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Primate and Murine Type-C Viral Nucleic Acid Association Kinetics: Analysis of Model Systems and Natural Tissues

E. M. SCOLNICK, W. PARKS, T. KAWAKAMI, DAVE KOHNE, H. OKABE, RAY GILDEN, AND M. HATANAKA

National Cancer Institute, Bethesda, Maryland 20014, University of California at Davis, California 95616, Scripps Institute, La Jolla, California 92037, and Flow Laboratories, Bethesda, Maryland 20014

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Hybridization studies employing single-stranded ^{3}H-DNA transcripts of type-C viruses isolated from a woolly monkey or gibbon ape failed to detect nucleic acid sequences homologous to these viruses in the DNA from a variety of uninfected primate species. The possible significance of these results for the epidemiology of type-C viruses in primates is discussed.

Several hybridization methods exist which are useful in searching for type-C viral nucleic acid sequences in cellular DNA. One approach is to hybridize, in solution, labeled viral RNA with an excess of cellular DNA (10). However, in the case of many type-C and type-B viruses, it has not been possible to obtain the quantities of labeled viral RNA required to perform such studies. In these cases, single-stranded or double-stranded ^{3}H-DNA transcripts of viral RNA have been used instead. In the DNA of various avian cells, regardless of whether or not these cells were producing virus, multiple copies of type-C viral nucleic acid sequences have been detected (2, 3, 10, 22). The exact number of copies, however, has varied with the type of probe employed. In hybridization studies on mammalian type-C viruses, by using double-stranded or single-stranded DNA transcripts of viral RNA, multiple copies of type-C viral nucleic acid sequences have also been found in the DNA of uninfected natural tissues of both murine (7, 8) and feline origin (15). In the case of type-B viruses of mice, the widespread occurrence of viral nucleic acid sequences was found even in mice not previously suspected of harboring mouse mammary tumor virus (M-MTV) (23).

Single-stranded viral DNA transcripts prepared by using actinomycin D are more representative of the entire viral RNA genome than double-stranded DNA prepared in the absence of actinomycin D (18). Thus, we chose to employ such single-stranded transcripts of viral RNA in cases where we could not obtain adequate quantities of labeled viral RNA itself. By using such single-stranded transcripts, it was possible with probes from mouse type-B virus to extend the observation (23) that the number of copies of type-B viruses is similar in cells derived from single-cell clones expressing low or high amounts of type-B virus (12).

A type-C virus has been identified in the original tumor tissue from a woolly monkey (19), and several isolates have been made of type-C viruses from gibbon monkeys (9) from diverse locations and times, and the viruses were found to be closely related immunologically (13). Because nucleic acid hybridization studies had proven useful for epidemiological studies of type-C viruses in chickens, mice, and cats, we used the single-stranded ^{3}H-DNA transcript of the type-C viruses isolated from the woolly monkey and gibbon ape to investigate the distribution of these viruses in primates, and we compared the results with similar studies employing type-B and type-C viruses in mice as model systems. The present report describes the results obtained with such probes in infected and control primate tissues.

MATERIALS AND METHODS

Viruses. The type-C virus derived from a spontaneous fibrosarcoma of a woolly monkey (WLV) (19) was grown in a human cell line, A204, derived from a human rhabdomyosarcoma (D. J. Giard et al., J. Nat. Cancer Inst., in press). The WLV together with the woolly monkey sarcoma virus was grown in NC-37 lymphocytes and was obtained from Pfizer Laboratories, Maywood, N.J., through Jack Gruber of the Office of Resources and Logistics of the Virus Cancer Program, National Cancer Institute (NCI), Bethesda, Md. The type-C virus (9) derived from a gibbon lymphosarcoma was grown in SV80 cells, an SV40-transformed human fibroblast cell (20), and was the gift of George Todaro, NCI. M-MTV was obtained from RIII mouse milk and purified as previously described

(12). The Kirsten strain of murine leukemia virus (Ki-MuLV) was grown in NIH 3T3 fibroblasts, also as previously described (5).

Preparation of ^{3}H-DNA. Single-stranded ^{3}H-DNA synthesized in endogenous reactions from density-banded, sucrose gradient-banded virus preparations. Each reaction mixture was incubated at 37 C and contained in from 5.0 to 20.0 ml: 0.02 M Tris-hydrochloride, pH 7.8; 0.06 M magnesium acetate, 0.02%; Triton X-100; 2×10^{-3} M dithiothreitol; 0.06 M potassium chloride; 1×10^{-4} M TTP, dGTP, dATP (P. L. Biochemicals, Milwaukee, Wisc.); 1×10^{-6} M ^{3}H-dCTP 20 Ci/mmol (New England Nuclear Corp., Boston, Mass.); 60 μg of actinomycin D per ml (Calbiochem, La Jolla, Calif.), and from 500 μg to 2.0 mg of virus preparations. Reaction mixtures were incubated for either 8 to 12 h or for 1 h. The 8- to 12-h reactions were deproteinized and processed to obtain the single-stranded ^{3}H-DNA as previously described (5). The 1-h reactions were deproteinized and processed to obtain the ^{3}H-DNA bound to 60 to 70*S* RNA as previously described (17). All studies were performed with products synthesized in both ways.

In addition, the ^{3}H-DNA product homologous to the RNA of the type-C virus isolated from a woolly monkey was synthesized in reactions containing partially purified avian myeloblastosis virus (AMV) polymerase and 60 to 70*S* RNA derived from a mixture of WLV and the woolly sarcoma virus. The methods of preparation of RNA (17) and the AMV polymerase have been detailed elsewhere (14). The ratio of type-C to focus-forming virus in such preparations ranged from 10 to 100/1 as determined on normal rat kidney (NRK) cells (16). These reactions were incubated for 4 h at 37 C and contained components including actinomycin D as noted above, 3 to 5 μg of 60 to 70*S* RNA per ml, and 10 to 20 μg of partially purified AMV polymerase. The single-stranded ^{3}H-DNA was deproteinized and processed in the same way as the ^{3}H-DNA synthesized in the 8- to 12-h endogenous reactions described above. Technical considerations secondary to the low yields of labelled wooly virus from infected cell lines made it impossible to prepare sufficient ^{32}P woolly viral RNA to determine the percentage of the viral genome represented in any of the ^{3}H-DNA products. However, for several probes of Ki-MuLV RNA synthesized similarly, 80% of the viral RNA was transcribed into a 2- to 10-fold molar excess of ^{3}H-DNA.

Tissues. A pool of liver and spleen from a colony of C57Bl/6 Cum mice, a low leukemia incidence strain, was obtained from an original stock from R. Salerno (Microbiological Associates, Bethesda, Md.) and was kindly provided by Ron Gillette, Meloy Laboratories, Springfield, Va. Primate tissues were collected in duplicate from different sources. Gibbon liver and woolly monkey liver were obtained from a colony at Davis, Calif., from an apparently normal animal. Gibbon liver was also obtained from Woodward Research Corp., Herndon, Va. Rhesus and African green monkey liver and spleens were from animals sacrificed for the preparation of kidney cell cultures and were from Flow Labs, Rockville, Md., and Primate Import Corp., Long Island, N.Y. Marmoset and squirrel monkey liver-spleen pools were prepared from animals (one of each species) which died spontaneously at Tarpon Zoo (Tarpon Springs, Fla.) and were shipped frozen to Bethesda, Md. Human tumor tissue was obtained from the following: acute lymphoblastic leukemic cells were from Robert McAllister (Children's Hospital, Los Angeles, Ca.) and acute myelocytic leukemia cells were received through J. Gruber's office.

Cells. The sources of NIH 3T3, BALB/c mouse cell lines and NRK rat cell line have been described (1, 11). A primary culture of a feral mouse embryo cell was the gift of Janet Hartley and Wallace Rowe, National Institute of Allergy and Infectious Diseases, Bethesda, Md. The cell line L8A, clone 11, derived from the Sykes mouse breast tumor line from a C57 × A F_1 hybrid mammary tumor which expresses M-MTV antigen and produces M-MTV particles, was previously described (12).

A nonproducer rat cell line derived from NRK cells transformed by the woolly sarcoma virus was obtained by microtiter and soft agar cloning procedures similar to those described for another strain of the woolly monkey sarcoma virus (16). This nonproducer cell line contained no detectable particles by electron microscopy, released no particles banding at 1.15 to 1.18 g/cm^3 in sucrose as determined by [^{3}H]uridine labeling, released no detectable viral reverse transcriptase in 1,000-fold concentrates of cell culture fluid supernatants, and contained intracellularly no woolly P30(gs) antigen by radioimmunoassay (13). Superinfection of the nonproducer cells with helper, nontransforming mouse or primate type-C virus rescued the woolly monkey sarcoma virus, as noted for our other isolate of the woolly monkey sarcoma virus (16).

Cultures of chimpanzee cell fibroblasts were obtained from Flow Laboratories, (Rockville, Md.). HBT-3, a human cell line derived from the tumor tissue of a patient with mammary adenocarcinoma (4) was provided by Paul Price, Microbiological Associates (Bethesda, Md.). All cell lines were grown in Eagle medium with amino acid and vitamin supplements as described by Dulbecco and Vogt supplemented with calf serum (murine and rat cells) or fetal calf serum (all others).

Hybridization. Total cellular RNA and DNA were prepared from tissues or cells by procedures previously described (5). RNA·^{3}H-DNA hybridization was performed and analyzed with the use of S1 nuclease as previously described (5), except that hybridization was performed at 66 C with 0.40 M NaCl instead of at 41 C with 39% formamide. DNA·^{3}H-DNA hybridization was also performed as previously described, except that reactions were incubated at 66 C in 0.75 M NaCl with no formamide instead of at 50 C with formamide. C_0t values were corrected for salt concentration to 0.18 M phosphate as described by Britten and Kohne (6).

RESULTS

Murine viral ^{3}H-DNA probes with murine cellular DNA sequences. To characterize viral ^{3}H-DNA probes, several natural tissues and cell

culture DNAs were examined for sequences homologous to single-stranded probes from the Kirsten strain of murine type-C virus, RIII M-MTV (type-B virus) and the type-C viruses derived from a woolly monkey and gibbon ape. The results with the mouse type-C virus and type-B virus are shown in Fig. 1 and 2.

As shown in Fig. 1, the ^{3}H-DNA transcript from M-MTV RNA hybridizes readily to all mouse tissue DNAs examined including L8A clone 11 DNA, BALB/c 3T3 DNA, NIH 3T3 DNA, and DNA derived from a feral mouse cell culture line. In each case, the rate and extent of the reaction is approximately the same. At the

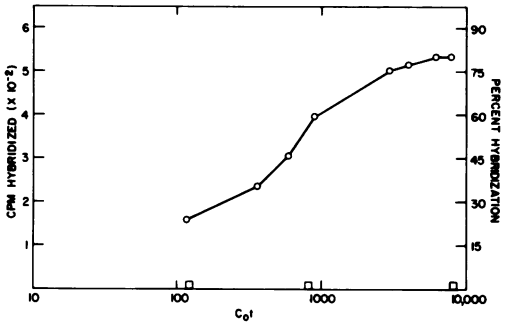

FIG. 1. *Hybridization of mouse mammary tumor virus (M-MTV) ^{3}H-DNA with various mouse cellular DNAs. Each reaction contained per 0.10 ml 700 trichloroacetic acid counts/min of ^{3}H-DNA from M-MTV. A background of 20 counts/min in the absence of DNA has been subtracted from all values. Details of hybridization methods are in Materials and Methods.* □, *DNA from calf thymus, HBT-3 cells, and normal rat kidney cells;* ○, *DNA from L8A clone 11 or NIH 3T3 or from BALB 3T3 or wild-mouse cell.*

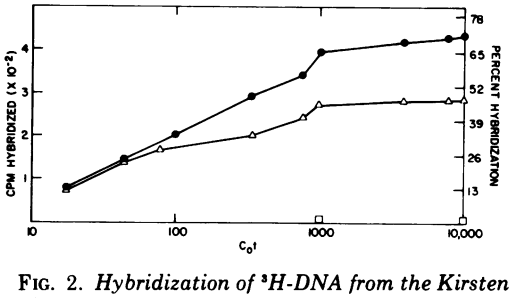

FIG. 2. *Hybridization of ^{3}H-DNA from the Kirsten strain of murine leukemia virus (Ki-MulV) to mouse cell DNAs. Each reaction mixture contained 600 trichloroacetic acid counts/min of ^{3}H-Ki-MuLV DNA product. Hybridization reaction mixtures were as described in Materials and Methods. A background of 43 counts/min in the absence of DNA has been subtracted from all values.* □, *Calf thymus DNA, HBT-3 DNA, and normal rat kidney DNA;* △, *NIH 3T3 or wild-mouse cell DNA;* ●, *DNA from BALB 3T3, C57 black, or NIH 3T3 infected with Ki-MuLV.*

maximal extent of the reaction, 75% of the ^{3}H-DNA is resistant to S1 nuclease. The 0.5 C_0t value for the observed reaction is approximately 5×10^2 to 7×10^2 for all of the strains of DNA tested. As controls for the positive reactions with mouse cells, rat cell DNA, calf thymus DNA, and human DNA from the HBT-3 cell line gave no detectable hybridization (fewer than 25 counts/min over a counter background of 25 counts/min).

A similar pattern of results was obtained with the Ki-MuLV type-C viral probe (Fig. 2). All of the mouse cell DNAs tested showed positive reactions. DNA from BALB/c, C57 Bl/6, and NIH 3T3 cells producing KiMuLV gave essentially indistinguishable rates and extents of the hybridization reaction. In contrast to the above, two cell lines, NIH 3T3 and the feral mouse line, gave significantly lower final extents of hybridization, although the early rates were similar to the other mouse cells tested. Again nonmurine DNAs from rat, human, and calf thymus gave no detectable hybridization with the murine type-C ^{3}H-DNA transcript.

Woolly monkey type-C viral ^{3}H-DNA probes with primate cellular DNA sequences. To compare murine viral ^{3}H-DNA-murine cellular DNA association kinetics with primate viral ^{3}H-DNA and primate cellular DNA association kinetics, woolly monkey and gibbon type-C viral ^{3}H-DNA transcripts were hybridized to a variety of cellular DNAs. The results with the woolly monkey probe are shown in Fig. 3. Sheared DNA from human cells infected with woolly monkey type-C virus or gibbon type-C virus readily hybridizes to the woolly monkey viral ^{3}H-DNA probe, indicating a significant degree of homology between woolly monkey and gibbon viral nucleic acids. At completion of the homologous observed reaction, 83% of the input counts were resistant to S1 nuclease digestion, and the 0.5 C_0t of this reaction was 5×10^2. However, in contrast to the "uninfected" mouse cellular DNA, with ^{3}H-DNA from mouse type-C virus, DNA from liver and spleen from clinically normal woolly monkey, gibbon, marmoset, rhesus, African green monkey, chimpanzee, and human acute lymphoblastic and myelocytic leukemic DNAs failed to hybridize (<5% of input counts/min) to the radioactive probe. Control tissues included rat cellular DNA and calf thymus DNA, both of which failed to hybridize significantly with the transcript.

Biological analysis of woolly monkey viral ^{3}H-DNA probe. To characterize further the woolly monkey viral ^{3}H-DNA probe, a biological system which might represent a single copy or

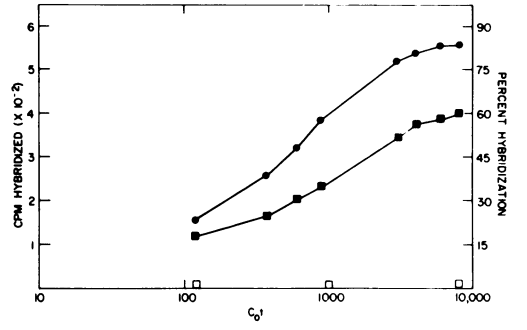

FIG. 3. *Hybridization with ^{3}H-DNA from woolly monkey type-C virus and various cellular DNAs. Each hybridization reaction contained 700 trichloroacetic acid counts per min per indicated point. Reactions were performed as detailed in Materials and Methods. The background in the absence of DNA was 34 counts/min and has been subtracted from all values. ●, DNA from NC-37 infected with woolly monkey leukemia and sarcoma viruses; ■, DNA from NC-37 infected with gibbon leukemia virus. □, DNA from calf thymus or normal rat kidney cells; DNA from NC-37; DNA from one case each of human acute myelocytic and lymphoblastic leukemia; DNA from liver and spleen from woolly monkey, gibbon ape, marmoset, rhesus monkey, African green monkey, and chimpanzee.*

less of woolly type-C viral information was analyzed. RNA and DNA sequences were studied from nonproducer rat cells transformed with woolly monkey sarcoma virus under conditions which ensured that the cell arose as a consequence of a single hit by the defective woolly monkey type-C transforming virus (16). The rationale for the search for woolly monkey type-C viral sequences in such sarcoma virus-transformed nonproducer cells was based on earlier studies in the murine model systems which show that nonproducer cells of both the HT-1 strain of Moloney sarcoma virus and the Kirsten sarcoma virus contain sequences homologous to murine type-C "helper" virus (5, 17). Therefore, the woolly nonproducer was studied under comparable conditions to determine whether sequences homologous to the "helper" woolly monkey type-C virus were present in these nonproducer cells. As controls, RNA from nontransformed rat cells producing infectious "helper" woolly monkey type-C virus, and RNA from rat cells producing rat type-C virus were also examined (Fig. 4 and 5). Both the nontransformed rat cell producing woolly type-C virus and the nonproducer rat cell transformed by the woolly sarcoma virus contain RNA (Fig. 4) and DNA (Fig. 5) homologous to the single-stranded ^{3}H-DNA copy of the nontransforming woolly monkey type-C virus. RNA or DNA from the rat cell producing rat type-C virus failed to hybridize with the same ^{3}H-DNA transcript of the woolly monkey type-C viral RNA. Thus, the woolly monkey sarcoma genome present in the HT-1 type of

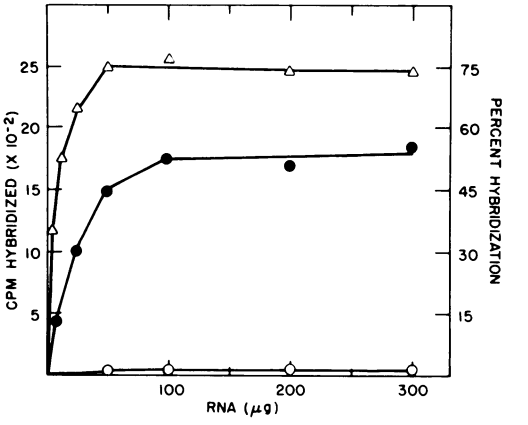

FIG. 4. *Hybridization of various rat cell RNAs with ^{3}H-DNA from woolly monkey type-C virus. Each hybridization reaction contained 3,200 trichloroacetic acid counts/min of ^{3}H-dCTP-labeled DNA product synthesized with the avian myeloblastosis virus polymerase and 60 to 70S RNA of the woolly monkey type-C virus. The virus preparation contained no detectable focus-forming (sarcoma) virus as assayed on either normal rat kidney cells or marmoset lung fibroblasts. The background in the absence of RNA was 43 trichloroacetic acid counts/min and has been subtracted from all values. ○, Total cellular RNA from rat cells producing rat type-C virus; △, total cellular RNA from rat cells producing woolly monkey type-C virus; ●, total cellular RNA from nonproducer rat cells transformed by woolly monkey sarcoma virus.*

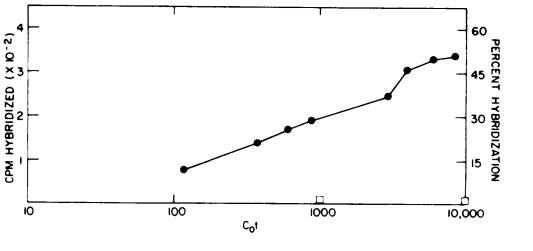

FIG. 5. *Hybridization with ^{3}H-DNA from woolly monkey type-C virus with DNA from woolly monkey sarcoma virus-transformed nonproducer cell. Each reaction mixture contained 700 trichloroacetic acid counts/min of ^{3}H-DNA. The background of 52 counts/min in the absence of DNA has been subtracted from all values. Details of hybridization are as described in Materials and Methods. ●, DNA from woolly monkey sarcoma virus-transformed nonproducer rat cell. □, DNA from calf thymus or rat cells producing rat type-C virus.*

nonproducer rat cells contains nucleic acid sequences homologous to the woolly monkey helper virus, and the ^{3}H-DNA transcript used will detect the sequences in the DNA of such cells. A comparable level of hybridization was not detected in liver and spleen DNA from a variety of uninfected primates including human tumor cell DNAs.

Gibbon type-C viral ^{3}H-DNA probe with primate tissues. Similar experiments were performed with a ^{3}H-DNA transcript of the type-C virus isolate from a gibbon ape. In this case the hybridization of the single-stranded ^{3}H-DNA to cellular DNA was monitored by fractionating the single-stranded ^{3}H-DNA from the double-stranded ^{3}H-DNA·cellular DNA by hydroxyapatite column chromatography (6, 7). Where indicated, the percentage of the cellular DNA annealing to itself was monitored in the same fractions by optical density at 260 nm. The results are summarized in Table 1. The ^{3}H-DNA probe again readily hybridized to DNA from cells infected with the gibbon virus, or to 60 to 70*S* RNA prepared from the gibbon virus. In contrast, uninfected gibbon liver DNA failed to give appreciable hybridization, even though the cellular DNA associated 83% to itself. Similarly, DNA from human placenta, fresh ALL or AML cells, or cells from rat, rabbit, or guinea pig liver failed to hybridize to the ^{3}H-DNA transcript.

DISCUSSION

The present work examined the DNA from several murine and primate tissues for sequences homologous to ^{3}H-DNA copies of mouse or primate RNA-containing type-B or type-C viruses. By using a ^{3}H-DNA copy of M-MTV RNA or Ki-MuLV RNA, the DNA from a variety of mouse cells, even those not producing type-B or type-C virus, gave readily detectable hybridization reactions. Interestingly, with the type-C ^{3}H-DNA probe, DNA from two of the mouse strains tested, NIH 3T3 and a feral mouse culture, had final extents of reaction that were lower than those noted with DNA from RIII, BALB/c, or C57 Bl/6 mouse DNA. The difference we noted with the NIH 3T3 DNA was not detected with double-stranded ^{3}H-DNA probes from Ki-MuLV (7, 8), indicating that the single-stranded transcript may be a more sensitive indicator of differences in cellular DNA sequences. The difference in viral DNA content within the murine species might represent structural differences (partial homology) between the viral genomes present in National

TABLE 1. *Hybridization of gibbon type-C virus ^{3}H-DNA with various DNAs*[a]

Source of unlabeled nucleic acid (NA)	Unlabeled NA/ ^{3}H-GaLV DNA	Unlabeled NA (C_0t)	Reassociation of unlabeled NA (%)	Hybridization of ^{3}H-GaLV DNA (%)
SV80 (GaLV) DNA	4×10^6	100		51.0
SV80 (GaLV) DNA		1,000		78.0
SV80 (GaLV) DNA		5,000	83	85.0
Gibbon DNA	4×10^6	288	60	0.5
Gibbon DNA	4×10^6	1,580	75	2.0
Gibbon DNA	4×10^6	5,000	83	2.0
Human placenta DNA Human AML, ALL DNA Human SV80 uninfected cell	6×10^6	1,900	70	4.0
Rat liver DNA	10^7	4,900	87	2.0
Guinea pig liver DNA	8×10^6	4,900	78	5.1
Rabbit liver DNA	6×10^6	3,200	82	4.2
GaLV-70*S* RNA	56	.012		81.0

[a] Hybridizations were performed as described in Materials and Methods, except that analysis of the hybridization reactions was done by hydroxyapatite column chromatography in water-jacketed columns at 60 C as described by Gelb et al. (8). This allowed analysis of the percentage of reassociation of unlabeled cellular DNA to itself as well as the ^{3}H-DNA present in the double-stranded form. Each reaction had approximately 500 trichloroacetic acid counts per min per assay point. Single-stranded DNA was eluted with 0.14 M sodium phosphate, 0.4% sodium dodecyl sulfate (SDS), and double-stranded DNA in 0.40 M sodium phosphate and 0.4% SDS. The specific activity of the ^{3}H-CH_3-TTP used to synthesize the DNA was 46 Ci/mmol; the specific activity of the ^{3}H-DNA product was 18,000 counts per min per ng. The percentage of hybridization of ^{3}H-gibbon lymphosarcoma (GaLV) DNA are values with only the counter background (20 counts/min) subtracted.

Institutes of Health (NIH) Swiss and feral mouse cells, and the Kirsten murine leukemia virus probe, and they might also be related to the fact that neither of these cells is inducible to form a complete type-C virus, although type-C viral proteins are present in each of these cells (11, unpublished data). The latter alternative would suggest that NIH Swiss and feral mouse cells lack an essential *viral* component necessary for complete virus expression. Additional work will be necessary to clarify and distinguish these alternatives. It is interesting that the ^{3}H-DNA copy of M-MTV showed identical final extents of reaction with BALB 3T3 and NIH 3T3 cellular DNA. Similar observations with the type-C DNA probes have been made independently and analyzed more extensively by using ^{3}H-DNA from AKR murine type-C virus by Chattopadhyay et al (Proc. Nat. Acad. Sci. U.S.A., in press).

Clearly, murine cells, even noninducible or wild-mouse cells, have DNA homologous to a type-C virus or type-B virus of mice. In the case of the type-C viruses isolated from primates, markedly different results were obtained. The ^{3}H-DNA transcript of the type-C virus isolated from gibbons or woolly monkeys readily hybridized to the cellular DNA from cells infected with the same virus, and the association kinetics indicated multiple copies of the virus in these exogenously infected cells. In contrast, the DNA from natural uninfected primate tissue and a variety of lower mammalian tissues failed to hybridize to these ^{3}H-DNA transcripts.

Although the theoretical kinetics of association imply that a single copy of viral nucleic acid sequences could have been detected, to assess the actual sensitivity of the assay using the single-stranded woolly monkey viral ^{3}H-DNA associating to cellular DNA, an analysis was made of the nucleic acid sequences in a nonproducer rat cell transformed by woolly monkey sarcoma virus. Based on Poisson distribution, such nonproducer cells are the result of an interaction with a single, infectious transforming virus during titration of the focus-forming virus on rat cells and, thus, might be a biological model for detection of a single DNA copy, in addition to the theoretical control of the rate of hybridization of unique sequence cellular DNA. Accordingly, an analysis of the DNA of the nonproducer cells was made and revealed sequences homologous to the nontransforming type-C woolly monkey virus. Thus, the actual viral ^{3}H-DNA probe used could have detected what probably is a single copy of woolly monkey type-C virus information in cellular DNA. We cannot exclude, however, that the woolly monkey type-C information in the nonproducer cell was reiterated to form multiple copies. Nevertheless, all uninfected natural primate tissues examined contained no detectable DNA sequences which hybridized to this viral probe. We conclude that these tissues contain less than one complete copy of either of the type-C viruses derived from a woolly monkey fibrosarcoma or gibbon ape lymphosarcoma.

The lack of reaction with the uninfected primate DNAs examined raises several questions about the origin of these type-C viruses in primates. The original primate tumors from which these viruses were isolated arose in widely different geographical locations, at different times, and had P30 (gs) and reverse transcriptase proteins which are immunologically closely related as determined by immunoassays (13). However, the present study suggests that these "primate" type-C viruses are not as widespread in their presumed natural hosts as the murine and chicken viruses are in their respective species. Two tentative explanations of this conclusion are possible. First, in contrast to mice, all primate cells may not contain complete copies of type-C viruses. Alternatively, the viruses thus far isolated from the woolly monkey and gibbon ape may have originated in another species and only rarely, if ever, undergo vertical transmission in primates. Other isolates of type-C viruses in primates and study of their epidemiology in natural primate tissues will be necessary to resolve these possibilities.

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