Differential Distribution of Mouse Mammary Tumor Virus-Related Sequences in the DNA's of Rats [1, 2]

W. Drohan [3] *and* J. Schlom [3, 4, 5]

ABSTRACT—Radioactively labeled mouse mammary tumor virus (MuMTV) 60-70S RNA, obtained from virions grown in both murine and feline cells, was employed in molecular hybridization experiments to detect MuMTV-related sequences in the DNA's of rats (*Rattus norvegicus*). With the use of relaxed conditions of hybridization and assay for RNA-DNA duplexes, all strains of laboratory rats and feral rats examined were shown to possess endogenous MuMTV-related DNA sequences in the low repetitive range. These sequences were related to approximately 20% of the MuMTV genome and exhibited a melting temperature (T_m) approximately 5° C lower than MuMTV-specific proviral sequences in murine (*Mus musculus*) DNA's. Certain colonies of the F344 strain of rat (Fischer) contained animals whose DNA's possessed additional MuMTV-related sequences. These sequences were related to the non-germ-line-transmitted, tumor-associated (TA) sequences of the highly oncogenic MuMTV (C3H). They were found in the DNA of some F344 rats and a cloned established F344 rat embryo cell line at a frequency of approximately one copy per haploid genome and exhibited a T_m 9° C lower than that of hybrid duplexes formed between radioactive MuMTV TA-sequence RNA and C3H mouse mammary tumor DNA. The DNA's of rats, therefore, contained two sets of sequences that were related to sequences of the MuMTV genome: One set was germ-line transmitted, whereas the other set appeared to be transmitted in some rats via a non-germ line or infectious process.—JNCI 62: 1279-1286, 1979.

We have recently demonstrated that different strains of mice may differ both qualitatively and quantitatively in MuMTV-specific proviral sequences (*1*). Whereas MuMTV is transmitted as a germinal provirus in GR mice (*1, 2*), the comparably high oncogenic MuMTV of C3H/He and RIII mice are not (*3*). The MuMTV sequences that are non-germ-line transmitted in C3H/He mice have recently been isolated by recycling radioactive MuMTV 60-70 S RNA against an excess of C3H liver DNA from apparently normal mice. These MuMTV TA sequences hybridize to the DNA of early occurring C3H/He and RIII mammary tumors but not to the DNA of normal C3H/He and RIII organs (*3*).

In view of the varied distribution of MuMTV proviral sequences in the mouse, we set out to determine if MuMTV-related proviral sequences are present in the DNA of the laboratory rat (*Rattus norvegicus*), inasmuch as this species is widely used in mammary cancer research (*4*). The occurrence of spontaneous mammary tumors in various rat strains ranges from less than 1 to 30% of all tumors (*5, 6*). Mammary tumors in the F344 rat have been reported to be as high as 11.5% in males and 19.6% in females (*7*).

We report here that MuMTV-related sequences are endogenous in the DNA's of all laboratory strains of rats tested as well as in feral rats. Certain animals of various colonies of F344 rats (*8*), however, contain additional proviral sequences related to the non-germ-line-transmitted MuMTV TA sequences found in the early mammary tumors of C3H/He and RIII mice. A cloned F344 rat embryo cell line that contains these sequences has also been characterized.

These findings demonstrate that all rats contain endogenous MuMTV-related DNA sequences, whereas some rats may be infected with an MuMTV-related virus.

MATERIALS AND METHODS

Viruses.—MuMTV was obtained from supernatants of the C3H mouse mammary tumor cell line Mm5mt/c_l (*9*) and was designated MuMTV(C3H). Propagation of cells and purification of supernatants were as previously described (*3, 10*).

The infection of feline embryo kidney cells (CrFK) with MuMTV(C3H) and the production of purified MuMTV 60-70S [^{3}H]RNA from supernatants of these cells have been described (*11*). The absence of type C virus by electron microscopy, radioimmunoassays, molecular hybridization, and DNA polymerase assays in the MuMTV preparations obtained from both feline cells and Mm5mt/c_l cells has been detailed previously (*3, 10, 11*). Murine leukemia virus from C3H mice was obtained and purified as previously described (*3*). Viral 60-70S RNA's from rat leukemia and sarcoma (Kirsten) type C viruses and a cloned FRE cell line were supplied by Dr. E. Scolnick, Laboratory of Tumor Virus Genetics, Division of Cancer Cause and Prevention, NCI. The FRE cell line was subjected to karyo-

---

ABBREVIATIONS USED: cpm = counts per minute; FCRC = NCI Frederick Cancer Research Center; FRE = Fischer rat embryo; MuMTV = murine mammary tumor virus(es); NaPB = sodium phosphate buffer; NCI = National Cancer Institute; NIH = National Institutes of Health; SLS = sodium lauryl sarcosinate; SSC = 0.15 M NaCl and 0.015 M Na citrate, pH 7.0; TA = tumor-associated; TCA = trichloroacetic acid; T_m = melting temperature.

---

[1] Received August 11, 1978; accepted October 30, 1978.

[2] Supported in part by Public Health Service contract N01-CP43223 from the National Cancer Institute.

[3] Laboratory of Viral Carcinogenesis, Division of Cancer Cause and Prevention, National Cancer Institute, National Institutes of Health, Public Health Service, U.S. Department of Health, Education, and Welfare, Bethesda, Md. 20014.

[4] *Address reprint requests to* Dr. Schlom.

[5] We thank Ms. S. Hamilton, Ms. E. Clark, Mr. L. Dreyer, Ms. D. Joiner, Ms. G. Gibson, Dr. D. Colcher, and Dr. D. Howard for their assistance.

type and immunologic analyses and was shown to be rat and not mouse.

Animals.—Hamsters [stock Lak:LVG(SYR)] were obtained from Lakeview Hamster Colony, Wilmington, Massachusetts. Guinea pigs (Wright strain 2) were received from the FCRC, Frederick, Maryland. SD (Sprague-Dawley), W (Wistar), ACI (Irish), LEW (Lewis), OM (Osborne-Mendel), and F344 (Fischer) rats were obtained through the Small Animal Section, Veterinary Resources Branch, NIH, Bethesda, Maryland. F344 rats were also obtained from the FCRC; Mason Research Institute, Worcester, Massachusetts; Charles River Breeding Laboratories, North Wilmington, Massachusetts; Sprague-Dawley, Inc., Madison, Wisconsin; and Harlan Industries, Inc., Indianapolis, Indiana. Tissues from germfree F344 rats (7) were supplied by Ms. M. Sacksteder, Leo Goodwin Institute for Cancer Research, Ft. Lauderdale, Florida. Feral rats were supplied by Mr. H. Lester, District of Columbia Bureau of Community Hygiene, Vector Control Division, Washington, D.C.; and Dr. R. Marsh, University of California, Davis, California.

F344 rats from NIH were tested for antibodies to adventitious viruses by either hemagglutination inhibition or complement fixation. The tests were performed at Microbiological Associates, Bethesda, Maryland, via the Office of Program Resources and Logistics, Virus Cancer Program, NCI. We tested for the following viruses: mouse pneumonia virus, reovirus, mouse encephalomyelitis virus, Kilham rat virus, Toolans H-I virus, Sendai virus, mouse adenovirus, mouse hepatitis virus, lymphocytic choriomeningitis virus, and rat coronavirus. All results were negative.

Purification of viral RNA's.—The procedure for the isolation of viral 60-70S RNA's was as described previously (*3*) and was the same for all viruses. The preparation of MuMTV(C3H) 60-70S [^{3}H]RNA was as described previously (*11*). The procedure for the iodination of MuMTV(C3H) 60-70S RNA was a modification of that of Commerford (*12*) as described by Colcher et al. (*13*). Iodinated RNA had a specific activity of approximately 2×10^7 cpm/μg, was 99% acid-precipitable, and was 98% RNase-sensitive.

Purification of cellular DNA.—DNA was extracted from tissues by means of the procedure of Drohan et al. (*3*). The sedimentation coefficient of the DNA, as determined by alkaline sucrose sedimentation, was between 6 and 9S.

Molecular hybridization.—Cellular DNA (first boiled in 0.001 M NaPB for 1 min in a bath of ethylene glycol), at a final concentration of 3 mg/ml in 0.4 M NaPB (pH 6.8) and 0.05% sodium dodecyl sulfate, was mixed with MuMTV(C3H) 60-70S ^{125}I-labeled RNA or [^{3}H]RNA (6,000 cpm/ml) and incubated at 68° C at variable times until the desired C_0t (concentration of nucleotides, in moles/liter, times the incubation time, in seconds) had been attained. C_0t values are corrected to 0.12 M NaPB (*14*). At appropriate times, aliquots of 0.17 ml (500 μg DNA and 1,000 cpm RNA) were removed and diluted into 10.5 ml two times SSC to a final concentration of 50 μg/ml. RNase A and RNase T_1 were added to one-half the sample at final concentrations of 15 μg/ml and 3 U/ml, respectively. Both tubes were then incubated at 37° C for 30 minutes. The samples were chilled for 10 minutes, and TCA was added to a final concentration of 10%. After being kept on ice for 30 minutes, the acid-insoluble material was collected on 0.45-μ nitrocellulose filters (Gelman Instrument Co., Ann Arbor, Mich.). A zero time control aliquot, which was boiled for 1 minute in a bath of ethylene glycol prior to the addition of RNase, was included in all experiments. The radioactivity (cpm) present in the RNase-treated portion of this sample was deducted from the amount obtained in all other samples. We determined the percent hybridization by dividing the cpm present in the RNase-treated sample by the cpm present in the untreated sample.

Relaxing conditions of hybridization.—Relaxed conditions of molecular hybridization and assay for radioactive RNA-DNA duplexes were detailed previously (*15*). Conditions of hybridization were the same as those described for standard hybridization conditions except that the hybridization was done at 54° C. To assay the RNase-resistant RNA-DNA duplexes, we diluted the sample as described above, but the final salt concentration for the RNase procedure was eight times SSC. RNase A (15 μg/ml) and RNase T_1 (3 U/ml) were added, and the solution was incubated for one-half hour at 37° C. RNase sensitivity of single-stranded radioactive RNA was the same in two times SSC and eight times SSC. The collection of samples on nitrocellulose filters and processing of filters were as described in (*3*).

Thermal stability of DNA-RNA hybrids.—The technique for the assay of the thermal stability of the DNA-RNA hybrids was as described in (*3*). The hybridization mixture contained 1 mg cellular DNA and 6,000 cpm MuMTV ^{125}I-labeled RNA. After hybridization to a C_0t of 20,000, the DNA was diluted to 50 μg/ml, and the solution was applied to a 4-ml column of packed hydroxyapatite 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. 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 increments of 5° C until a temperature of 100° C was attained. We measured the thermal dissociation of the DNA-RNA hybrids by monitoring the optical density at 260 nm in the effluent fractions and that of the ^{125}I-labeled RNA-DNA hybridization by determining the TCA-precipitable cpm in the effluent fractions. We have previously demonstrated (*15*) that these are the proper "window conditions" as defined by Martinson and Wagenaar (*16*) for measuring the true dissociation of RNA-DNA duplexes.

Isolation of MuMTV(C3H) TA sequences.—The technique was a modification of that of Shoyab et al. (*17*) and has been recently reported with the use of MuMTV (*3*). MuMTV(C3H) 60-70S ^{125}I-labeled RNA (300,000 cpm) was hybridized to 30 mg of apparently normal C3H liver DNA to a C_0t of 15,000 under the

standard conditions described above. The sample was then diluted to 150 μg DNA/ml, heated to 60° C, and applied to a column of 150 ml packed hydroxyapatite at 60° C. The sample that came through was reloaded, and the column was rinsed with a solution of 0.01 M NaPB (pH 6.8) and 0.01% SLS until no material eluted which absorbed at 260 nm. Single-stranded RNA was then eluted with a solution of 0.14 M NaPB (pH 6.8) in 0.01% SLS and dialyzed against three changes of 6 liters of water containing 0.01% SLS. After dialysis, 1-2 mg of yeast carrier RNA was added, and RNA was precipitated with 2 volumes of alcohol. The RNA was then pelleted, dissolved in 1 ml of 0.001 M NaPB, and stored at −20° C until use.

RESULTS

Standard Hybridization Conditions

MuMTV-iodinated 60-70S RNA was prepared as described in "Materials and Methods." As detailed previously (*3*), with the use of standard conditions of hybridization, this RNA hybridized approximately 60% to C3H murine mammary tumors and approximately 30% to apparently normal organs of C3H mice (table 1). When this RNA was annealed to DNA obtained from tissues of several strains of laboratory rats, hamsters, guinea pigs, or other mammals under these same standard conditions, no hybridization above background was observed (table 1).

Relaxed Hybridization Conditions

To determine if sequences related to, but not identical to, the MuMTV could be detected in the DNA of rats, we relaxed the conditions of hybridization by lowering the temperature at which hybridizations were performed from 68 to 54° C (*15*). To assay RNA-DNA duplexes formed at 54° C, we raised the salt concentration used for RNase treatment from the standard conditions of two times SSC to 20 times SSC in several increments. Maximum hybridization of radioactive MuMTV RNA to rat DNA's was observed in RNase conditions of eight times SSC. This result was the same as that detailed previously in another retrovirus system (*15*). Under these conditions, the percent hybridization obtained between MuMTV 60-70S ^{125}I-labeled RNA and DNA from various rat tissues increased from background levels to approximately 17%. When conditions of hybridization were relaxed and the salt concentration during RNase treatment was raised, specificity was still maintained; i.e., no hybridization was observed to DNA obtained from guinea pig, hamster, bovine, and canine tissues, as well as to *E. coli* (table 1).

TABLE 1.—*Hybridization of MuMTV 60-70S ^{125}I-labeled RNA to rat DNA's under standard and relaxed conditions*

Source of DNA	Percent hybridization	
	Standard conditions[a]	Relaxed conditions
C3H murine mammary tumor	60	60
C3H murine liver	30	34
Rats		
SD (*R. norvegicus*)	7	16
W (*R. norvegicus*)	4	17
ACI (*R. norvegicus*)	5	18
LEW (*R. norvegicus*)	4	17
OM (*R. norvegicus*)	5	15
Feral (*R. rattus, R. norvegicus*)	3	16
Hamster	4	5
Guinea pig	2	7
Bovine	2	3
Canine	3	4
Escherichia coli	4	3

[a] Standard and relaxed conditions of hybridization are detailed in "Materials and Methods." Hybridizations are to a C_0t of 20,000.

TABLE 2.—*Hybridization of MuMTV 60-70S ^{125}I-labeled RNA to the DNA's of feral rats under standard and relaxed conditions*[a]

Source of DNA	Percent hybridization	
	Standard	Relaxed
Rat #1:		
Liver	5	16
Kidney	6	15
Lung	6	16
Spleen	5	15
Rat #2: Pooled organs	5	16
Rat #3: Pooled organs	6	17
Hamster	4	6
Feline	5	5
Feline cells infected with MuMTV	66	64
C3H mouse mammary tumor	54	56

[a] MuMTV RNA was obtained from virions from supernatants of feline cells infected with MuMTV (C3H) as described in "Materials and Methods" and (*11*).

MuMTV-Related DNA in Rats

All 5 laboratory strains of rats tested contained MuMTV-related information in their DNA's (table 1). To determine if this information was endogenous, we examined DNA from each organ of feral rats for MuMTV-related information using relaxed conditions of hybridization and RNase assay. As can be seen in table 2, all tissues of all feral rats (*R. norvegicus*) assayed contained the same degree of MuMTV-related information; these sequences thus appeared to be endogenous. The MuMTV 60-70S [^{3}H]RNA used in these experiments was obtained from virions from the supernatants of MuMTV-infected feline cells (*11*). We used this RNA to rule out the possibility that normal murine or feline cellular RNA or DNA was contaminating the MuMTV 60-70S RNA preparations. No hybridization was obtained when this RNA was hybridized to the uninfected feline cells (table 2). These experiments further demonstrated that the hybridizations observed to rat DNA's were the result of the presence of nucleic acid sequences coded by the MuMTV genome.

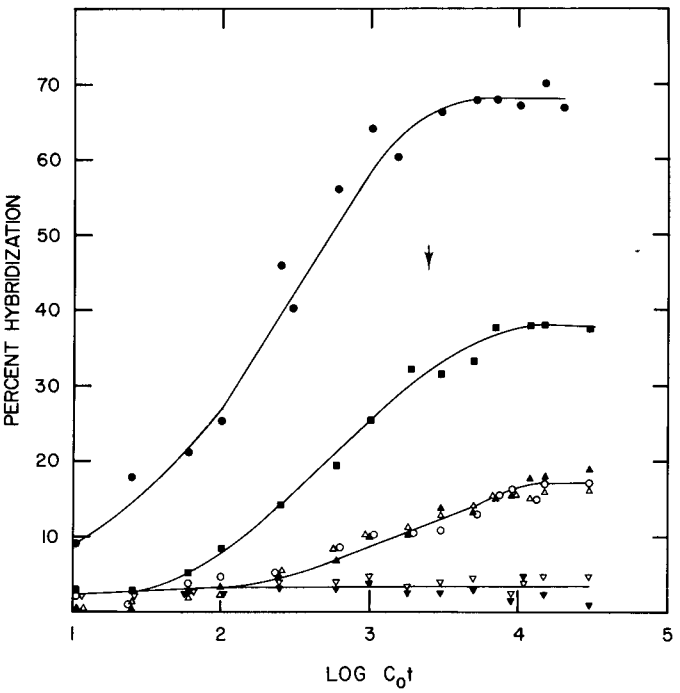
Kinetics of Hybridization

To determine at what frequency the MuMTV-related

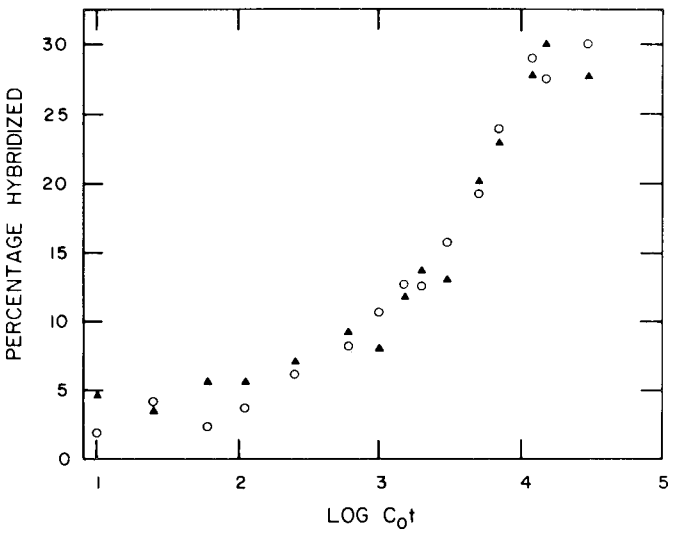
sequences were present in rat DNA, we hybridized the radioactive MuMTV 60-70S RNA to the DNA's of 3 laboratory strains of rats as well as to C3H mouse mammary tumor and liver DNA. The kinetics of hybridization to the DNA's of all rat strains were extremely similar (text-fig. 1); the $C_0t_{1/2}$ values obtained for all strains were approximately 800. The $C_0t_{1/2}$ values for the hybridization between ^{125}I-labeled polyadenine-selected normal rat RNA and rat DNA were approximately 2,600 (text-fig. 1, *arrow*). Consequently, the endogenous MuMTV-related sequences were present in the "low repetitive" range of rat cellular sequences (*3*). Hybridization to murine C3H liver and mammary tumor DNA's was shown for comparison. No hybridization was observed to hamster or guinea pig DNA's.

Hybridization to F344 Rat DNA's

Whereas the DNA of all laboratory strains and feral rats tested exhibited approximately 17% hybridization to MuMTV 60-70S RNA, DNA's from pooled organs of some F344 rats gave levels of hybridization as high as 30-35%. Text-figure 2 shows the kinetics of hybridization (under relaxed conditions) between two such F344 rat DNA's and MuMTV 60-70S ^{125}I-labeled RNA. Of 115 tissues tested from F344 rats, 48 showed this higher level of hybridization. To determine if this additional hybridization was due to MuMTV-related sequences, we hybridized MuMTV 60-70S [^{3}H]RNA, obtained from purified MuMTV virions grown in feline cells, to F344 rat DNA and obtained comparable results. Furthermore, this hybridization was abrogated with the addition of 50 ng purified, unlabeled, MuMTV(C3H) 60-70S RNA. Amounts up to 1,000 ng 60-70S RNA added from murine leukemia virus, rat leukemia virus, or rat sarcoma virus (Kirsten), however, did not compete for this hybridization. These results help to rule out the possibility that the hybridization observed was due to mouse or rat type C viral information.



TEXT-FIGURE 1.—Kinetic analysis of hybridization between MuMTV 60-70S ^{125}I-labeled RNA and rat cellular DNA's under relaxed conditions of hybridization and RNase assay. *Each point* represents the hybridization between 1,000 cpm MuMTV 60-70S ^{125}I-labeled RNA and 500 μg DNA from the following: C3H mammary tumor cell line (●), C3H liver (■), ACI rat (▲), LEW rat (○), W rat (△), hamster (▼), and guinea pig (▽). *Arrow* indicates the $C_0t_{1/2}$ for the hybridization between ^{125}I-labeled polyadenine-selected normal rat RNA [selected on Poly U Sepharose 4B (*3*)] and rat DNA under relaxed conditions of hybridization.



TEXT-FIGURE 2.—Kinetic analysis of hybridization of MuMTV ^{125}I-labeled RNA and F344 rat DNA under relaxed conditions of hybridization and RNase assay. *Each point* represents the hybridization between 1,000 cpm MuMTV 60-70S ^{125}I-labeled RNA and 500 μg DNA from pooled organs of an F344 rat obtained from NIH (○) and from FCRC (▲).

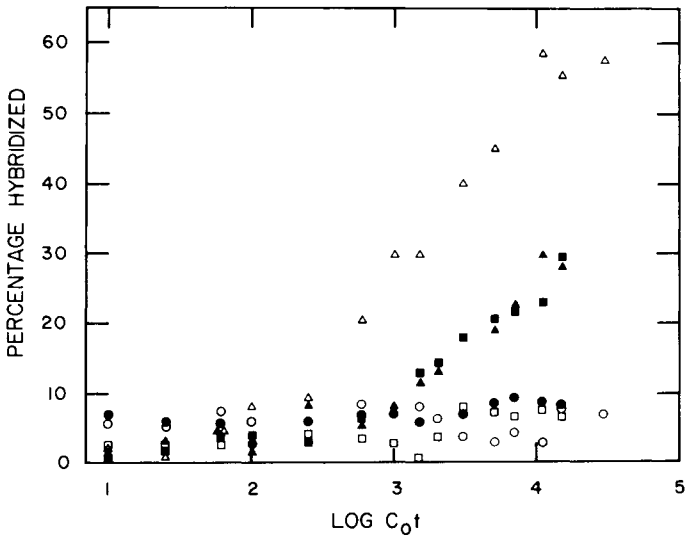
Hybridization of MuMTV(C3H) TA Sequences to F344 Rat DNA's

We have previously detailed the isolation of the TA sequences of the highly oncogenic MuMTV(C3H). These sequences were found in the DNA of early occurring C3H/He mammary tumors and not in normal C3H/He DNA (*3*) and were therefore transmitted via a non-germ-line mechanism; they were, however, transmitted via the germ line in GR mice (table 3). The DNA's of several strains of laboratory rats (excluding F344) and feral rats were tested for the presence of these sequences under relaxed conditions and were all found to be negative (text-fig. 3). Those DNA's of F344 rats that showed higher levels of hybridization with MuMTV 60-70S RNA, however, also showed annealing with MuMTV(C3H) TA sequences when conditions of hybridization were relaxed. Kinetics of this hybridization are shown in text-figure 3. Because TA-sequence RNA did not hybridize to normal murine DNA (text-fig. 3), these results ruled out the possibility that

TABLE 3.—*Hybridization of MuMTV (C3H) TA sequence RNA to the DNA's of mice and F344 rats*[a]

Source of DNA	Percent hybridization	No. of animals tested
F344 rat—type A:		
Liver	4	18
Spleen	5	
Lung	2	
Kidney	2	
F344 rat—type B:		
Liver	6	5
Spleen	27	
Lung	14	
Kidney	3	
F344 rat—type C:		
Liver	29	8
Spleen	31	
Lung	34	
Kidney	30	
C3H/He mouse:		
Mammary tumor	53	10
Liver	4	
Spleen	5	
Lung	5	
Kidney	4	
GR mouse:		
Liver	30	5
Spleen	30	
Lung	29	
Kidney	35	

[a] All hybridizations were to a C_0t of 20,000. Type A F344 rats were obtained from colonies at Nova University, NIH, Charles River Breeding Laboratories, and Harlan Farms. Type B F344 rats were obtained from the NIH colony. Type C F344 rats were obtained from colonies at NIH, FCRC, and Mason Research Institute.



TEXT-FIGURE 3.—Hybridization of MuMTV TA-sequence ^{125}I-labeled RNA to rat DNA's. MuMTV-iodinated 60-70S RNA was hybridized to normal C3H liver DNA to a C_0t of 20,000 under standard conditions. The unhybridized fraction was recovered by hydroxyapatite column chromatography as described in "Materials and Methods." This recycled TA-sequence RNA was then hybridized, under relaxed conditions, to cellular DNA's from the following: C3H murine mammary tumor (△), C3H liver (●), OM rat (○), SD rat (□), F344 rat from NIH (▲), and F344 rat from FCRC (■).

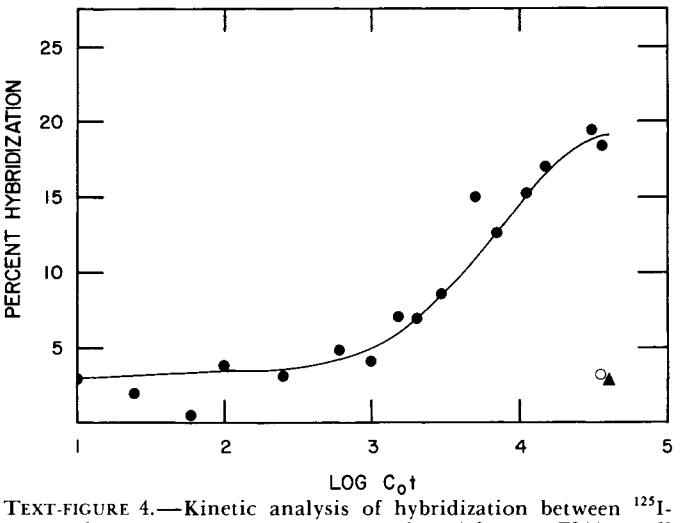
the hybridization observed between F344 rat DNA and MuMTV(C3H) TA-sequence RNA was due to normal murine sequences.

The distribution of sequences related to MuMTV (C3H) TA sequences in F344 rats was surprising. Most F344 rats from most colonies were negative (table 3, F344 rats—type A). DNA's from some rats obtained from colonies at NIH and FCRC were positive, and two distinct types of hybridization were observed (table 3). Rats of the type B group appeared to be infected with an MuMTV-related virus, whereas these sequences appeared to be integrated as endogenous provirus in rats of the type C group. Therefore, 13 of 31 F344 rats tested appeared to contain additional MuMTV-related information.

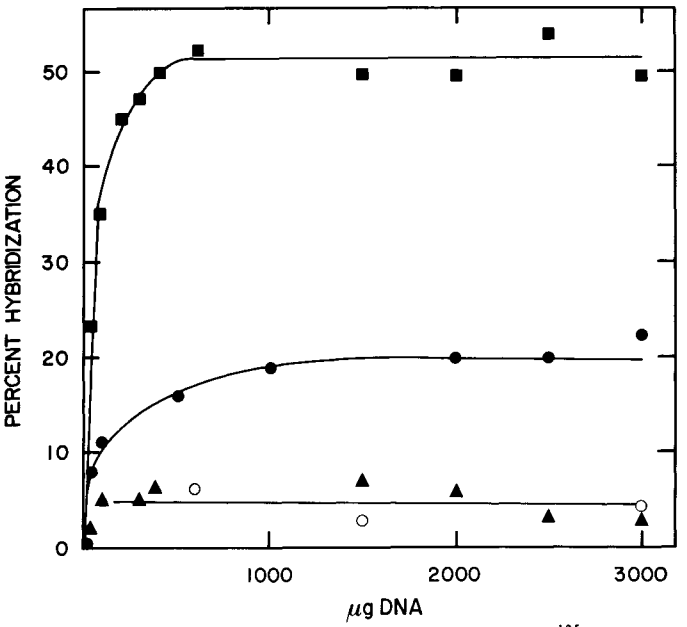
In view of the unexpected nature of these findings, various F344 rat DNA's were also hybridized to radioactive unique-sequence rat RNA, iodinated to the same specific activity as MuMTV RNA. DNA's of all rats gave virtually identical results when hybridized to poly A-enriched normal rat cellular RNA. C_0t curves of hybridizations between rat unique-sequence RNA's and DNA's from all F344 rats tested were also identical. These experiments were conducted to rule out the possibility that the differential degree of hybridization observed with MuMTV RNA was due to differential qualities of rat DNA's as a result of extraction procedures. The above experiments ruled out that possibility.

MuMTV-Related Sequences in Established Rat Cell Lines

In view of the surprising nature of the distribution of MuMTV-related sequences in rat tissues, we obtained DNA's from several established rat cell lines (supplied under code by Dr. E. Scolnick). When these



TEXT-FIGURE 4.—Kinetic analysis of hybridization between ^{125}I-labeled MuMTV TA-sequence RNA and DNA from an F344 rat cell line under relaxed hybridization conditions. *Each point* represents the hybridization between 1,000 cpm of MuMTV TA-sequence ^{125}I-labeled RNA and 500 μg DNA. DNA from a cloned FRE cell line (●) and DNA from the pooled organs of an OM rat (▲) and from an SD (○) rat.



TEXT-FIGURE 5.—Saturation hybridization between ^{125}I-labeled MuMTV TA-sequence RNA's and rodent DNA's. ^{125}I-labeled MuMTV TA-sequence RNA (1,000 cpm) was hybridized to increasing amounts of cellular DNA's at a constant DNA concentration (3 mg/ml). All samples were hybridized under relaxed conditions as detailed in "Materials and Methods" and incubated to a C_0t of 35,000. Hybridization to DNA from: an F344 rat embryo cell line (●), a spontaneous C3H mouse mammary tumor (■), pooled organs of a normal OM rat (○), and normal liver of a C3H/He mouse (▲).

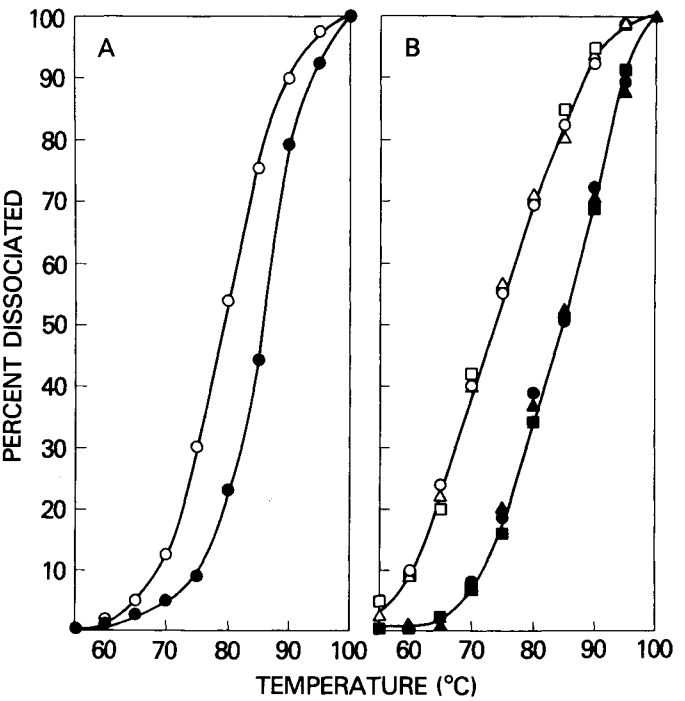
DNA's were hybridized with MuMTV(C3H) TA-sequence RNA under relaxed conditions, 1 of 2 F344 rat cell lines was positive. Two other rat cell lines (SD and OM) as well as cell lines from dogs and minks were negative. The positive line, designated FRE, was a cloned, established cell line from an F344 rat embryo. DNA from the FRE line was used for further characterization.

Hybridizations were performed under relaxed conditions between FRE cellular DNA and MuMTV(C3H) TA-sequence ^{125}I-labeled RNA. The $C_0t_{1/2}$ value obtained was approximately 3,500 (text-fig. 4). To determine if the 20% hybridization value obtained by annealing MuMTV(C3H) TA-sequence ^{125}I-labeled RNA and FRE cellular DNA was the maximum that could be achieved, DNA saturation experiments were performed. Addition up to 3,000 μg DNA from murine C3H liver or OM rat DNA resulted in no increase in hybridization above background levels (text-fig. 5). Hybridization to FRE cellular DNA, however, did plateau at 20% at approximately 1,000 μg input. These results argue against the possibility that FRE DNA was contaminated with murine cellular DNA.

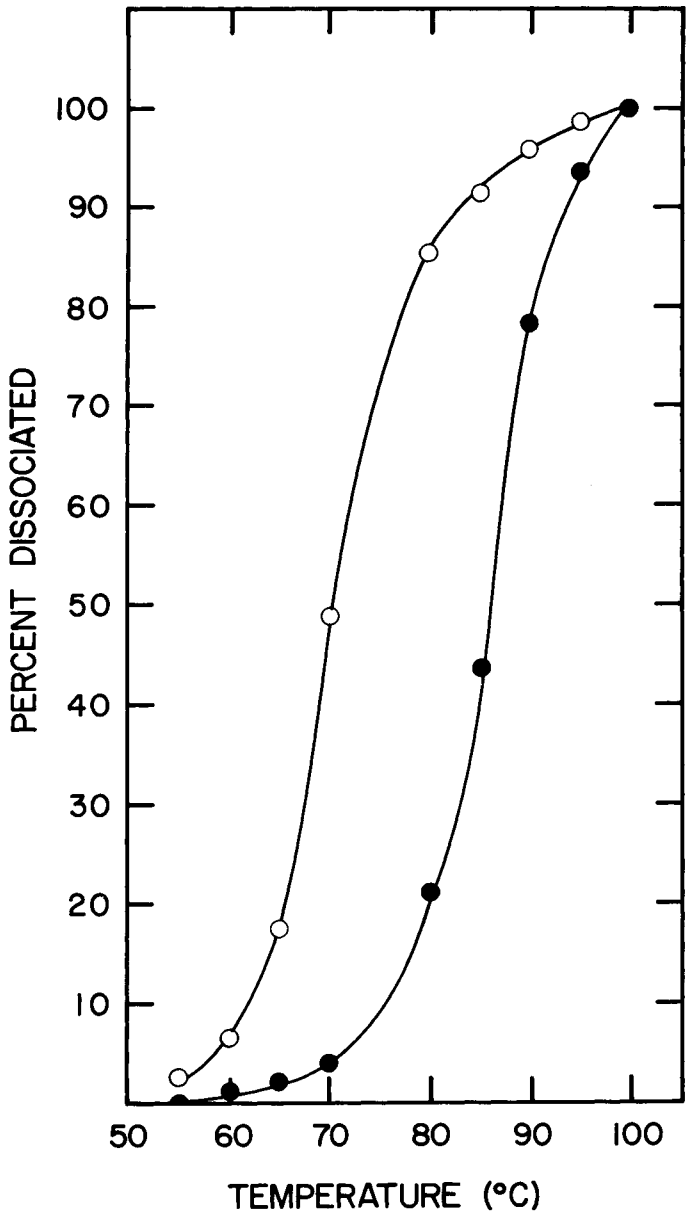
Thermal Analysis of Hybrids

To further characterize the hybridizations observed between the MuMTV genome and rat DNA's, employing hydroxyapatite chromatography, we conducted thermal analyses of hybrid duplex structures. The differential between the 50% elution of DNA-DNA duplexes versus radioactive RNA-DNA duplexes in the homologous system, i.e, MuMTV 60-70S ^{125}I-labeled RNA versus C3H mammary tumor DNA, was 6.3° C (text-fig. 6A). The hybrid duplexes formed between MuMTV(C3H) 60-70S ^{125}I-labeled RNA and OM or SD rat DNA's displayed a ΔT_m of 11.6° C, i.e., 5.3° C lower than the homologous ΔT_m (text-fig. 6B). The ΔT_m of the hybrid duplex formed between MuMTV 60-70S RNA and F344 rat DNA, obtained from animals at Nova University, Ft. Lauderdale, Florida (*see* table 3, F344 rat type A), was also 11.6° C.

We also conducted thermal analyses of hybrid structures formed with MuMTV(C3H) TA-sequence ^{125}I-labeled RNA. Duplexes formed between this RNA and C3H mammary tumor DNA displayed a ΔT_m of approximately 7° C. However, duplex structures formed between this RNA and FRE cellular DNA displayed a ΔT_m of 15.5° C, i.e., a value approximately 8.5° C lower than that for the homologous hybrid structure (text-fig. 7). The DNA's from organs of F344 rats that gave increased percent hybridization (text-fig. 3, table 3) also displayed similar ΔT_m's of 15.5° C.



TEXT-FIGURE 6.—Thermal stability of hybrids formed between MuMTV 60-70S ^{125}I-labeled RNA and rat DNA's. Relaxed hybridization conditions and thermal elution from hydroxyapatite were performed as described in "Materials and Methods." ●, ▲, ■: DNA-DNA dissociation profiles as monitored by absorbance at 260 nm. ○, △, □: ^{125}I-labeled RNA-DNA dissociation as measured by acid-precipitable radioactivity in each fraction. *Panel A*: Hybridization between MuMTV 60-70S ^{125}I-labeled RNA and DNA from C3H mouse mammary tumors. *Panel B*: Hybridization between MuMTV 60-70S ^{125}I-labeled RNA and DNA from organs of OM rats (●, ○), DNA from organs of SD rats (▲, △), and DNA from a germfree F344 rat from Nova University (■, □).



TEXT-FIGURE 7.—Thermal stability of hybrids formed between MuMTV(C3H) TA-sequence ^{125}I-labeled RNA and F344 rat embryo cell line DNA. Relaxed hybridization conditions and thermal elution from hydroxyapatite were performed as described in "Materials and Methods." Approximately 10,000 cpm ^{125}I-labeled RNA was hybridized to 1,000 μg F344 rat cell line DNA to a C_0t of 20,000. ●: DNA-DNA dissociation profiles as monitored by absorbance at 260 nm. ○: Radioactive RNA-DNA dissociation as measured by acid-precipitable radioactivity in each fraction.

DISCUSSION

The studies reported here demonstrated that sequences related to approximately 20% of the MuMTV genome are endogenous to laboratory as well as to feral rats. We detected these sequences by employing a lower temperature of hybridization (54° C) and by raising the salt concentration (eight times SSC) in which RNA-DNA duplexes were scored for RNase resistance. To rule out the possibility that contaminating normal murine cellular sequences were responsible for this hybridization, we also did experiments using MuMTV 60-70S RNA from virions grown in feline cells; this RNA gave results comparable to those obtained with viral RNA from MuMTV grown in murine cells.

MuMTV-related sequences were not detected in the DNA's of hamsters and guinea pigs. Because hamsters diverged from the mouse-rat evolutionary line approximately 20 million years ago (*18, 19*), the MuMTV-related sequences most probably became integrated into the mouse-rat germ line within the last 20 million years. These sequences are present in the DNA of all rats as endogenous sequences present in the low repetitive range.

The observation of additional MuMTV-related sequences in the DNA of some F344 rats and a cloned, established F344 rat cell line was unexpected. These additional sequences are present in the nonrepetitive range and are related to the sequences of the MuMTV genome that are non-germ-line transmitted in the C3H/He mouse and RIII mouse (*3*). This was demonstrated by the use of radioactive RNA that was first recycled against C3H/He liver DNA. Using this probe, we detected no homologous sequences in normal C3H/He mouse tissues or in the DNA of tissues of other strains of rats. However, these sequences were detected in the DNA of C3H/He mammary tumors induced by the highly oncogenic milk-transmitted MuMTV and in the DNA of tissues of some F344 rats (text-fig. 3).

There are several possible explanations for the presence of these extra MuMTV sequences in the DNA of F344 rats and perhaps in other strains or colonies of rats not yet as thoroughly investigated. One explanation is that a rat virus related to the MuMTV, but as yet unknown, has infected and in some instances has integrated into the genome of F344 rats. This integration can be occurring in a manner similar to the integration demonstrated by mouse leukemia virus into mouse embryo DNA as described by Jaenisch (*20*). The apparent integration of MuMTV(C3H) TA sequences in some F344 rats has a parallel in mice. As previously shown, these sequences are non-germ-line transmitted in C3H/He mice (*3*); however, a recent study (*21*) demonstrated the integration of these sequences as endogenous provirus in colonies of C3H/Bi mice. A second explanation of the results reported here involves a similar infection as described above but with a xenotropic or amphotropic variant of the MuMTV. This explanation is possible in light of the isolation of host range variants of MuMTV's that have the ability to infect heterologous cells in culture (*11, 22, 23*). Other explanations are, of course, possible.

These findings reported here demonstrate that laboratory strains of rats commonly used in mammary cancer research contain at least one set and sometimes two different sets of MuMTV-related information in their DNA's. The first set is present in the DNA's of all rats in the low repetitive range and is related to approximately 20% of the MuMTV genome. The second set of sequences, found thus far only in F344 rats,

is slightly more diverged from the MuMTV genome than the endogenous sequences. These sequences are related to the part of the MuMTV genome that is non-germ-line transmitted in several mouse strains such as RIII and C3H/He. The role of these two sets of MuMTV-related sequences in the etiology of mammary neoplasia and other neoplastic diseases of the rat obviously merits further study.

REFERENCES

(1) SCHLOM J, COLCHER D, DROHAN W, et al: Differences in mouse mammary tumor viruses: Relationship to early and late occurring mammary tumors. Cancer 39:2727-2733, 1977

(2) BENTVELZEN P: The biology of the mouse mammary tumor virus. Int Rev Exp Pathol 11:259-297, 1972

(3) DROHAN W, KETTMANN R, COLCHER D, et al: Isolation of the mouse mammary tumor virus sequences not transmitted as germinal provirus in the C3H and RIII mouse strains. J Virol 21:986-995, 1977

(4) RUSSFIELD AB: Mammary gland. *In* Tumors of Endocrine Glands and Secondary Sex Organs (Taylor J, Schumacher AM, eds), chapt XII. Public Health Serv, DHEW Publ No. (PHS) 1332. Washington, D.C.: U.S. Govt Print Off, 1966, pp 87-116

(5) NOBLE RL, CUTTS JH: Mammary tumors of the rat: A review. Cancer Res 19:1125-1139, 1959

(6) JACOBS BB, HUSEBY RA: Neoplasms occurring in aged Fischer rats, with special reference to testicular, uterine, and thyroid tumors. J Natl Cancer Inst 39:303-309, 1967

(7) SACKSTEDER MR: Occurrence of spontaneous tumors in the germfree F344 rat. J Natl Cancer Inst 57:1371-1373, 1976

(8) CURTIS MR, BULLOCK FD: Strain and family differences in susceptibility to cysticercus sarcoma. Cancer Res 8:1-17, 1924

(9) OWENS RB, HACKETT AJ: Tissue culture studies of mouse mammary tumor cells and associated viruses. J Natl Cancer Inst 49:1321-1332, 1972

(10) SCHOCHETMAN G, SCHLOM J: Independent polypeptide chain initiation sites for the synthesis of different classes of proteins for an RNA tumor virus: Mouse mammary tumor virus. Virology 73:431-441, 1976

(11) HOWARD DK, COLCHER D, TERAMOTO YA, et al: Characterization of mouse mammary tumor viruses propagated in non-murine cells. Cancer Res 37:2696-2704, 1977

(12) COMMERFORD SL: Iodination of nucleic acids in vitro. Biochemistry 10:1993-1999, 1971

(13) COLCHER D, DROHAN W, SCHLOM J: Mason-Pfizer virus RNA genome: Relationship to the RNA of morphologically similar isolates and other oncornaviruses. j Virol 17:705-712, 1976

(14) BRITTEN RJ, SMITH J: A bovine genome. Carnegie Inst Washington Yearbook 68:378-386, 1970

(15) DROHAN W, COLCHER D, SCHLOM J: The use of standard and relaxed conditions to detect two classes of sequences related to type-D retroviruses in the DNAs of primates. Biochim Biophys Acta 518:1-15, 1978

(16) MARTINSON HG, WAGENAAR EB: Thermal elution chromatography of nucleic acids on hydroxyapatite. Biochim Biophys Acta 474:445-455, 1978

(17) SHOYAB M, EVANS RM, BALUDA MA: Presence in leukemia cells of avian myeloblastosis virus-specific DNA sequences absent in normal chicken cells. J Virol 14:47-49, 1974

(18) RICE NR: Change in repeated DNA in evolution. *In* Evolution of Genetic Systems (Smith HH, ed). New York: Gordon & Breach Science, 1972, pp 44-79

(19) BENVENISTE RE, CALLAHAN R, SHERR CJ, et al: Two distinct endogenous type C viruses isolated from the Asian rodent *Mus cervicolor*: Conservation of virogene sequences in related rodent species. J Virol 21:849-862, 1977

(20) JAENISCH R: Germ line integration and Mendelian transmission of the exogenous Moloney leukemia virus. Proc Natl Acad Sci USA 73:1260-1264, 1976

(21) SCHLOM J, COLCHER D, DROHAN W, et al: Systematics of murine mammary tumor viruses. *In* Workshop on the Origins of Inbred Mice (Morse HC III, ed). New York: Academic Press, 1978, pp 343-368

(22) LASFARGUES EY, LASFARGUES JC, DION AS, et al: Experimental infection of a cat kidney cell line with the mouse mammary tumor virus. Cancer Res 36:67-72, 1976

(23) HOWARD D, SCHLOM J: Isolation of mouse mammary tumor virus host range variants that efficiently infect cells in vitro. Proc Natl Acad Sci USA 75:5718-5722, 1978