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Mason-Pfizer Virus RNA Genome: Relationship to the RNA of Morphologically Similar Isolates and Other Oncornaviruses

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The 60-70S RNA of Mason-Pfizer virus (MPV) was iodinated in vitro and used in both direct and competitive molecular hybridization studies. MPV proviral sequences are present at a frequency of approximately one to two copies per haploid genome in the DNA of experimentally infected human cells. By nucleic acid competition hybridization, MPV RNA was found to be indistinguishable from the RNA of a virus (X381) isolated from a rhesus mammary gland and from RNA isolated from the cytoplasm of AO cells (Parks et al., 1973) and HeLa cells (Gelderblom et al., 1974), both previously reported to produce MPV-related particles. No homology was observed, however, between MPV RNA and the RNA, or the DNA, from two clones of HeLa cells obtained from the American Type Culture Collection. Hybridization of MPV 60-70S RNA to the DNA of normal tissues of humans and to the DNA of 11 other species revealed that MPV is not an endogenous virus of any of these species. Competition hybridization revealed no detectable sequence homology between the RNA of MPV and the RNAs of simian sarcoma virus, murine mammary tumor virus, murine leukemia virus, BUdR-induced guinea pig virus, or avian myeloblastosis virus. These nucleic acid studies substantiate previous ultrastructural and immunological findings that MPV and morphologically similar isolates constitute a distinct group of oncornavirus.

Mason-Pfizer virus (MPV) was isolated from a tumor in the breast of an 8-year-old female rhesus monkey (10, 25) and has been propagated successfully both by co-cultivation with rhesus embryo cells and by cell-free infection of human and subhuman primate cell lines (1, 11, 21). Recently, particles have been detected in cultures of various rhesus tissues, that are morphologically and immunologically related to MPV (2). Furthermore, cell lines producing these particles have been established from a normal rhesus lactating mammary gland (X381) and from normal rhesus placental tissue (2, 41).

MPV has the biochemical and biophysical properties of an oncornavirus (24, 31, 34-36). It contains a 60-70S RNA with an apparent molecular weight of 8×10^6, that can be converted into subunits of molecular weight 2.8×10^6 (37). The reverse transcriptase and major internal protein of MPV (p27) both cross-react with disrupted X381 virus but do not cross-react with the known type C and type B oncornaviruses tested (38, 40, 41). Finally, the ultrastructure of MPV is different in several ways from both type B and type C RNA tumor viruses (10, 11, 22).

Recently, isolates from a number of human cells have been reported that have a morphology similar to MPV, immunologically cross-react with MPV, and have some nucleic acid sequences in common with MPV. These cell lines include HeLa clones as described by Gelderblom et al. (3, 16, 39) and AO cells (32), as well as a variety of other cell lines (8, 17-20, 28, 42, 43). Some of these cell lines, however, have been shown to contain HeLa markers (30).

We report here no detectable differences in nucleotide sequences among the RNAs of MPV, X381, and cytoplasmic RNA isolated from HeLa (16) and AO (32) cell lines producing particles morphologically similar to MPV. No MPV-related sequences, however, were found in the RNA or in the DNA of two clones of HeLa cells obtained from the American Type Culture Collection. Furthermore, no detectable nucleic acid sequence homology was observed between MPV 60-70S RNA and either the RNAs of a variety of type B and type C viruses or the DNAs of a variety of species.

MATERIALS AND METHODS

Virus and cells. MPV was grown in suspension cultures of the normal human lymphocyte cell line NC-37 (J. L. Smith Memorial Laboratories, Pfizer,

Inc.) and was purified from culture supernatant fluids by equilibrium density gradient centrifugation and concentrated as described previously (35). Avian myeloblastosis virus was purified from the plasma of leukemic chickens (Life Sciences, Inc.). Murine leukemia virus (Rauscher) was obtained from plasma of infected BALB/c mice (University Laboratories). Simian sarcoma virus 1 was grown in NC-37 cells. The above-mentioned viruses were obtained through the National Cancer Institute, Virus Cancer Program Office of Resources and Logistics. BUdR-induced guinea pig virus (14) was kindly supplied by J. Dahlberg. Murine mammary tumor virus was purified from culture supernatant fluid from primary cultures of RIII mammary tumors (9; P. Kimball, M. B. Truitt, G. Schochetman, and J. Schlom, J. Natl. Cancer Inst., in press). X381 virus was kindly supplied by M. Ahmed. The AO cell line was supplied by W. Parks. HeLa cells producing an MPV-related virus (3, 16, 39) were obtained from the laboratory of H. Bauer via G. Vande Woude. HeLa cells (clones CCL2 and CCL2.1) were obtained from the American Type Culture Collection, Rockville, Md. All cell lines were grown in Dulbecco-modified Eagle medium with 10% heat-inactivated fetal calf serum.

Tissues from various species were obtained from Pel Freeze (Rogers, Ark.) and R. Gillette, Meloy Laboratories.

Viral RNA extraction. The procedure used for isolation of viral RNA was as described previously (34, 37) and was the same for all viruses. Viral pellets were suspended in a solution of 0.01 M Tris-hydrochloride (pH 8.3), 0.1 M NaCl, and 0.01 M EDTA and lysed by the addition of sodium dodecyl sulfate (SDS) 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 incubated at 37 C for 30 min. This mixture was then extracted twice with 2 volumes of PCC (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, 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 adsorbance at 260 nm, was precipitated with ethanol as described above.

Cellular DNA extraction. Cell pellets were dissolved in 2 volumes of 5% sucrose in TNE, disrupted with 10 strokes of a tight Dounce homogenizer, and centrifuged for 10 min at 4 C at 3,000 × *g*. The resulting pellet was suspended in 20 volumes of 1% SDS and 1 M sodium perchlorate in TNE. The mixture was extracted twice with PCC and four times with chloroform containing 1% isoamyl alcohol. One volume of cold ethanol was added to the aqueous phase, and the DNA was then spooled out and dissolved in 3 mM EDTA. The DNA was then adjusted to 0.4 N NaOH and incubated at 37 C for 2 h. Nitrogen was bubbled through the DNA solution, and then the DNA was fragmented (6-7*S* as measured by alkaline sucrose gradient centrifugation) by sonic oscillation. The DNA solution was adjusted to 0.1 M Tris-hydrochloride (pH 8.3) and neutralized with HCl. The DNA was then reextracted with PCC and precipitated with 2 volumes of cold ethanol. After storage at −20 C the DNA was pelleted at 17,000 × *g* for 30 min at −20 C, dissolved in 0.003 M EDTA, and stored at −70 C.

Cytoplasmic RNA extraction. The supernatant of the 3,000 × *g* centrifugation (as described above) was recentrifuged at 8,000 × *g*, and the supernatant was adjusted to 1% SDS. This mixture was then extracted twice with PCC and twice with chloroform containing isoamyl alcohol. The aqueous phase was then adjusted to 0.4 M LiCl, and the nucleic acids were precipitated as described above. The RNA was then dissolved in 0.003 M EDTA and stored at −70 C until used.

Iodination of viral RNA. The iodination procedure is a modification of that of Commerford (13), worked out in collaboration with D. L. Kacian, Columbia University. Carrier-free ^{125}I was obtained from Amersham/Searle (pH 8 to 11, 100 mCi/ml). Thallium percholate, obtained from Alfa products, was dissolved at 4×10^{-3} M in 0.05 M sodium acetate buffer (pH 4.2). Sodium sulfite, obtained from Fisher, 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 (10 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 (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.

Hybridization reactions. Two different annealing reactions were used. (i) Approximately 2,000 counts/min of ^{125}I-labeled 60-70*S* RNA from MPV was annealed to various amounts of denatured (2 min, 100 C) nuclear DNA at a concentration of 5 mg/ml. The reaction conditions were adjusted to 0.12 M sodium phosphate (pH 6.8), 0.4 M NaCl, 0.1% (wt/vol) SDS, and 0.05 M EDTA. The mixture was incubated at 68 C to a C_ot of 35,000 (C_ot is defined as the product of the DNA concentration in moles of deoxyribonucleotides per liter and the incubation time in seconds). C_ot values are corrected to a standard of 0.12 M sodium phosphate to compensate for the change in rate due to varying salt concentrations (7). (ii) Approximately 1,000 counts/min of

^{125}I-labeled 60-70S RNA from MPV was annealed to 1 mg of denatured cellular DNA (2 min, 100 C) at a concentration of 3 mg/ml in 0.4 M sodium phosphate (pH 6.8) plus 0.05% (wt/vol) SDS. The reaction mixture was incubated at 68 C for varying amounts of time to reach the desired C_0t, adjusting for salt concentration (7). All products of the reaction mixtures were assayed for acquisition of RNase resistance. The hybridization reaction mixtures were adjusted to a DNA concentration of 50 μg/ml in 2× SSC (0.3 M NaCl plus 0.03 M sodium citrate). The sample was split in half, and to one fraction 50 μg of RNase A per ml and 100 U of RNase T_1 per ml were added. Both samples were incubated at 37 C for 30 min, precipitated with 10% trichloroacetic acid, and collected on nitrocellulose filters.

Competition hybridization. Hybridization procedure i, as described above, was employed. The competitor RNA was added to the hybridization mixture (containing 2,000 counts/min of MPV ^{125}I-labeled 60-70S RNA and 300 μg of DNA from MPV-infected NC37 cells) in varying amounts (0.1 ng to 800 μg). The hybridization reaction mixtures were incubated to a C_0t of 35,000 and assayed for the acquisition of RNase resistance. Competition was determined by comparing the percentage of hybridization in the presence of competitor RNA with that of the control sample (MPV ^{125}I-labeled 60-70S RNA hybridized to DNA of NC37 cells infected with MPV) after subtracting a background hybridization of 4.5% (MPV ^{125}I-labeled 60-70S RNA annealed to *Escherichia coli* DNA).

RESULTS

Hybridization of MPV RNA to DNA from MPV-infected cells. MPV 60-70S RNA was purified and iodinated in vitro to a specific activity of approximately 2×10^7 to 5×10^7 counts/min per μg. This RNA was 100% acid precipitable and 99% sensitive to RNase and banded as a sharp peak in cesium sulfate at a density of 1.63 g/ml. Iodinated MPV RNA was hybridized for various amounts of time to the DNA (3 mg/ml) that had been purified from MPV-infected NC-37 cells (Table 1). The hybrids were assayed by RNase sensitivity as described above. The DNA exhibited a $C_0t_{\frac{1}{2}}$ of approximately 3,000, indicating that the MPV-related proviral information is in the nonrepeated class of DNA and is present at approximately one to two copies per haploid cellular genome (33). The same $C_0t_{\frac{1}{2}}$ was obtained by using tritiated MPV 60-70S RNA prepared as described previously (37). With ^{125}I-labeled 60-70S RNA, a maximum hybridization of greater than 60% at a C_0t of 75,000 was achieved, whereas less than 6% hybridization was observed with the DNA of either uninfected NC37 cells or *E. coli* (Table 1). This assured us that the vast majority of ^{125}I-labeled RNA used in these studies was MPV specific and did not contain significant amounts of RNA or DNA of NC37 cells, the cells in which the virus was grown.

TABLE 1. *Hybridization of MPV ^{125}I-labeled 60-70S RNA to DNA of MPV-infected NC37 cells*[a]

Cellular DNA	C_0t	% Hybridization
NC37, MPV-infected	100	4.1
	600	14.4
	1,000	19.9
	5,000	32.8
	10,000	40.3
	50,000	55.4
	75,000	64.0
NC37, uninfected	10,000	4.6
	75,000	5.1
E. coli	10,000	4.1
	75,000	3.2

[a] 1,000 counts/min of ^{125}I-labeled 60-70S MPV RNA was annealed to 1 mg of cellular DNA from MPV-infected NC37 cells as described in Materials and Methods (technique ii). The hybridization mixture was incubated for appropriate periods of time to achieve the desired C_0t values. The counts per minute hybridized by a sample that was quenched immediately after boiling has been subtracted from all samples as a zero-time background. Hybrids were scored as indicated in Materials and Methods.

Saturation hybridizations. To develop conditions for a sensitive competitive hybridization assay, it was important to determine the minimum amount of DNA from MPV-infected NC37 cells needed to obtain maximum hybridization. Therefore, increasing amounts of DNA from MPV-infected NC37 cells (at a constant DNA concentration of 5 mg/ml) were annealed to a constant amount of ^{125}I-labeled MPV 60-70S RNA. The annealing reaction mixtures were incubated at 68 C to a C_0t of 35,000 and analyzed for RNase sensitivity. Higher C_0t values were not used to preclude the possible degradation of RNA. No substantial increase in percent hybridization was seen using DNA inputs greater than 0.3 mg, with the amount of ^{125}I-labeled 60-70S MPV RNA used (Fig. 1). A small amount of hybridization to the DNA of uninfected NC37 cells was detected, but this was only slightly greater than that detected with DNA from calf thymus or *E. coli* (Fig. 1). This DNA/RNA ratio, i.e., 0.3 mg of DNA from MPV-infected NC37 cells and 2,000 counts/min of ^{125}I-labeled MPV 60-70S RNA, was therefore employed in the competitive molecular hybridization experiments described below.

Competitive molecular hybridizations. Various amounts (0.1 to 1,000 ng) of unlabeled

MPV 60-70S RNA were added to the standard hybridization reaction between ^{125}I-labeled MPV 60-70S RNA and DNA from MPV-infected NC37 cells. Approximately 95% of the ^{125}I-labeled 60-70S RNA from MPV was competed out with 400 ng of unlabeled MPV RNA (Fig. 2). The midpoint of this curve was obtained with an input of 6.3 ng of MPV RNA. When the same competition reaction was performed with cytoplasmic RNA from NC37 cells infected with MPV (Fig. 2), competition was again observed to a level of approximately 95%, but this time with an input of 10^5 ng of cellular RNA. The midpoint of the competition reaction was achieved at approximately 9,500 ng of competitor RNA. A comparison of the midpoints using viral-versus-cytoplasmic competitor RNA indicates that approximately 0.07% of the total cytoplasmic RNA of MPV-infected cells is MPV related. This is in good agreement with the percentage obtained by C_rt analysis of the hybridization between cytoplasmic RNA

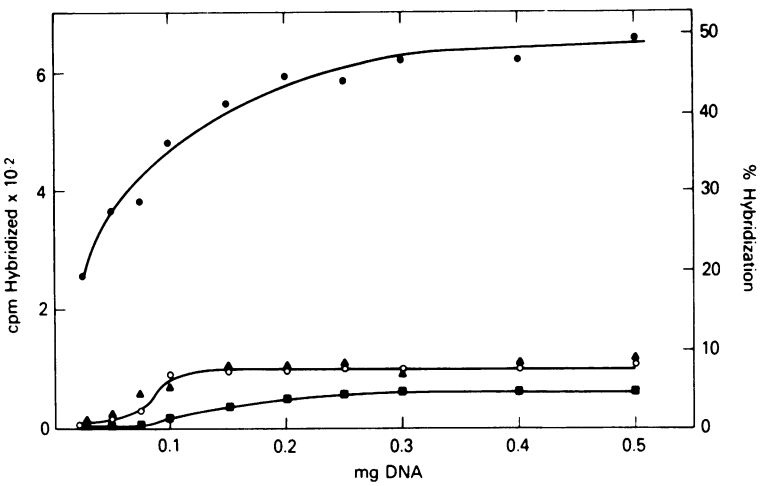

FIG. 1. *Saturation hybridization between ^{125}I-labeled 60-70S MPV RNA and cellular DNA. Two thousand counts per minute of ^{125}I-labeled 60-70S MPV RNA was hybridized to increasing amounts of cellular DNAs (technique i). All samples were hybridized to a C_0t of 35,000 and assayed for the acquisition of RNase resistance. Symbols: ●, DNA from MPV-infected NC37 cells; ○, DNA from uninfected NC37 cells; ▲, calf thymus DNA; ■, E. coli DNA.*

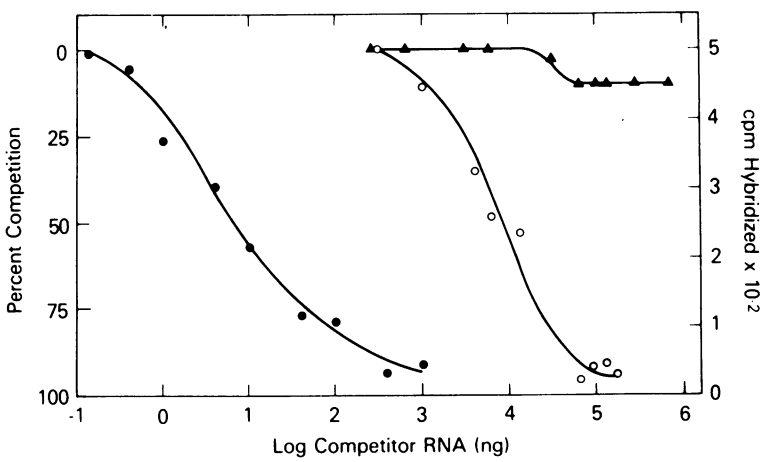

FIG. 2. *Competition hybridization of the MPV genome. Two thousand counts per minute of ^{125}I-labeled 60-70S MPV RNA was hybridized to 300 μg of DNA from MPV-infected NC37 cells. To this reaction, increasing amounts of unlabeled viral or cytoplasmic RNAs were added. The samples were incubated at 68 C in a solution of 0.12 M sodium phosphate (pH 6.8), 0.4 M NaCl, 0.1% SDS, 0.05 M EDTA to a C_0t of 35,000, and then assayed for acquisition of RNase resistance. Symbols: ●, MPV 60-70S RNA; ○, cytoplasmic RNA from MPV-infected NC37 cells; ▲, cytoplasmic RNA from uninfected NC37 cells.*

and ^{3}H-labeled DNA made from MPV RNA (12). Slight differences in slope of the competition reactions may be due to different lengths of competitor RNA molecules (4). A small amount of competition was observed with competitor RNA from uninfected NC37 cells up to an input of 5×10^{5} ng of RNA (Fig. 2). This competition may be due, in part, to the 4S, 4.5S, and 7S RNAs that are found to be associated with the 60-70S MPV genome (37).

The competition of the hybridization between the ^{125}I-labeled MPV RNA and the DNA of MPV-infected cells was then used to determine the extent of relatedness, if any, between MPV RNA and the 60-70S RNAs of type B and type C RNA tumor viruses. No competition was detected with viral RNA (from 0.1 to 1,000 ng) from the following type C viruses examined: avian myeloblastosis virus, murine leukemia virus (Rauscher), and simian sarcoma virus (Fig. 3). Furthermore, no competition of hybridization was observed with 0.1 to 1,000 ng of viral RNA from RIII murine mammary tumor virus of BUdR-induced guinea pig virus. Guinea pig virus has been shown previously to be similar to murine mammary tumor virus and MPV in several stages of morphogenesis (14). Reciprocal competition hybridization experiments have also been carried out in our laboratory with murine mammary tumor virus (26) and guinea pig virus (27). In these experiments, unlabeled MPV 60-70S RNA showed no detectable competition in hybridizations between (i) murine mammary tumor virus (RIII) radioactive 60-70S RNA and DNA of RIII mouse mammary tumors, or (ii) radioactive BUdR-induced guinea pig virus 60-70S RNA and DNA of guinea pig embryos. The RNAs from avian myeloblastosis virus, murine leukemia virus (Rauscher), and simian sarcoma virus were also tested for hybridizability and showed specific hybridization with their respective complementary DNA probes.

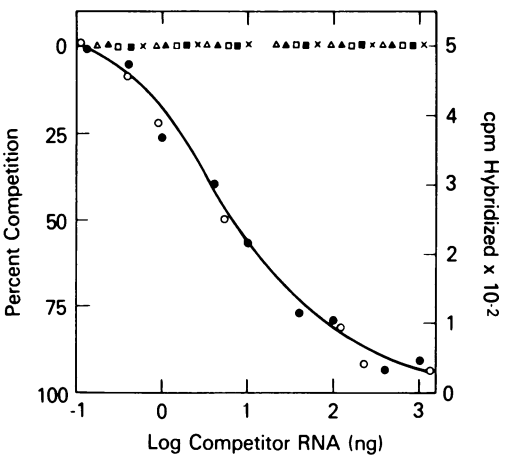

FIG. 3. *Competition of the MPV genome with viral RNAs. Competition hybridizations between ^{125}I-labeled MPV 60-70S RNA and DNA of MPV-infected NC37 cells were as described in the legend to Fig. 2. Competition reactions were performed with viral RNA as competitor from: ●, MPV; ○, X381; △, murine mammary tumor virus RIII; ▲, guinea pig virus B; □, simian sarcoma virus 1; ■, avian myeloblastosis virus; ×, murine leukemia virus (Rauscher).*

A virus (X381) morphologically similar to MPV was isolated from a cultured rhesus lactating mammary gland (2, 41). To determine the extent of nucleic acid sequence homology between MPV and X381, 0.1 to 1,000 ng of X381 RNA was added to the standard MPV competition hybridization reaction. As can be seen in Fig. 3, unlabeled X381 RNA showed the same degree of competition as did the homologous unlabeled MPV RNA. It can be concluded, therefore, that X381 and MPV share at least 95% sequence homology.

Experiments were then undertaken to determine the relatedness between MPV and morphologically similar isolates produced from HeLa cells (16) and AO cells (32). Sufficient quantities of unlabeled 60-70S RNA from particles produced by these cell lines could not be obtained due to their low level of virus production. We therefore determined whether any MPV-related sequences could be found in the cytoplasmic RNA of these cells. Increasing amounts of cytoplasmic RNA (from 1×10^{2} to 5×10^{5} ng) were introduced into the hybridization reaction between ^{125}I-labeled MPV RNA and the DNA from MPV-infected NC37 cells. Greater than 90% competition was obtained with the RNA from the HeLa clone described by Gelderblom et al. (16) and approximately 90% competition was obtained with the RNA from the AO cell line (32) (Fig. 4). Two to five times more cytoplasmic RNA from these two lines was required to reach the 50% point of the competition curve than RNA from the NC37 cells producing MPV. These two cell lines, therefore, contain RNA that is at least 90% related to the RNA of MPV. No MPV-related information was found, however, in the RNA purified from two clones of HeLa cells (CCL2 and CCL2.1) obtained from the American Type Culture Collection (Fig. 4).

The presence of MPV information in the RNA of one clone of HeLa cells (16) and its absence from two other clones of HeLa cells may be explained by one of two alternatives. First, the MPV-related information is present in the DNA of all HeLa cells and has been activated in

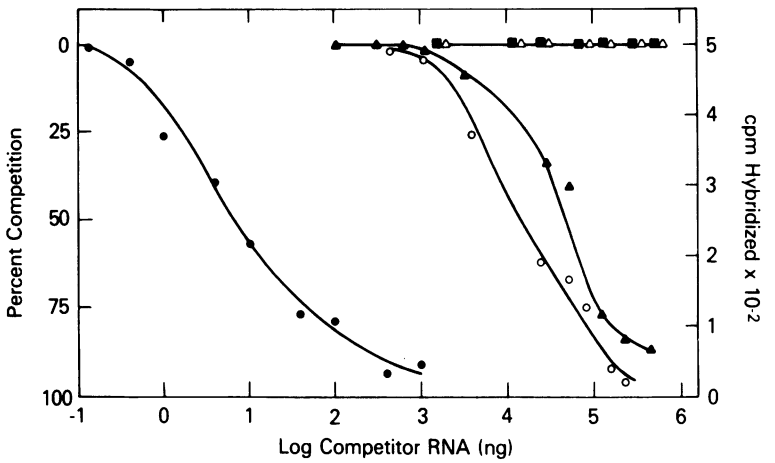

FIG. 4. *Competition hybridization of the MPV genome with cellular RNAs. Competition hybridizations were performed as described in the legend to Fig. 2. The following RNAs were used as competitor RNA:* ●, *MPV 60-70S RNA;* ○, *cytoplasmic RNA from HeLa cells (16);* ■, *cytoplasmic RNA from HeLa cells (ATCC CCL2);* △, *cytoplasmic RNA from HeLa cells (ATCC CCL2.1);* ▲, *cytoplasmic RNA from AO cells (32).*

certain clones. Second, the viral information is not in the DNA of all HeLa clones, and some clones contain MPV information that may have been introduced by laboratory manipulation. To answer this question, ^{125}I-labeled MPV 60-70*S* RNA was annealed to 300 μg of nuclear DNA from the three clones of HeLa cells in question, using technique i as described in Materials and Methods. The hybridization reaction was incubated to a C_0t of 35,000. Significant hybridization (greater than 80% of the control hybridization to MPV-infected NC37 cells) was detected with the clone of HeLa cells (16) producing MPV-related particles. However, no MPV-related sequences were detected in the DNA of the two HeLa clones from the American Type Culture Collection. This finding is evidence for the second explanation put forth above.

Hybridization of ^{125}I-labeled MPV RNA to the DNA of various species. The lack of appreciable hybridization of MPV ^{125}I-labeled RNA to the DNA of normal NC37 cells and two clones of HeLa cells demonstrates that MPV is not an "endogenous" virus of humans; i.e., it is not present as a provirus in the DNA of all cells of that species. ^{125}I-labeled MPV 60-70*S* RNA shows a low degree of hybridization to normal rhesus tissue (Table 2). It appears therefore that MPV, although indigenous to rhesus monkeys (2, 10, 21), is not a true "endogenous" virus of that species. Table 2 also demonstrates that MPV is not an "endogenous" virus of any of the 11 other species tested, nor is it appreciably related in nucleic acid sequences to an "endogenous" virus of any of these species.

TABLE 2. *Hybridization of MPV ^{125}I-labeled 60-70S RNA to cellular DNA of various species*[a]

Source	Tissue	% Hybridization
Human	NC37 cells infected with MPV	59.4
	NC37 cells, uninfected	6.7
	Liver	6.9
Monkey (rhesus)	Liver	13.1
Cat	Mammary gland (malignant)	1.0
Chicken	Embryo (whole)	6.3
Cow	Thymus	6.9
Dog	Mammary gland (normal)	5.1
Guinea pig	Pool of organs	4.3
Hamster	Pool of organs	5.0
Mouse	Embryo (whole)	3.2
Pig	Embryo (pool of organs)	2.2
Rabbit	Liver	4.9
Rat	Pool of organs	3.6
Sheep	Spleen	6.4

[a] 1,000 counts/min of ^{125}I-labeled 60-70*S* MPV RNA was annealed to 1 mg of cellular DNA from various sources, using technique ii as described in Materials and Methods. The hybridization reactions were incubated to a C_0t of 35,000 and assayed for RNase resistance.

DISCUSSION

The technique of competitive molecular hybridization used in these studies considers the entire viral RNA genome and avoids inconclusive results that may result if incomplete or preferential cellular DNA transcripts are employed. The disadvantage of molecular compe-

tition hybridization, in which a radioactively labeled RNA is hybridized to cellular DNA, is that the hybrid formation does not include all of the input radioactive RNA. This is most likely due to the different rate constants for DNA:DNA renaturation versus DNA:RNA renaturation (5). The extent of hybrid formation between MPV RNA and the DNA of MPV-infected NC37 cells (Table 1) is in agreement with the extent of hybrid formation between radioactively labeled 60-70*S* RNA or mRNA's and cellular DNAs in other systems (6, 23, 29). We therefore feel confident that the radioactively labeled MPV RNA that entered into a DNA:RNA hybrid is representative of the total MPV RNA genome.

Numerous reports have appeared recently concerning the detection of viruses morphologically related to MPV in a variety of human cell lines including clones of HeLa (16) and AO cells (32). The AO cells in question have been reported to contain HeLa-like markers (30). The greater than 90% sequence homology between MPV RNA and the RNA from these cells and the lack of any detectable MPV-related information in the RNA or in the DNA of two clones of HeLa cells from the American Type Culture Collection provide evidence that at least some isolates may be the result of either viral or cellular contamination.

The fact that MPV is not an "endogenous" virus of humans (Fig. 1, Tables 1 and 2) does not mean that any of the MPV-related isolates reported (8, 16-20, 28, 32, 42, 43) are not indeed human isolates. The possibility has not been ruled out that MPV is being transmitted in either rhesus or human populations by some mechanism other than a germinal provirus. There are lines of evidence that, in fact, support this possibility. Some MPV-related proviral sequences have been found in the DNA of rhesus monkeys, while the DNA of some organs contain additional sequences (W. Drohan, D. Colcher, G. Schochetman, and J. Schlom, submitted for publication).

MPV and the BUdR-induced guinea pig virus (14) have some features of morphogenesis similar to those of murine mammary tumor virus, such as the presence of intracytoplasmic A particles and budding with complete nucleoids formed. The studies presented here, however, show no detectable nucleic acid sequence homology between MPV, guniea pig virus, and murine mammary tumor virus. Nucleic acid sequence homologies of less than 5% of the viral genome could not be detected, however, by any of the methods employed here.

The nucleic acid studies reported here substantiate previous ultrastructural studies that MPV and morphologically related isolates constitute a distinct group of oncornavirus. A recent publication (15) suggests the classification of "genus candidate oncornavirus D" for MPV, to distinguish it from the established type A, B, and C oncornaviruses. Since MPV and morphologically related isolates appear to have an RNA genome distinct from those of any of the type B or type C viruses examined, our results are in agreement with this classification.

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