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Distribution of Mason-Pfizer Virus-Specific Sequences in the DNA of Primates

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Iodinated Mason-Pfizer virus (MPV) 60-70S RNA has been used in molecular hybridization experiments to determine the distribution of MPV-specific proviral sequences in the DNAs of primates. Approximately 20% of the MPV genome is present as endogenous provirus in rhesus monkeys. Competitive hybridization experiments showed no homology between MPV 60-70S RNA and the 60-70S RNAs of M7, RD-114, and the simian sarcoma virus. No MPV-specific proviral sequences were detected in the DNAs of apparently normal tissues of various species of New World monkeys, apes, and humans. The part of the MPV genome that is endogenous to rhesus is also endogenous to the other species of Old World monkeys examined: baboon, African green, and patas. This was determined as a result of the following observations: (i) $C_0t_{1/2}$ values and final extent of hybridization were the same for all four species. (ii) T_m values of MPV 60-70S RNA and DNA of all four species were identical. (iii) The removal of MPV sequences endogenous to rhesus tissues by recycling against rhesus DNA resulted in the loss of any hybridizable MPV RNA to the DNAs of baboon, African green, and patas tissues. (iv) Mixing experiments of rhesus, African green, and baboon DNAs resulted in the same kinetics of hybridization as did rhesus DNA alone, when hybridized with MPV 60-70S RNA. These findings demonstrate that sequences that constitute an integral part of the MPV genome are conserved in the DNAs of several different species of Old World monkeys.

The Mason-Pfizer virus (MPV; 8) and morphologically similar viruses (1, 2) have been isolated from a carcinoma, lactating mammary gland, and placenta of rhesus monkeys. Ahmed et al. (3) have shown that Mason-Pfizer antigens, or particles detected by electron microscopy, are also found in a variety of rhesus tissues.

We report here, using radioactive 60-70S RNA of MPV in RNA-DNA hybridization experiments, that approximately 20% of the MPV genome is present as endogenous provirus in rhesus tissues. The MPV is, therefore, being transmitted in the primate population by a mechanism other than as a germinal provirus. We also demonstrate that the identical part of the Mason-Pfizer genome that is endogenous to rhesus is also endogenous to the other species of Old World monkeys examined.

MATERIALS AND METHODS

Viruses and cells. MPV was grown in suspension cultures of the normal human lymphocyte cell line NC-37 (J. L. Smith Memorial Laboratories, Chas. Pfizer & Co., Inc., New York, N.Y.), purified from culture supernatant fluids by equilibrium density gradient centrifugation, and concentrated as described previously (15). Simian sarcoma virus (SSV-1) was grown in NC-37 cells. The endogenous virus of baboons (M7) was grown in BKCT cells (5, 11), and the endogenous virus of cats (RD-114) was induced from and grown in feline cells (12). The above viruses were obtained through the Office of Resources and Logistics of the Virus Cancer Program of the National Cancer Institute.

African green monkey tissue and rhesus monkey tissue were obtained from Pel Freeze, Inc. (Rogers, Ark.). Patas and rhesus monkey placentas were kindly supplied by D. Sly (Meloy Laboratories Inc., Springfield, Va.). Baboon tissue was kindly supplied by T. Barker and S. Kalter (Southwest Foundation for Research and Education, Austin, Tex.). Gibbon ape and howler monkey tissues were kindly supplied by R. Benveniste and G. Todaro (National Cancer Institute, Bethesda, Md.).

Viral RNA extraction. The procedure used for the isolation of viral RNA was described previously (9, 14) and was the same for all viruses. Viral pellets were suspended in a solution of 0.01 M Tris-hydrochloride (pH 8.3), 0.15 M NaCl, and 0.01 M EDTA and lysed by the addition of sodium dodecyl sulfate to a 1% final concentration. After the suspension cleared, 0.75 mg of self-digested Pronase (2 h, 37°C) per ml and 1% mercaptoethanol were added, and the suspension was incubated at 37°C for 30 min.

This mixture was then extracted twice with 2 volumes of phenol-cresol-chloroform (7:1:8, vol/vol/vol, containing 8-hydroxyquinoline [0.185 g/100 ml], pH 8.3). The aqueous phase was removed and adjusted to 0.4 M LiCl, and the RNA was precipitated by the addition of 2 volumes of cold ethanol. After 16 h at −20°C, the RNA was pelleted at 17,000 × *g* for 30 min at −20°C and dissolved in 100 μl of TNE buffer (0.01 M Tris-hydrochloride [pH 8.3], 0.15 M NaCl, and 0.002 M EDTA), and the 60-70*S* component was isolated by glycerol gradient sedimentation in a 10 to 30% linear glycerol gradient in TNE (centrifuged at 200,000 × *g* for 3 h at 4°C [Spinco SW41 rotor]). Fractions were collected dropwise, and the 60–70*S* RNA peak of the gradient, determined by absorbance at 260 nm, was precipitated with ethanol as described above.

Iodination of viral RNA. Carrier-free ^{125}I (pH 8 to 11, 100 mCi/ml) was obtained from Amersham/Searle, Des Plaines, Ill. Thallium perchlorate (Alfa Products) was dissolved at 4×10^{-3} M in 0.05 M sodium acetate buffer (pH 4.2). Sodium sulfite (Fisher Products Co., Lewes, Del.) was dissolved at 4×10^{-4} M in 0.05 M sodium acetate (pH 4.2).

Reactions were performed in siliconized 50-μl capillary pipettes. Six hundred microcuries of ^{125}I was mixed with 1 μl of sodium sulfite and incubated at 25°C for 15 min. To this solution, 2 μl of thallium perchlorate and 1 μl of MPV 60-70*S* RNA (5 μg/μl) were added and mixed well. The capillary was then sealed, and the reaction was allowed to proceed at 68°C for 15 min. The unstable intermediates were destroyed by further incubation of this mixture for 45 min at 68°C after dilution with 200 μl of 0.5 M sodium phosphate buffer (NaPB; pH 6.8) containing 0.01 M β-mercaptoethanol. The RNA was separated from free iodine by column chromatography using Sephadex G-50 (10-ml column) in 0.05 M sodium phosphate (pH 6.8). The RNA sample was then adjusted to 30% ethanol in TNE and layered on a 2-ml column of CF11 (Whatman) equilibrated in this buffer. The column was washed with 20 ml of the same buffer, and the RNA was eluted with TNE. MPV RNA produced in this way had a specific activity of approximately 3×10^7 cpm/μg and was 99% trichloroacetic acid precipitable and 98% RNase sensitive.

Hybridization of ^{125}I-labeled MPV RNA to cellular DNA. The technique of hybridizing viral RNA to excesses of cellular DNA is essentially the same as previously described (9). Cellular DNA was hybridized to iodinated 60-70*S* MPV RNA under the following conditions: cellular DNA at 3 mg/ml in 0.4 M NaPB (pH 6.8), 0.05% sodium dodecyl sulfate, and 3,000 cpm of labeled MPV RNA per ml. The DNA (at 6 mg/ml) was first boiled for 1 min in a bath of ethylene glycol and then put in a water bath at 68°C. The salt and labeled RNA were mixed with the DNA, and the entire solution was incubated at 68°C until the desired C_0t had been attained. At appropriate times, 335-μl portions (1,000 μg) were removed and diluted into 10.5 ml of 2× SSC (1× SSC is 0.15 M NaCl with 0.015 M Na citrate). The sample was divided in half and added to each of two tubes containing an additional 5 ml of 2× SSC. (DNA concentration is now 50 μg/ml.) RNases A and T_1 were added to one half of the sample at final concentrations of 15 μg/ml and 3 U/ml, respectively. Both tubes were then incubated at 37°C for 30 min. The samples were chilled for 10 min, and trichloroacetic acid was added to a final concentration of 10%. After being kept on ice for 30 min, the acid-insoluble material was collected on Gelman filters (0.45 μm). A zero time control, which was boiled for 1 min in a bath of ethylene glycol prior to the addition of RNase, was included in all experiments. The percent hybridization was determined by dividing the counts per minute in the RNase-treated sample by the counts per minute in the untreated sample after deducting the zero time background. C_0t values are corrected to a standard of 0.12 M sodium phosphate.

Extraction of cellular DNA. DNA was extracted from tissues using methods similar to those previously described (4). Approximately 1 g of wet tissue was added to 9 ml of extraction buffer (0.1 M NaCl, 0.01 M Tris [pH 8.5], 0.001 M EDTA), and this mixture was disrupted by homogenization in a Waring blender for 5 min. The solution was then adjusted to 1% sodium dodecyl sulfate and 1% mercaptoethanol and incubated for about 30 min at 37°C. Predigested Pronase was added at a final concentration of 0.5 to 1.0 mg/ml. The solution was incubated an additional 2 to 12 h. Then, a one-half volume of chloroform and a one-half volume of extraction buffer-saturated phenol were added to the DNA solution, and the solution was shaken for 15 to 30 min. The aqueous phase was separated by centrifugation for 15 min at 4,000 × *g*. The aqueous phase was adjusted with 0.2 M Na acetate, and 2 volumes of ethanol were added. The DNA was then spooled out on a glass rod, dissolved in 0.3 N KOH, and incubated at 37°C for 16 h to hydrolyze any contaminating RNA. The solution was neutralized with a solution of 2 N HCl in 0.4 M Tris and sonically treated (Sonifer cell disruptor, model W185, Heat Systems-Ultrasonic Inc.; highest setting) for 3 min at room temperature. The DNA solution was precipitated with 0.2 M Na acetate and 2 volumes of alcohol, pelleted, and dissolved in extraction buffer. At this time, the solution was extensively reextracted with a phenol-chloroform mixture until no interphase was observed between the aqueous and organic phases. The aqueous phase was separated from the phenol phase, and the DNA was precipitated with 0.2 M Na acetate and 2 volumes of alcohol and then dissolved in 0.001 M NaPB (pH 6.8) at a concentration of 6 mg/ml; DNA prepared in this way had a 260/280-nm ratio of at least 1.70, usually above 1.80. The sedimentation coefficient as determined by alkaline sucrose sedimentation was between 6 and 9*S*.

Recycling of ^{125}I-labeled MPV 60-70*S* RNA against normal rhesus DNA. The technique is a modification of that previously described (10, 16). DNA was treated with 0.01% diethyl pyrocarbonate (Calbiochem, San Diego, Calif.) and mixed for 5 min at 4°C. The diethyl pyrocarbonate was removed by heating the DNA at 68°C for 1 h. A total of 300,000 cpm of ^{125}I-labeled MPV 60-70*S* RNA was hybridized to 30 mg of normal rhesus placenta DNA to a C_0t of 35,000 under the conditions described above. At the completion of hybridization, the sample was diluted

to 50 μg of DNA per ml with sterile water and then heated to 60°C. The solution was applied to a column of 150 ml of packed hydroxylapatite (DNA grade, Bio-Gel, HTP, lot no. 14557, Bio-Rad Laboratories, Richmond, Calif.) at 60°C. The sample which came through was reloaded, and the column was rinsed with a solution 0.01 M NaPB (pH 6.8) and 0.01% sodium *N*-lauryl sarcosinate (SLS) until no material eluted that absorbed at 260 nm. Single-stranded DNA and RNA were then eluted with a solution of 0.14 M NaPB (pH 6.8) and 0.01% SLS. The double-stranded DNA and hybridized RNA were eluted with a solution of 0.4 M NaPB (pH 6.8) and 0.01% SLS. The single-stranded fraction, termed "recycled RNA," was dialyzed (Spectrapor no. 1 or no. 3) against three changes of 6 liters of water containing 0.1% SLS. After dialysis, 1 to 2 mg of yeast carrier RNA was added, and the RNA was precipitated with 2 volumes of alcohol. The RNA was pelleted and dissolved in 1 ml of 0.001 M NaPB and stored at −20°C until it was hybridized to cellular DNAs under conditions identical to those described above using ^{125}I-labeled 60-70*S* MPV RNA.

Thermal stability of DNA-RNA hybrids. The technique for studying the thermal stability of the DNA-RNA hybrids is similar to that described previously (7). The hybridization mixture contained 1 mg of cellular DNA and 6,000 cpm of ^{125}I-labeled MPV RNA. The conditions of hybridization are the same as described above. After hybridizing to a C_0t of 35,000, the DNA concentration was diluted to 50 μg per ml, and the solution was applied twice to a 4-ml column of packed hydroxylapatite maintained at 60°C in 0.006 M NaPB (pH 6.8). Under these conditions, approximately 95% of the cellular DNA remains bound to the column. While at 60°C, the column was washed twice with 10-ml samples of 0.12 M NaPB (pH 6.8) containing 0.01% SLS. This procedure was repeated at 5°C increments until a temperature of 100°C was attained. The thermal dissociation of the DNA-DNA hybrids was measured by monitoring the optical density at 260 nm in the effluent fractions, and that of the ^{125}I-labeled RNA-DNA hybridization was measured by determining the trichloroacetic acid-precipitable counts per minute in the effluent fractions.

RESULTS

Kinetics of hybridization of ^{125}I-labeled MPV 60-70*S* RNA and primate DNAs. MPV was purified from supernatant fluids of MPV-infected NC-37 cells. Purified 60-70*S* RNA was iodinated in vitro to a specific activity of approximately 3×10^7 cpm/μg. The iodinated RNA was 99% acid precipitable, 98% sensitive to RNase, and banded as a sharp peak in a cesium sulfate density gradient at 1.63 g/ml.

Figure 1 shows the kinetics of hybridization between ^{125}I-labeled MPV RNA and cellular DNAs. Since the MPV used in the experiment was grown in human NC-37 cells, the ^{125}I-labeled RNA was hybridized to the DNA of uninfected NC-37 cells. No hybridization above 6%

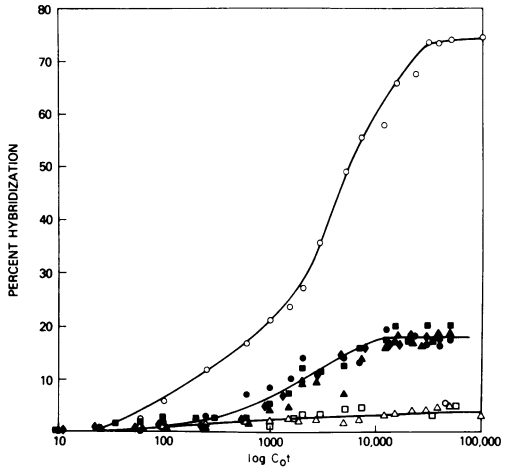

FIG. 1. *Hybridization of ^{125}I-labeled MPV 60-70S RNA to various primate DNAs. Hybridization conditions were as described in Materials and Methods. Hybridization mixtures contained ^{125}I-labeled MPV 60-70S RNA and DNA from the following tissues: the human lymphoblastic cell line, NC-37, infected with MPV (○); uninfected NC-37 cells (△); rhesus placenta (▲); baboon liver (■); patas placenta (●); African green liver (◆); and sheep lung (□).*

(background) was observed with NC-37, ovine, bovine, and *Escherichia coli* DNAs (Fig. 1 and Table 1). This demonstrates that the ^{125}I-labeled RNA was viral specific and did not contain any detectable RNA or DNA from the cell in which it was grown. Hybridization to the DNA of MPV-infected NC-37 cells was greater than 70%, with a $C_0t_{1/2}$ value of approximately 3,000 (Fig. 1).

The MPV RNA hybridized approximately 18% (Fig. 1) to normal rhesus placenta. Similar results were observed with DNAs from apparently normal rhesus liver, spleen, lung, kidney, and brain. Tissues were obtained from monkeys that had recently arrived from India and had not been held in any laboratory in which virus research was being conducted. DNAs from all the apparently normal tissues of baboon, African green, and patas also showed the same final percent hybridization, and $C_0t_{1/2}$ values of approximately 3,000 (Fig. 1). This $C_0t_{1/2}$ value indicates that the viral RNA is hybridizing with low frequency cellular DNA sequences.

When ^{125}I-labeled MPV 60-70*S* RNA was hybridized to DNAs of apparently normal tissues of a variety of New World monkeys, no significant hybridization (between 3 and 7%) was observed. These tissues included livers of marmoset (*Saguinus oedipus* and *Saguinus fuscicollis*), howler (*Alouatta* sp.), squirrel (*Saimiri scuireus*), *Aotus trivirgatus*, and cebus, as well

as cebus placenta. DNA from gibbon ape (*Hylobates lar*) liver, chimpanzee (*Pantroglodytes*) spleen, and a variety of apparently normal human tissues also showed no significant hybridization above background.

Thermal stability of RNA-DNA hybrids. The thermal stability of nucleic acid hybrids is a good indication of the extent of mismatching between base pairs of a DNA-RNA duplex. Therefore, the DNA-RNA hybrids formed between ^{125}I-labeled MPV 60-70*S* RNA and Old World monkey DNAs were analyzed for thermal stability by hydroxylapatite column chromatography. Figure 2 depicts the dissociation of hybrids formed between ^{125}I-labeled MPV 60-70*S* RNA and rhesus, baboon, African green, and patas DNAs. A T_m of 80.5°C (Fig. 2A) was obtained for the dissociation of hybrids formed between MPV RNA and control DNA from NC-37 cells infected with MPV. The hybrids formed between MPV RNA and all the Old World DNAs showed T_m values of approximately 80.2°C (Fig. 2B-E), indicating that the hybrids formed between MPV RNA and the different Old World monkey DNAs demonstrate little, if any, mismatching. As expected, the dissociation of reannealed DNA-DNA duplexes for all four tissues had a T_m of 87°C.

TABLE 1. *Hybridization of ^{125}I-labeled MPV 60-70S RNA to primate DNAs before and after recycling against normal rhesus placenta DNA*[a]

Source of DNA	% Hybridization	
	Before recycling	After recycling
Rhesus	18	3
Baboon	18	4
Patas	16	5
African green	20	4
NC-37, infected with MPV	60	54
NC-37, uninfected	5	5
Normal human liver	6	6
Bovine ovary	6	2
E. coli	6	5

[a] All values refer to an average percent hybridization of duplicate points assayed at a C_0t of 35,000.

Competitive molecular hybridizations with ^{125}I-labeled MPV RNA. To determine if there is any nucleic acid sequence relationship between MPV and other primate retroviruses, competitive molecular hybridization experiments were conducted. Various amounts of unlabeled 60-70*S* RNA from M7, RD-114, and SSV-1 were added to hybridization reactions between ^{125}I-labeled MPV RNA and DNA from infected NC-37 cells. Unlabeled 60-70*S* RNA from MPV competed completely in the hybridization reactions (Fig. 3). No significant competition was observed, however, with 60-70*S* RNAs from SSV-1 or the endogenous viruses of baboons (M7) or cats (RD-114). These results also rule out the possibility that the sequence homology observed between MPV RNA and Old World monkey DNAs is due to a contamination of

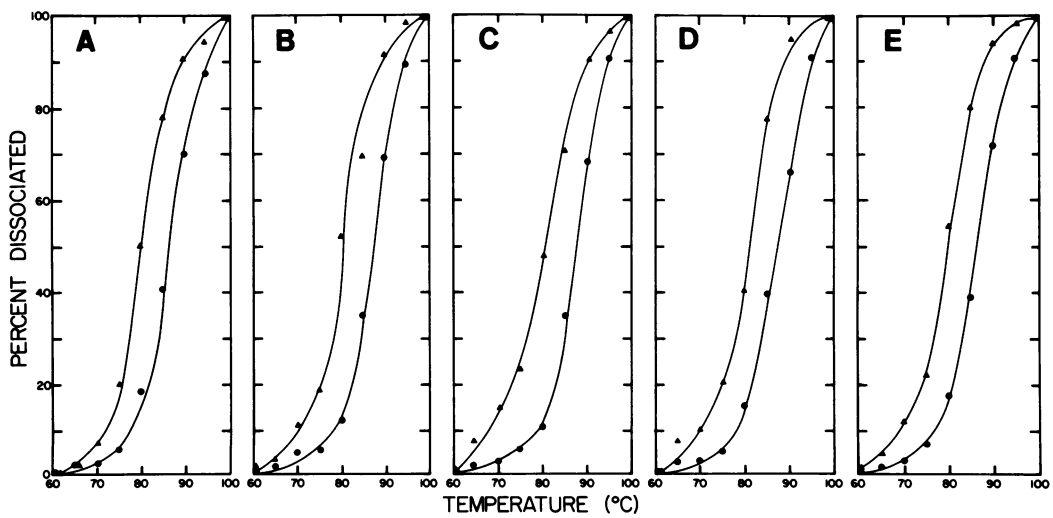

FIG. 2. *Thermal stability of hybrids formed between ^{125}I-labeled MPV RNA and primate DNAs. Hybridization conditions and thermal elution from hydroxylapatite were performed as described in Materials and Methods. Solid circles represent DNA:DNA dissociation profiles as monitored by absorbance at 260 nm. Solid triangles represent DNA:RNA dissociation as measured by acid-precipitable radioactivity in each fraction. Hybridization between ^{125}I-labeled MPV RNA and DNAs from the following tissues: NC-37 cells infected with MPV (A); rhesus placenta (B); patas placenta (C); baboon liver (D); and African green liver (E).*

MPV RNA with RNA of the M7 or RD-114 viruses.

Recycling of ^{125}I-labeled MPV 60-70S RNA. The approximately 18% hybridization observed between the DNA of Old World monkeys and MPV RNA (Fig. 1) could reflect: (i) a part of the MPV genome as provirus in all rhesus cells or (ii) the entire MPV genome as provirus in only a limited number of cells. To answer this question, recycling experiments were performed. Iodinated MPV 60-70S RNA was first annealed to 30 mg of normal rhesus monkey DNA at 68°C to a C_0t of 35,000; the unhybridized, single-stranded, ^{125}I-labeled RNA eluting from the hydroxylapatite column at 0.14 M sodium phosphate was termed "recycled RNA." This RNA was then concentrated and reannealed to DNA of NC-37 cells infected with MPV and to DNA from normal rhesus tissue. The recycled MPV RNA did not hybridize to the DNA of normal rhesus tissue above background levels (Fig. 4). This demonstrates that the recycling effectively removes all portions of the ^{125}I-labeled MPV RNA that are complementary to the DNA of normal rhesus tissue. The recycled ^{125}I-labeled RNA, however, still hybridized more than 65% with DNA from NC-37 cells infected with MPV (Fig. 4), thus demonstrating that the first possibility stated above is correct.

To determine whether or not the MPV-specific sequences found in the DNA of each species of Old World monkey are the same, this recycled ^{125}I-labeled MPV RNA was then hybridized to the DNA of normal rhesus, baboon, African green, and patas tissues. ^{125}I-labeled MPV RNA hybridized to the DNAs of all Old

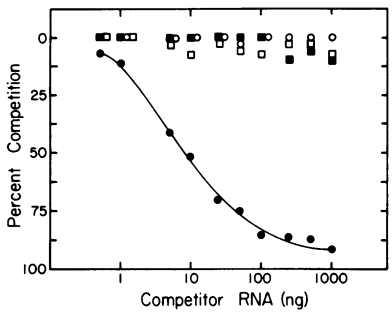

FIG. 3. *Competition molecular hybridizations with MPV RNA. ^{125}I-labeled MPV 60-70S RNA (1,500 cpm) was hybridized to 400 μg of cellular DNA (for maximum sensitivity [9]) from MPV-infected NC-37 cells as described in Materials and Methods. To this hybridization mixture increasing amounts of various viral RNAs were added. The hybridizations were incubated to a C_0t of 35,000 and then assayed for the acquisition of RNase resistance. The percent hybridization to DNA from NC-37 cells infected with MPV was 43%. A background hybridization of 5% to calf thymus DNA has been subtracted from all values. Competitor RNAs are as follows: MPV RNA (●); M7 RNA (■); RD-114 RNA (○); and SSV-1 (□).*

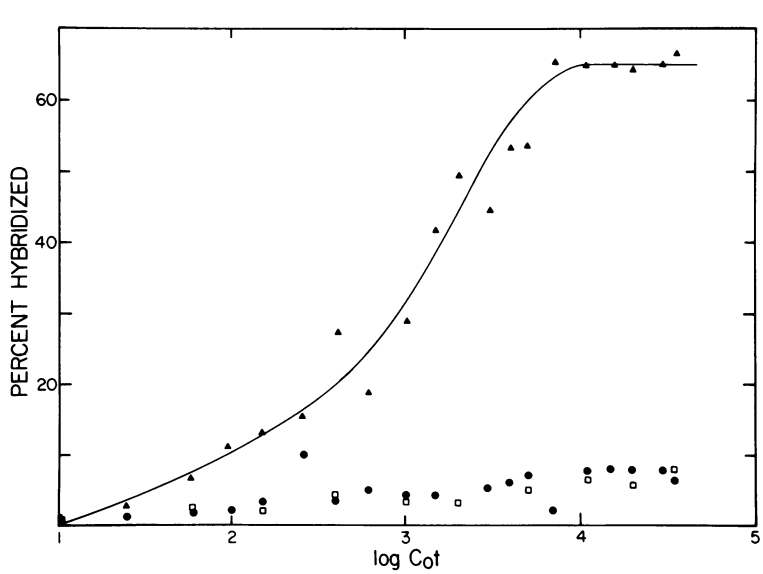

FIG. 4. *Hybridization of recycled ^{125}I-labeled MPV RNA to primate DNAs. Iodinated MPV 60-70S RNA was extensively hybridized to DNA from normal rhesus placenta, and the unhybridized fraction was recovered by hydroxylapatite column chromatography as described in Materials and Methods. The recycled RNA was then hybridized to the following cellular DNAs: NC-37 cells infected with MPV (▲), normal rhesus placenta (●), and sheep lung (□).*

World monkeys before recycling, but did not hybridize above background levels to these same DNAs after recycling (Table 1). This demonstrates that the viral-specific sequences removed from the DNA of rhesus tissue by the recycling procedure are the same viral-specific sequences contained in the DNA of the three other Old World monkeys tested.

^{125}I-labeled MPV RNA hybridized to a mixture of Old World monkey DNAs. As an additional method to determine if the same MPV-specific sequences are contained in the DNA of several species of Old World monkeys, equal amounts of rhesus, baboon, and patas DNAs were mixed together and hybridized to ^{125}I-labeled MPV 60-70S RNA. Since each DNA individually hybridizes approximately 18% to MPV RNA, when they are mixed together in a DNA excess-liquid hybridization experiment, they should hybridize approximately 54% of the viral genome if each DNA contains a different portion of the MPV genome. Furthermore, the $C_0t_{1/2}$ of the hybridization should be increased by a factor of three due to the dilution of viral sequences with different cellular DNAs. To demonstrate that the $C_0t_{1/2}$ is actually increased when viral-specific sequences in an Old World monkey DNA are diluted with an unrelated DNA, one part rhesus monkey DNA was mixed with two parts unrelated DNA (bovine ovary) and hybridized to ^{125}I-labeled MPV 60-70S RNA. The final percent hybridization, as seen in Fig. 5, was the same as that obtained when DNA from rhesus tissue alone was hybridized to MPV RNA. The $C_0t_{1/2}$, however, was shifted from 3,000 to approximately 9,000, reflecting the threefold dilution of viral sequences with bovine DNA.

The mixture of the DNAs from three different Old World monkey species hybridized to the same percentage as did each individual DNA (Fig. 5). Furthermore, the $C_0t_{1/2}$ of 3,000 was the same for the mixture as seen with the hybridization between the viral RNA and each individual Old World monkey DNA (Fig. 1). This further demonstrates that the same MPV-specific sequences are contained in the DNA of the different species of Old World monkey examined.

DISCUSSION

MPV was isolated from a carcinoma in the breast of a rhesus monkey, and viruses with a morphology similar to that of MPV have also been isolated from the mammary gland and rhesus placenta (1, 2, 8). We have recently shown (9) that there is a greater than 90% sequence homology between the RNA of MPV and the RNA of X381, the virus isolated from rhesus mammary glands. Antigens related to MPV and particles morphologically similar to

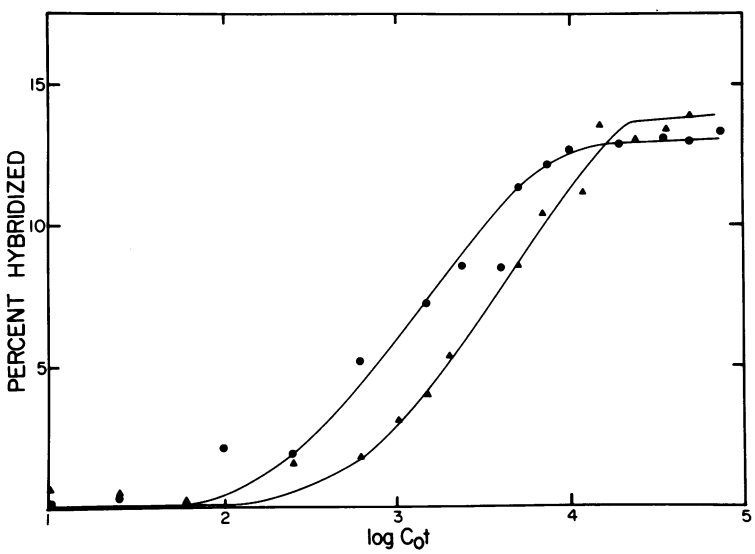

FIG. 5. *Hybridization of ^{125}I-labeled MPV 60-70S RNA to a mixture of Old World monkey DNAs. Solid circles represent the hybridization of ^{125}I-labeled MPV RNA to an equal mixture of rhesus, patas, and baboon DNAs. The hybridization was performed as described in Materials and Methods, with 1-mg portions of DNA and 1,000 cpm of ^{125}I-labeled MPV RNA removed at the indicated C_0t's. Solid triangles represent the hybridization of ^{125}I-labeled MPV RNA to a mixture of 1 part normal rhesus placenta DNA (0.33 mg) to 2 parts bovine ovary DNA (0.66 mg). Hybridizations were carried out in excess DNA as described previously (9) and in Materials and Methods.*

MPV have also been found in a variety of biopsies and cultures from rhesus placentas, breast cultures, and fetal tissues (3). The studies reported here demonstrate that the entire RNA genome of MPV is not endogenous to rhesus monkeys and, therefore, MPV is being transmitted in the rhesus population by a nongerm line mechanism. Approximately 20% of the MPV genome is found as endogenous provirus of rhesus monkeys (Fig. 1, 2, and 4). As shown by competitive molecular hybridization experiments (Fig. 3), MPV 60-70*S* RNA is not related to the 60-70*S* RNAs of M7 (the endogenous virus of baboons), RD-114 (the endogenous virus of cats), and SSV-1. This finding demonstrates that the endogenous rhesus sequences related to MPV are not the same as those previously shown to be endogenous to Old World monkeys and related to the M7 virus (5), RD-114 (6), or SSV-1 (17).

Some apparently normal tissues of some rhesus monkeys contain more than 20% of the MPV genome. This was shown by experiments employing direct hybridization of ^{125}I-labeled MPV 60-70*S* RNA and rhesus DNAs and by competitive molecular hybridization experiments using ^{125}I-labeled MPV 60-70*S* RNA; these preliminary observations are currently under investigation in our laboratory.

Proviral sequences related to MPV were not found in DNAs from a variety of New World monkeys, apes, and apparently normal human tissues. Among the New World monkeys tested and found to be negative was the squirrel monkey. This is of interest, since a virus with a morphology similar to that of MPV has been isolated from squirrel monkeys (R. L. Heberling, S. T. Barker, R. J. Helmke, G. C. Smith, and S. S. Kalter, Abstr. Annu. Meet. Am. Soc. Microbiol. 1976, S69, p. 216).

A previous study (13) reported no MPV-related proviral sequences in rhesus DNA; these studies, however, were conducted with a ^{3}H-labeled complementary DNA ([^{3}H]cDNA) probe that may not have represented the entire MPV genome. Studies in our own laboratory (data not shown) conducted with [^{3}H]cDNA probes also did not detect MPV-related provirus in rhesus DNA. However, these probes were shown to represent only a small portion of the MPV genome.

Of particular interest is the finding that the MPV-specific sequences that are found as endogenous provirus in rhesus are the same as are found as endogenous provirus in baboon, African green, and patas DNAs, the other species of Old World monkeys tested. The fact that these MPV-specific proviral sequences are the same in all four species is shown by the following observations. (i) $C_0t_{1/2}$ values and final extent of hybridization were identical for all four species examined (Fig. 1). (ii) T_m values of hybrids formed between MPV 60-70*S* RNA and rhesus, African green, baboon, and patas DNAs were all identical (Fig. 2). (iii) When the MPV sequences endogenous to rhesus DNA were removed by recycling against rhesus DNA (Fig. 4), the remaining RNA did not hybridize to baboon, African green, or patas DNA (Table 1). (iv) Mixing experiments of rhesus, African green, and baboon DNAs resulted in the same kinetics of hybridization as did the rhesus DNA alone, when hybridized with MPV 60-70*S* RNA. If each of these different species contained a different part of the MPV genome, hybridization of MPV RNA to the mixture of DNAs would result in approximately 54% hybridization and an increase in the $C_0t_{1/2}$ value by a factor of 3. However, this did not occur, again indicating that all these sequences are the same (Fig. 5). Further studies involving the expression of the conserved MPV-related sequences in Old World monkey DNAs are currently in progress.

ACKNOWLEDGMENTS

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