:DNA hybridization studies were carried out at an incubation temperature 19 to 23 C below the T_m of the homologous viral DNA probe-cellular DNA hybrids. Lowering the tem-

TABLE 2. *Hybridization of primate and nonprimate DNAs to various [³H]DNA baboon viral probes*

Cell DNA[a]	Hybridization (% [³H]DNA[b])	
	M7/FCf2Th	M7/FCf2Th[c]
Old World monkeys		
Baboon		
Placenta no. 1[d]	68	
Liver no. 2	70	
Lung no. 3	70	
Spleen no. 3	71	79
Patas		
Liver no. 1	34	35
Liver no. 2	33	
African Green liver	18	21
Stumptail liver	25	
Rhesus		
Liver no. 1	23	
Liver no. 2	21	
New World monkeys		
Spider liver	4.5	
Woolly liver	3.5	
Marmoset liver	3.5	
Capuchin liver	5.0	
Apes		
Gibbon liver	4.0	
Chimpanzee liver[e]	4.5	
Human		
Spleen no. 1	4.0	0.0
Placenta no. 2	4.0	
Nonprimates		
Cat		
Spleen no. 1	21	18
Liver no. 2	17	
Dog liver	4.5	0.5
Pig liver	3.1	
Mouse liver	2.5	
Calf thymus	2.2	0.0
Salmon sperm	0	
Virus-producing cultures		
M7/FCf2Th[f]	70	

[a] DNA was extracted from these tissues as described in Materials and Methods.

[b] Each [³H]DNA product was hybridized to the cellular DNAs as described in Materials and Methods. Each reaction contained approximately 500 to 1,000 counts per min per assay point. The percentage of hybridization listed represents the average final saturation values to the [³H]DNA probes from several experiments; all hybridizations were carried out to a C_0t of at least 5×10^3.

[c] This baboon probe has been preannealed to 35*S* baboon viral RNA and the unhybridized sequences digested away with S_1 nuclease as described in Materials and Methods.

[d] Each number represents a separate animal (see Materials and Methods).

[e] The source of chimpanzee DNA is a chimpanzee liver cell line.

[f] DNA was extracted from the FCf2Th canine cell line productively infected with M7 virus.

perature or the salt concentration during hybrid formation might potentially allow more distantly related sequences to pair, resulting in a higher percentage of mismatched bases. The use of hydroxylapatite instead of nuclease S_1 to assay for hybrid formation is also believed to allow detection of weakly paired regions since adjacent single-stranded sequences can be eluted with the DNA:DNA hybrid (21). However, none of these techniques has allowed us to detect homology between the baboon viral [^{3}H]DNA probes and either New World monkey or human cellular DNA.

The DNA of various nonprimates was also examined for homology to M7 [^{3}H]DNA. There is a striking degree of homology to DNA extracted from tissues of normal domestic cats (17 to 21%). Mouse, pig liver, and calf thymus DNA hybridize to about 2 to 3%, and dog tissue DNA to about 5% of the M7 probe. No hybridization was detected with salmon sperm DNA.

The last column in Table 2 shows data obtained with a [^{3}H]M7/FCf2Th DNA probe that was annealed to a saturating amount of 35*S* viral M7/A204 RNA to remove any nonviral sequences present in the probe, as described in Materials and Methods. Baboon, patas, and African green monkey DNA contain information that is homologous to 35*S* viral M7 RNA. Human spleen, dog liver, and calf thymus DNA no longer hybridize to the probe. However, 18% of M7-related viral information was still detected in normal cat spleen DNA.

The hybridization between DNA probes prepared from M7 (endogenous baboon) and CCC (endogenous cat) and normal cellular DNA is shown in Fig. 2. Figure 2A shows the kinetics of annealing of the M7 [^{3}H]DNA probe to baboon lung and cat liver DNA. Figure 2B shows the reciprocal experiment, by using a [^{3}H]DNA probe prepared from CCC virus. These data show that there is information in normal cat DNA that is partially related (approximately 15 to 20%) to the M7 virus probe; similarly, there are nucleic acid sequences in baboon tissue homologous to the CCC viral DNA. Mouse liver, calf thymus, and salmon sperm DNA all hybridize to less than 4.0% of either probe at a C_0t value of 8×10^3.

Table 3 summarizes data obtained with baboon (M7) and feline (CCC and RD-114) viral [^{3}H]DNA probes and various DNAs. A [^{3}H]DNA probe prepared from an endogenous feline virus grown in canine cells (CCC/FCf2Th) and a probe prepared from the RD-114 virus grown in human (RD) cells yield essentially the same data; namely, there is informa-

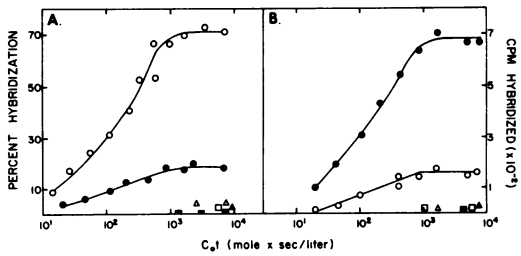

FIG. 2. *Hybridization of endogenous feline and baboon viral [^{3}H]DNA products to DNA extracted from various mammals. 1,000 counts per min per 0.05 ml of hybridization reaction sample of either DNA product was added; the details of the hybridization are described in Materials and Methods. (A) The [^{3}H]DNA product was prepared from M7/FCf2Th virus and hybridized to cellular DNA extracted from: O, baboon lung; ●, cat liver; Δ, dog liver; ▲, mouse liver; □, calf thymus; ■, salmon sperm. (B) The [^{3}H]DNA product was prepared from CCC/FCf2Th virus. Symbols are the same as in (A).*

TABLE 3. *Hybridization of various tissue DNAs to feline and baboon viral [^{3}H]DNA probes*

Cell DNA[a]	Hybridization (% [^{3}H]DNA[b])			
	M7/FCf2Th	M7/A204	CCC/FCf2Th	RD114/RD
Cat spleen	21	15	68	60
Cat liver	22		68	
Baboon spleen	71	67	12	14
Patas liver	33	31	8	8.4
Rhesus liver	18		9	10
Capuchin liver	5.0	6.0	1.5	
Human spleen	4.0	7.0	0.5	0.2
Dog liver	4.5		2.5	0.0
Calf thymus	2.2	5.0	1.9	
Lion kidney	4.0			0.7
Snow leopard liver	3.5			0.7
Salmon sperm	0.0	2.0		
Baboon spleen[c] plus Patas liver			13	14
Rhesus liver[d] plus cat liver	21			

[a] DNA was extracted from these tissues as described in Materials and Methods.

[b] [^{3}H]DNA:DNA hybridization was performed as described in Materials and Methods. See legend to Table 2.

[c] Baboon spleen and patas liver DNA were annealed simultaneously to the [^{3}H]DNA probes listed; the final C_0t value was greater than 5×10^3 for each DNA added.

[d] Rhesus liver and normal domestic cat liver were hybridized simultaneously to the [^{3}H]M7/FCf2Th probe; the final C_0t value was greater than 6×10^3 for each DNA added.

tion related to these endogenous feline viruses in all the Old World primates examined, although such information cannot be detected in DNA from humans or New World monkeys. Two members of the cat family (lion and Asian snow leopard) were also examined for homology to RD-114 and M7. There is no detectable information in the DNA of these animals that is related to either RD-114 or to M7.

Several laboratories (3, 24, 27) have examined human DNA for RD-114 related sequences, and no nucleic acid homology has been detected. However, the present results demonstrate that information related to the endogenous feline viruses of the RD-114/CCC group can readily be detected in Old World primates. Although saturating hybridization levels are relatively low (8 to 15%), they are significantly above background levels (0 to 2%) using these probes. Although the viruses used in these studies were propagated in either dog or human cell cultures, the low levels of hybridization to dog or human cell DNA indicate that the probes are relatively free of contaminating cellular sequences.

To determine whether two different Old World monkeys share the same nucleic acid sequences with RD-114 or CCC viral RNA, baboon and patas tissue DNA were simultaneously hybridized to a C_0t of 6×10^3 (for each DNA) to both the CCC and RD-114 viral [^{3}H]DNA probes. The final level of hybridization achieved at saturation was not additive, implying that common nucleic acid sequences related to each endogenous cat probe are present in the DNAs from both Old World monkeys. Parallel experiments were performed by simultaneously hybridizing rhesus monkey and cat tissue DNA to the baboon viral DNA probe. Again, the levels of hybridization were not additive, indicating that the DNAs from rhesus monkeys and domestic cats share common sequences related to baboon viral genes.

Thermal stability of viral [^{3}H]DNA probe-cell DNA hybrids. The duplexes formed between M7 viral [^{3}H]DNA probe and baboon, rhesus, and cat cellular DNA were also analyzed for their thermal stability. As shown in Fig. 3, the hybrid formed between M7 viral [^{3}H]DNA and baboon cellular DNA has a T_m of approximately 88 C. The hybrid formed with rhesus monkey cellular DNA has a T_m of 79 C, thus revealing a significant degree of mismatching. The hybrid formed between M7 viral [^{3}H]DNA and cat DNA exhibits a T_m of 77 C. In the reciprocal determinations, the hybrid formed between RD-114 viral [^{3}H]DNA probe and cat cellular DNA had a T_m of 84 C. The T_m of the hybrid formed between RD-114 DNA product and Old World monkey (rhesus) cellular DNA was significantly lower (76 C). The endogenous cat and baboon viruses can thus be readily distinguished from one another both by the extent of hybridization of the viral DNA products to normal cell DNA and by the degree of mismatching as determined by T_m between homologous and heterologous cellular DNA.

Hybridization of gibbon ape and woolly monkey type C [^{3}H]DNA probes to various primate and nonprimate cellular DNAs. Type C viruses isolated from the tumor tissue of the woolly monkey (35) and gibbon ape (19) have been shown to be related to each other immunologically (30) and to share extensive nucleic acid sequence homology (7). [^{3}H]DNA transcripts of these two type C viruses were employed to examine for nucleic acid sequences related to these viruses in the DNA of various primate and nonprimate tissues.

Figure 4 shows representative data obtained with [^{3}H]DNA probe obtained from the gibbon type C virus grown in human (SV clone 80) cells. This probe hybridizes readily to the DNA extracted from cells infected with the gibbon

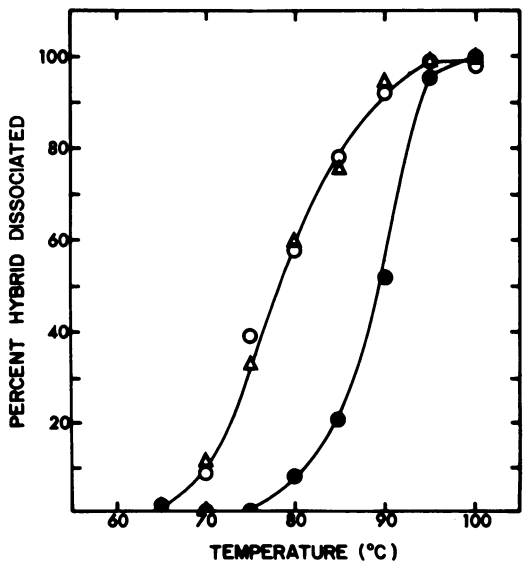

FIG. 3. *Thermal stability of hybrids formed between a baboon viral [^{3}H]DNA probe (M7/FCf2Th) and baboon, rhesus, and cat cell DNA. The hybridizations of baboon [^{3}H]DNA probe and baboon testis DNA (●), rhesus liver DNA (○), and cat spleen DNA (△) were carried out to a C_0t of 8×10^3 at 65 C as described in Materials and Methods. Samples of the hybridization mixture were heated in 0.75 M NaCl at the temperatures listed and then digested with S_1 nuclease.*

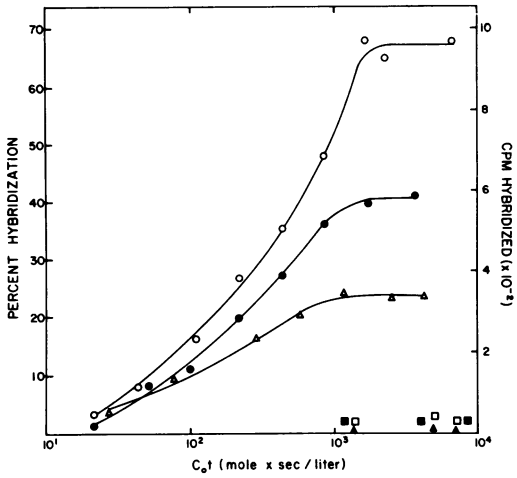

FIG. 4. *Hybridization of gibbon ape type C virus [^{3}H]DNA probe to DNA extracted from various mammals. There were 1,400 counts/min of [^{3}H]DNA added per hybridization reaction sample. Cellular DNA was extracted from: O, SV80 cells infected with gibbon type C virus; ●, A204 cells infected with woolly type C virus; △, mouse liver; ▲, gibbon liver; □, baboon spleen; ■, human spleen.*

type C virus and partially to the DNA of cells infected with the woolly monkey type C virus. This result is expected, since these two viruses have previously been shown to be 40 to 50% related by viral RNA: [^{3}H]DNA hybridization (7). There is no detectable hybridization with the DNA extracted from gibbon liver, baboon spleen, or human spleen. However, there are nucleic acid sequences in normal mouse DNA that hybridize to 25% of the gibbon [^{3}H]DNA probe.

Table 4 shows that none of the primates examined (including gibbon ape and woolly monkey) contain DNA that hybridizes to a significant extent to either the woolly monkey or the gibbon ape type C viral [^{3}H]DNA probes. The nonprimate DNAs examined (dog, cat, calf, and salmon) all hybridize to less than 2.5% of these [^{3}H]DNA probes. However, normal mouse liver DNA extracted from seven-week-old BALB/c mice hybridizes to 19% of the woolly monkey type C probe and to 25% of the gibbon ape type C [^{3}H]DNA probe at saturation. The woolly viral [^{3}H]DNA:mouse cellular DNA hybrid has a T_m of 80 C (data not shown). This murine-woolly monkey and murine-gibbon ape homology has been observed before using RNA:DNA hybridization: viral RNA extracted from an endogenous N-tropic mouse virus (S2CL3) hybridizes 15 to 25% to either the woolly monkey or the gibbon ape type C viral [^{3}H]DNA probes. In fact, these primate type C viruses are as related to certain mouse type C viruses as different murine type C viruses are related to each other (7).

Baboon viral information in various baboon tissues and infected cells. To determine the relative frequency of baboon type C sequences in various tissues, the kinetics of annealing of unique sequence baboon cellular DNA was established. Figure 5 shows the results of an experiment in which [^{3}H]thymidine-labeled baboon cellular DNA was mixed with a large excess of non-radioactively labeled baboon spleen DNA, denatured, and self-annealed. A C_0t of 8×10^2 to 9×10^2 is required to reanneal 50% of this baboon DNA. By comparing this half C_0t value to that required to hybridize half of the various baboon DNAs to the M7 baboon viral [^{3}H]DNA probe, the relative quantities of

TABLE 4. *Hybridization of primate and nonprimate cellular DNAs to gibbon ape and woolly monkey type C viral probes*

Cell DNA[a]	Hybridization (% [^{3}H]DNA[b])	
	Gibbon/SV80	Woolly/A204
Old world monkeys		
Baboon spleen	2.9	
Patas liver	3.3	5.0
New world monkeys		
Woolly liver		3.0
Spider liver	1.5	2.0
Marmoset liver		2.0
Capuchin liver		2.0
Owl liver		2.0
Apes		
Gibbon liver	0.5	
Chimpanzee liver[c]	2.6	
Human spleen	3.2	5.5
Nonprimates		
Mouse liver	25.0	19.0
Dog liver	1.0	
Cat spleen		2.5
Calf thymus	0.8	2.0
Salmon sperm	1.5	0.0
Virus-producing cultures		
Woolly/A204[d]	42.0	71.0
Gibbon/SV80[e]	68.0	

[a] DNA was extracted from these tissues as described in Materials and Methods.

[b] [^{3}H]DNA:DNA hybridization was performed as described in Materials and Methods. See legend to Table 2.

[c] The source of chimpanzee DNA is a chimpanzee liver cell line.

[d] DNA was extracted from the human A204 cell line infected with woolly type C virus.

[e] DNA was extracted from the SV80 cell line infected with gibbon type C virus.

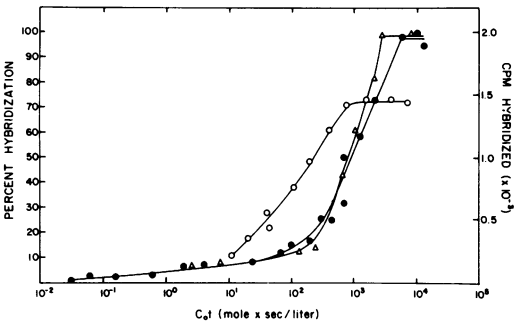

FIG. 5. *Annealing of baboon spleen and dog liver DNA. Symbols:* ●, *self-annealing of baboon spleen DNA;* △, *self-annealing of dog liver DNA;* ○, *hybridization of M7/FCf2Th baboon [³H]DNA probe to baboon spleen DNA.*

viral genomes in baboon tissues can be estimated. Figures 1 and 2A show that the half C_0t values (35% hybridization) for the M7 [³H]DNA:baboon cellular DNA reassociation ranges between 9×10^1 for baboon spleen to 2×10^2 for lung and liver DNA. Since the rates of these reactions are 5 to 10 times faster than the rate of reassociation of unique sequence baboon cellular DNA, there are probably multiple copies of baboon type C virus information in normal baboon tissues.

The half C_0t value for the detection of M7-related information in the FCf2Th line that is productively infected with M7 is 5×10^2. This rate of reassociation is almost two times faster than the rate of reassociation of unique sequence dog DNA (see Fig. 5). There thus appear to be less copies of baboon viral information in productively virus infected dog cells than in normal baboon tissue DNA.

By using the M7 [³H]DNA probe that was purified by preannealing to M7 35S viral RNA, the extent of transcription of M7 related information was examined in several baboon tissues from four animals. The data are plotted in Fig. 6 as a function of C_rt as suggested by Birnsteil et al. (9) and describe RNA:DNA hybridizations with RNA in vast excess. Cytoplasmic RNA extracted from an M7-infected FCf2Th culture hybridizes to 100% of this [³H]DNA probe. Although the RNA:DNA hybridizations were not carried out to a high enough C_rt to reveal full viral expression in any baboon tissue, the majority of the tissues examined transcribe significant amounts of baboon viral-specific information. At a C_rt of 10^4, 70% of the baboon viral information present in the [³H]DNA probe can be shown to be expressed in the baboon placenta from which M7 was originally isolated. Baboon testicular, splenic, lung, and uterine tissue all transcribe more than 30% of baboon viral information. The liver transcribes the least amount of information—10% of the genome that is represented in the M7 [³H]DNA probe. No information that is M7 specific could be detected in cytoplasmic RNA extracted from a normal first trimester human placenta.

A C_rt of 10^{-2} to 3×10^{-2} is required to hybridize 50% of [³H]baboon M7 DNA to 35S viral RNA (data not shown). This value can be used to estimate the frequency of virus-specific nucleotide sequences in virus-producing cells. A limitation on the interpretation of these results rests on the fact that approximately 25% of the baboon viral RNA was not transcribed to [³H]DNA. The C_rt value required to hybridize 50% of M7 [³H]DNA to cytoplasmic RNA extracted from an M7-infected FCf2Th culture is 7×10^1 (Fig. 6). Thus, approximately 0.04% of the cytoplasmic RNA in infected cells is viral. The half C_rt value cannot be determined with accuracy for any of the natural tissues tested since saturating values were not obtained, but it can be estimated from the data in Fig. 6 that the amount of M7 viral RNA in the baboon placenta would be a maximum of about 1% of that found in the productively infected canine thymus cells.

DISCUSSION

The examination of various tissues obtained from three baboons for DNA sequences homologous to a [³H]DNA copy of baboon (M7) viral RNA reveals the presence of multiple copies of baboon type C viral information in each of the

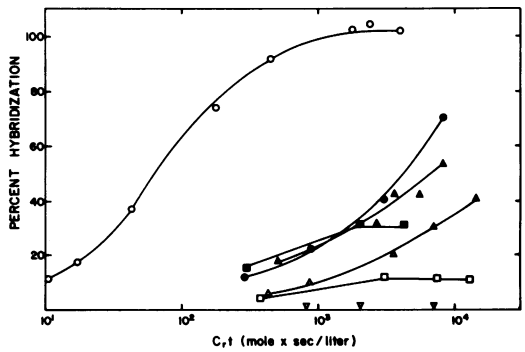

FIG. 6. *Expression of baboon type C viral information in various baboon tissues. The M7/FCf2Th [³H]DNA probe that had been preannealed to M7 35S viral RNA was hybridized to cellular RNA extracted from:* ○, *an FCf2Th culture infected with M7 virus;* ●, *the baboon placenta from which M7 was originally isolated (4);* ▲, *baboon testis;* △, *baboon spleen;* ■, *baboon lung or uterus;* □, *baboon liver; and,* ▽, *normal human spleen.*

normal tissues tested. With an M7 [^{3}H]DNA probe purified so as to contain only 35S baboon viral RNA-specific sequences, it was shown that baboon testicular, splenic, lung, uterine, placental, and liver tissues transcribe anywhere from 10% to at least 70% of M7 viral-related information. The lowest level of viral transcription was present in the liver, whereas the highest levels were found in placental, testicular, and lung tissue. These data are consistent with the isolation of complete, infectious type C virus from cell cultures of baboon testicular, kidney, and lung cells. The latter viruses are indistinguishable by multiple criteria from the baboon type C virus first isolated from a baboon placenta (37). A low level of transcription of avian (18) and murine (8) type C viral sequences has previously been reported in normal avian and mouse cells, but the expression of viral-specific information in the baboon tissues is quite extensive and is similar to the levels of expression of endogenous feline (RD-114/CCC group) viral-specific information in certain cat tissues (28).

Among the primates, M7-related viral-specific sequences can be found in those animals most closely related evolutionarily to the baboon. The patas monkey shows the greatest extent of homology, but other Old World monkeys such as African green and two species of macaques (rhesus and stumptail monkeys) also clearly contain viral-related sequences in their DNA. The baboons and macaques are believed to have diverged from one another approximately 5 million years ago (reviewed in 31). The presence of type C viral-specific information in tissues from these species as well as in baboon tissues suggests that a virus related to the baboon type C viruses is present in the genomes of these other Old World monkeys, even though infectious virus has yet to be isolated from them. By using the same baboon viral [^{3}H]DNA probes, however, no significant amount of M7-related viral-specific information can be detected in DNA extracted from tissues of apes, humans, or New World monkeys, primates that are more distantly related to the baboon (30 to 50 million years) (31).

A striking degree of hybridization (15 to 21%) was observed between normal domestic cat DNA and the baboon viral [^{3}H]DNA probe. This finding extends previous observations that viruses of the RD-114/CCC virus group are the most closely related to the baboon type C viruses (4, 34). In reciprocal experiments using a [^{3}H]DNA probe prepared from RD-114 virus and from an endogenous cat virus (CCC) which was never grown in primate cells, a significant degree of homology is also found with several Old World monkey tissue DNAs but, again, not with ape or New World monkey tissue DNA. Human DNA has been examined for RD-114-related nucleic acid sequences and the results have been uniformly negative (3, 27, 32). However, it is apparent from the data presented here that there is information related to RD-114 and CCC type C viruses that can readily be detected in Old World primate DNA. This homology between cat and Old World monkey type C viral information clearly would not have been expected on evolutionary grounds, and suggests the possibility of transmission from one species to another with the subsequent integration of that information into the DNA and its perpetuation through the germ line. If this hypothesis is correct, it is not possible at present to conclude in which direction the genetic information was transmitted. A primate virus could have infected cat tissues and become integrated into the genome, a cat virus in some progenitor of the domestic cat might have spread to the primates, or ancestors of both species might have been exogenously infected with the same or closely related viruses.

The failure to find any baboon type C viral-specific sequences in the higher apes or in the New World primates could be explained in either of two ways. First, the baboon viruses may have evolved to a degree that makes it impossible to detect homology with DNA from less related primates. This is consistent with the observation that mice, rats, and hamsters have endogenous type C viruses distinct enough so that these hybridization techniques do not detect homology between their genomes (7). (These rodents are believed to have diverged from each other about 10 million years ago [25].) The second possibility, however, is that a virus related to the baboon type C viruses was introduced into the primate stock at some point after the Old World monkeys diverged from the apes and New World monkeys. This would place the time of infection at less than 30 million years (31). If the latter possibility is correct, baboon type C viral-related information would be present only in the Old World monkeys and not in other primates.

The thermal stability of hybrids formed between the unique sequence cellular DNA of various species has been shown by several investigators to correlate well with the classical paleontological view of evolution (reviewed in 20). Baboons and rhesus monkeys diverged approximately 5 million years ago. The T_m of the DNA:DNA hybrid formed by annealing the unique sequence DNAs of two Old World mon-

keys is 82 C, 2 C lower than the T_m of the hybrid formed after annealing unique sequence DNA of any one old World monkey species to itself (20). As reported here, the T_m of the hybrid formed between the baboon type C viral [^{3}H]DNA probe and rhesus cellular DNA is 77 C, considerably lower than the T_m of the homologous baboon [^{3}H]DNA viral probe:-baboon cellular DNA hybrid. Thus, the partial hybridization between baboon viral [^{3}H]DNA and rhesus cellular DNA is not due to a segment of the viral genome that has remained constant in these two species, but rather to a high degree of mismatching as a result of evolutionary divergence. Since apes and Old World monkeys have diverged for 30 to 50 million years (6 to 10 times longer than baboons and rhesus monkeys), the failure to detect a hybrid between the baboon type C viral probe and human cellular DNA under our hybridization conditions (65 C, 0.75 M NaCl) may reflect the extensive evolutionary divergence between human and baboon type C genetic information.

The T_m of a hybrid formed between Old World monkey and cat unique sequence cellular DNA is less than 50 C (20) and would not be detected under our conditions. However, the T_m of the baboon viral DNA probe:cat cellular DNA hybrid as well as the T_m of the RD-114 viral DNA:baboon cellular DNA hybrid are 77 C and 76 C, respectively. In this case, the T_m of the hybrid formed between a type C viral DNA probe and cellular DNA is much higher than the T_m of the hybrid formed between the unique sequence cellular DNA of the two species. These data suggest that these type C viral genes may have been acquired after the species diverged.

[^{3}H]DNA probes prepared from the type C viruses isolated from a gibbon ape and a woolly monkey, type C viruses that replicate readily in primate cells, show no detectable nucleic acid homology with the cellular DNA extracted from any primates, including gibbon and woolly. Thus, these two viruses cannot be considered to be endogenous viruses of primates. Scolnick et al. (33) have also reported similar results. Although the gibbon and woolly type C viruses have been shown to be related to one another immunologically (30) and to share extensive nucleic acid sequence homology (7), they are not related by any immunologic or hybridization criterion to any of the baboon type C viruses thus far isolated (4, 34).

The observation that the viral RNAs isolated from the woolly monkey and gibbon ape type C viruses share a significant nucleic acid sequence homology with several murine type C viruses (7) prompted us to examine the DNA from nonprimate tissues, and especially mouse tissue, for woolly monkey and gibbon viral-related sequences. The only significant level of hybridization that was detected was with mouse cellular DNA. The hybrid formed between the woolly monkey virus DNA probe and mouse cellular DNA shows a relatively low degree of mismatching since it exhibits a T_m of 80 C. At least three endogenous mouse type C viruses can be distinguished by their host range and by their nucleic acid sequence homology both from each other and from the infectious laboratory strains of mouse leukemia virus (5). One of these viruses, the S-tropic or xenotropic virus (5, 22), grows readily in several nonmouse cells, including primate cells. The degree of nucleic acid homology between normal mouse cell DNA and [^{3}H]DNA probes prepared from the viruses isolated from gibbon and woolly monkey suggests the possibility that these "primate" viruses were derived from endogenous mouse viruses or from a type C virus of a rodent closely related to the laboratory mouse. Although the viruses isolated from both woolly monkeys and gibbon apes are not endogenous to those species, they are associated with tumors in these animals, and may well play an etiologic role in tumors of other primates.

To determine the natural hosts of type C viruses, either labeled viral RNA or a single-stranded [^{3}H]DNA copy of viral RNA can be annealed to cellular DNA extracted from the tissues of various species. These kinds of studies have revealed the presence of murine (15, 16), avian (2, 26, 39), porcine (36), and feline (3, 27, 32) type C viral information in the DNAs of their respective species. However, the discovery of type C viral sequences shared in common between species that are evolutionarily distant may have an important bearing on our understanding of both the origin and modes of transmission of type C viruses.

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