Conserved Region of Mammalian Retrovirus RNA

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Received for publication 8 May 1979

The viral RNAs of various mammalian retroviruses contain highly conserved sequences close to their 3' ends. This was demonstrated by interviral molecular hybridization between fractionated viral complementary DNA (cDNA) and RNA. cDNA near the 3' end (cDNA_{3'}) from a rat virus (RPL strain) was fractionated by size and mixed with mouse virus RNA (Rauscher leukemia virus). No hybridization occurred with total cDNA (cDNA_{total}), in agreement with previous results, but a cross-reacting sequence was found with the fractionated cDNA_{3'}. The sequences between 50 to 400 nucleotides from the 3' terminus of heteropolymeric RNA were most hybridizable. The rat viral cDNA_{3'} hybridized with mouse virus RNA more extensively than with RNA of remotely related retroviruses. The related viral sequence of the rodent viruses (mouse and rat) showed as much divergence in heteroduplex thermal denaturation profiles as did the unique sequence DNA of these two rodents. This suggests that over a period of time, rodent viruses have preserved a sequence with changes correlated to phylogenetic distance of hosts. The cross-reacting sequence of replication-competent retroviruses was conserved even in the genome of the replication-defective sarcoma virus and was also located in these genomes near the 3' end of 30S RNA. A fraction of RD114 cDNA_{3'}, corresponding to the conserved region, cross-hybridized extensively with RNA of a baboon endogenous virus (M7). Fractions of similar size prepared from cDNA_{3'} of MPMV, a primate type D virus, hybridized with M7 RNA to a lesser extent. Hybridization was not observed between Mason-Pfizer monkey virus and M7 if total cDNA's were incubated with viral RNAs. The degree of cross-reaction of the shared sequence appeared to be influenced by viral ancestral relatedness and host cell phylogenetic relationships. Thus, the strikingly high extent of cross-reaction at the conserved region between rodent viruses and simian sarcoma virus and between baboon virus and RD114 virus may reflect ancestral relatedness of the viruses. Slight cross-reaction at the site between type B and C viruses of rodents (mouse mammary tumor virus and RPL virus, 58-2T) or type C and D viruses of primates (M7, RD114, and Mason-Pfizer monkey virus) may have arisen at the conserved region through a mechanism that depends more on the phylogenetic relatedness of the host cells than on the viral type or origin. Determining the sequence of the conserved region may help elucidate this mechanism. The conserved sequences in retroviruses described here may be an important functional unit for the life cycle of many retroviruses.

The genome of RNA tumor viruses is an RNA of 10,000 nucleotides with a polyadenylic acid [poly(A)] sequence of about 200 nucleotides at its 3' terminus (3). Four genetic regions have been identified: *gag*, coding for internal structural proteins; *pol*, coding for RNA-dependent DNA polymerase; *env*, coding for viral glycoproteins; and a gene for oncogenic transformation termed *onc* (3). In avian sarcoma viruses, these genes have been mapped by Wang et al. (42, 44) and Joho et al. (20, 21) by analysis of RNA of various mutant viruses. Recently, a fifth region has been found which is a common sequence of

unknown function, located at the 3' terminus in RNAs of both avian leukosis and sarcoma viruses (16, 36, 43, 46). Mammalian RNA tumor viruses are thought to consist of a similar set of genetic regions (3). Although sequences common to the RNAs of Moloney sarcoma and leukemia viruses have been shown by heteroduplex analysis (18), there have been no reports of common sequences in the genomes of RNA tumor viruses whose origins are distant in birds and mammals.

It is generally accepted that under stringent conditions of molecular hybridization, type C viruses of different mammalian species rarely

cross-react (6, 15, 28, 29). Two exceptions are woolly monkey-gibbon ape virus (28) and feline RD114-baboon isolates (4). A common ancestral origin has been proposed which could account for these exceptions (7). Several reports that mention slight cross-hybridization between feline leukemia viruses and rodent viruses (6, 28) or between simian viruses and rodent viruses (6) have used complementary DNA (cDNA) of endogenous reactions. Benveniste et al. constructed a pedigree of the viruses (5). It remains to derive from cross-hybridization studies how a phylogeny arose through chromosomal localization, distribution, mosaicism, and sequence divergence.

We have synthesized cDNA near the 3' end (cDNA₃) by using an oligodeoxythymidylic acid [oligo(dT)] primer and fractionated it by size and have found that some of the mammalian retroviruses have a shared region in their genomes. This conserved region is located between 50 and 400 nucleotides from the 3' terminus of retrovirus genomic RNA.

(Preliminary reports of these findings were presented at the 78th Annual Meeting of the American Society for Microbiology, Las Vegas, Nev., 14–19 May 1978, abstr. S164, p. 240, and at the Meeting on RNA Tumor Viruses, Cold Spring Harbor, N.Y., 25–29 May 1978, abstr., p. 103.)

MATERIALS AND METHODS

Virus and viral RNA. Viruses were supplied from the Frederick Cancer Research Center, Life Sciences Research (Gulfport, Fla.) and University Laboratories, Inc. (Highland Park, N.J.). The viruses were isolated by double-gradient centrifugation from supernatant fluids of chronically infected cultures.

Purified viruses were incubated for 30 min at 37°C in the presence of 200 µg of proteinase K per ml and 0.5% sodium dodecyl sulfate (SDS) and then extracted three times with a phenol-chloroform-isoamyl alcohol mixture (1:1:0.01, vol/vol/vol) containing 0.05% 8-hydroxyquinoline. RNA was precipitated with 95% ethanol containing 2% potassium acetate (11, 16).

The precipitated RNA was dissolved in NTE buffer (0.1 M NaCl, 10 mM Tris-hydrochloride [pH 7.4], and 1 mM EDTA) and centrifuged on a 10 to 30% (wt/vol) linear sucrose gradient. Fractions containing 60 to 70S RNA were pooled, and RNA was precipitated with ethanol.

To obtain poly(A)-containing RNA [poly(A) (+) RNA], viral RNA was dissolved in 0.5 M NaCl and 0.01 M Tris-hydrochloride (pH 7.6) and applied to a column of oligo(dT)-cellulose (P-L Biochemicals) which had been equilibrated in the buffer (2). All steps were performed at room temperature. The column was washed successively with 10 bed volumes of 0.5 M NaCl and 10 mM Tris-hydrochloride (pH 7.6) and 5 volumes of 0.1 M NaCl and 10 mM Tris-hydrochloride (pH 7.6).

Poly(A) (+) RNA bound to oligo(dT)-cellulose was eluted in 1 ml of 10 mM Tris-hydrochloride buffer, although some poly(A) (–) RNA was still present in this fraction. Poly(A) (+) RNA was repurified by a second oligo(dT)-cellulose chromatography.

Synthesis of retroviral cDNA. (i) **Total cDNA (cDNA_{total}).** Single-stranded viral cDNA's complementary to the RNAs of retroviruses were synthesized according to Taylor et al. (37). Reaction mixtures contained 50 mM Tris-hydrochloride (pH 8.3), 60 mM NaCl, 6 mM MgCl₂, 1 mM dithiothreitol, 1 mM each dATP, dCTP, and dGTP, 0.05 mM [³H]dTTP (55 Ci/mmol; New England Nuclear Corp.), 20 µg of 70S viral RNA per ml, 50 µg of actinomycin D per ml, 200 µg of avian myeloblastosis virus (AMV) reverse transcriptase per ml, and 0.5 mg of calf thymus DNA partially digested by DNase I per ml. After incubation for 40 min, mixtures were adjusted to 0.3 M NaOH, heated at 80°C for 30 min, neutralized, and desalted through a Sephadex G-50 column (0.6 by 15 cm). The excluded fraction was precipitated by ethanol in the presence of 100 µg of yeast tRNA as carrier.

(ii) **Synthesis of cDNA₃.** cDNA₃ was synthesized by the method of Friedman and Rosbach (12, 36). The reaction mixture was: 50 mM Tris-hydrochloride (pH 8.3), 60 mM NaCl, 6 mM MgCl₂, 20 mM dithiothreitol, 100 µg of actinomycin D per ml, 10 µg of oligo(dT)₁₀ per ml, 50 µg of 70S viral RNA per ml, and 100 U of AMV reverse transcriptase per ml. Three unlabeled deoxynucleotides were present at 1 mM, and tritium-labeled dXTP ([³H]dATP, -dCTP, -dGTP, or -dTTP) was present at 200 µM.

The reaction was initiated in 100-µl volumes by the addition of reverse transcriptase and incubated at 37°C for 20 to 40 min.

The reaction mixture was treated with 0.05 vol of 2 M NaOH at 80°C for 30 min to hydrolyze RNA. After neutralization with 0.05 volume of 2 M HCl, the DNA product was applied on a Sephadex G-50 column (0.7 by 20 cm) to eliminate excess [³H]dXTP. The excluded fraction was extracted with phenol-chloroform and precipitated with ethanol and 50 µg of yeast tRNA as carrier.

The yield of Rauscher leukemia virus (RLV) cDNA in control reactions without added primer was 2% of cDNA generated by the primed reaction. Radioactivity incorporated in primed reactions of RLV poly(A) (–) RNA was 0.2% of incorporation dependent on poly(A) (+) RNA. Poly(A) (–) RNA used here was the fraction not retained on an oligo(dT)-cellulose column after mild alkali digestion of RLV 70S RNA. The average size of the RNA was 18S.

Vertical slab gels of 4% polyacrylamide-bisacrylamide (20:1) with or without 7 M urea were prepared as described by Loening (25). Ethanol-precipitated [³H]cDNA samples were dissolved in 20 µl of E buffer (40 mM Tris-acetate, 5 mM sodium acetate [pH 7.8], and 1 mM EDTA) containing 20% sucrose and bromophenol blue, with or without 7 M urea. Electrophoresis was performed at room temperature for 3 to 4 h at 2 V/cm.

One slot contained single-stranded DNA fragments of φX174 digested by restriction enzyme *Hae*III as markers of molecular weight (33). The gel was cut into five fractions from the top to the position of bromo-

phenol blue marker. The average sizes of cDNA were calculated based on the position of the markers. The nucleotide lengths of cDNA_{3'} on urea gels were: 2,000 to 1,100 in fraction 1; 440 to 1,100 in fraction 2; 200 to 440 in fraction 3; 105 to 200 in fraction 4; and 56 to 105 in fraction 5.

Due to transcription of part of the 3' poly(A) sequence from viral RNA, cDNA contained short 5'-terminal oligo(dT) tracts. To estimate the size of the oligo(dT) tract before fractionation of cDNA by size, hybrids were formed with [³H]dTTP-labeled RLV cDNA, unlabeled RLV RNA, poly(A), and yeast tRNA. The mean size of the oligo(dT) tract, 20 nucleotides, has been subtracted from the lengths of cDNA fractions.

More than 90% of radioactivity in cDNA_{3'} formed thermally stable triple-stranded complexes with poly(A) and polyuridylic acid, thereby demonstrating that oligo(dT) tracts were linked to cDNA_{3'} (36) (data not shown).

cDNA was extracted from the gel by incubation in 4 ml of E buffer containing 0.3 M NaCl, 0.5% SDS, and 0.1 mg of tRNA at room temperature overnight. Of radioactivity recovered from RLV cDNA_{3'}, 29% was in fraction 1, 23% was in fraction 2, 24% was in fraction 3, 14% was in fraction 4, and 9% was in fraction 5.

cDNA/RNA hybridization reactions. Duplicate samples, in siliconized glass capillaries, contained 1,000 cpm of [³H]cDNA (specific activity, 1×10^7 to 5×10^7 cpm/ μ g) and viral RNA at a concentration of 50 μ g/ml in 0.05 ml of 0.72 M NaCl, 0.05 M PIPES [piperazine-*N,N'*-bis(2-ethanesulfonic acid)] (pH 6.8), 10 mM EDTA, 0.5% SDS, and 50% formamide. The solution was annealed for 17 h at 37°C. The contents of the capillary was expelled into 1 ml of S1 nuclease buffer (0.034 M sodium acetate [pH 4.6], 0.18 M NaCl, and 0.14 mM zinc acetate) containing 10 μ g each of calf thymus native and denatured DNA. S1 nuclease (100 U) was added. After digestion for 2 h at 43°C, the samples were precipitated with yeast tRNA carrier and 2 vol of 10% trichloroacetic acid, collected on hydroxyapatite (HA) filters (Millipore Corp.), and counted. The background, in the presence of tRNA, was 2.3 to 2.7% of the total input radioactivity of cDNA (500 to 1,000 cpm). Under conditions of hybridization and assay described above, radioactivity levels at least 300% of the background are considered significant. To verify this, some replicates were assayed by a less stringent HA method without S1 nuclease treatment. More than 15% of the radioactivity was retained on HA as a duplex in those samples in which 5 to 10% of the radioactivity resisted digestion by S1 nuclease. Furthermore, cDNA_{3'} of mammalian retroviruses formed duplexes with avian viral RNA (AMV or Rous sarcoma virus [RSV]) by the HA method when tRNA was used as control, although the S1 nuclease method failed to show any cross-hybridization between mammalian and avian retroviruses (this paper and unpublished data).

Hybrids were eluted from HA to determine thermal denaturation profiles, as described previously (28). The hybridization mixtures were diluted to 5 ml in warm buffer (0.12 M sodium phosphate [pH 6.8]–0.4% SDS) and loaded onto a water-jacketed column of HA (1 by 2 cm). After two washes with 5 ml of the buffer,

the hybrid molecules were eluted by raising the column temperature by 5°C increments to 100°C. The radioactivity of each eluate was precipitated with 50% trichloroacetic acid in the presence of 20 μ g of calf thymus DNA, collected onto an HA filter (Millipore Corp.), and counted in a liquid scintillation counter.

Enzymes and radiochemicals. Sources of enzymes were as follows: proteinase K, EM Laboratories; RNase T1, Sigma Chemical Co.; RNase A and S1 nuclease, Worthington Biochemicals Corp. Sources of radiochemicals were: [³H]dATP, -dCTP, -dGTP, and -dTTP (10 to 50 Ci/mmol), New England Nuclear Corp. Sources of chemicals were: calf thymus DNA, Worthington Biochemical Corp.; yeast tRNA, Miles Research Products; deoxynucleoside triphosphates, P-L Biochemicals. Other chemicals were purchased from Sigma Chemical Co.

RESULTS

Homology between size-fractionated rat virus (RPL) cDNA and viral RNAs. We began to search for a nucleotide sequence homologous in type C RNA viruses originating from closely related rodent species, mouse and rat. Rat RPL cDNA was selected for attempts to detect homology to other viral RNAs because RPL cDNA made in the endogenous reaction is known not to cross-hybridize significantly with other viral RNAs, nor are any reciprocal reactions obtained (30).

cDNA_{3'} was prepared from RPL 70S RNA with oligo(dT)₁₀ as primer, using AMV reverse transcriptase. The cDNA was fractionated by acrylamide gel electrophoresis into five size classes, and each fraction was used for molecular hybridization to various viral 70S RNAs (Table 1). cDNA of fraction 1 represented 2,000 to 1,100 nucleotides of RPL RNA from the 3' end, and those of fraction 2 to fraction 5 exhibited 1,100 to 440, 440 to 200, 200 to 105, and 105 to 56 nucleotide sequences from the 3' end, respectively. All hybridizations were carried out at high *C*_t values (>5). In Table 1, S1 nuclease-resistant radioactivities are expressed as a percentage of the value obtained in homologous reactions. Although mouse virus (RLV) RNA hybridized slightly with the smallest cDNA (fraction 5), the extent of relative reaction increased with the size of cDNA up to fractions 3 and 4 (Table 1). This effect may have been due to a sequence that is different from the terminal redundant sequence observed at the 3' end of the viral genomes (10). When larger cDNA's (fractions 1 and 2) were used, the relative extent decreased with size, implying that the homologous sequence in RPL and RLV was most enriched at fractions 3 and 4. The extent of hybridization was not affected by the extensive sonication of the fractionated cDNA probes. The distribution of reactions with other viral RNAs

TABLE 1. Homology between size-fractionated rat RPL cDNA and viral RNAs

Viral RNA	% Hybridization ^a				
	Fraction 1, 2,000-1,100 nucleotides	Fraction 2, 1,100-440 nucleotides	Fraction 3, 440-200 nu- cleotides	Fraction 4, 200-105 nu- cleotides	Fraction 5, 105-56 nu- cleotides
Rat RPL (type C)	100 ^b	100	100	100	100
Mouse RLV (type C)	5.8	16	31	23	7.0
Simian SSV (type C)	7.7	14	26	24	7.6
Mouse MMTV (type B)	1.6	4.1	9.1	6.4	2.7
Cat RD114 (type C)	0.7	1.1	2.1	3.0	2.0
Cat FeLV ^c (type C)	1.9	4.4	4.8	4.4	3.3
Baboon M7 (type C)	1.3	1.8	1.6	2.4	1.1
Avian RSV (type C)	0.2	0.1	0.4	0.2	0.2

^a Oligo(dT)-primed cDNA from PRL was synthesized by using [³H]dCTP and separated by size into five fractions on a 4% polyacrylamide gel with 7 M urea as described in the text. cDNA (~1,000 cpm) from each of the fractions was hybridized with 50 µg of viral 70S RNAs and tRNA per ml. C_t values were 5 to 10 mol s liter⁻¹.

^b Radioactivity in cDNA RNA hybrids was determined after S1 nuclease assay. The radioactivities are expressed as a percentage of the corresponding homologous hybrid. The actual radioactivities in hybrids between cDNA and RNAs were 1,464 to 520 cpm. The reaction extents of these homologous hybrids were 80 to 90%. The values obtained in hybridizations between cDNA and tRNA were 12 to 40 cpm, and were subtracted before calculation of virus-specific values.

^c Feline leukemia virus.

also showed a similar pattern to RLV and peaked at fractions 3 and 4 (Table 1). This demonstrates that the related or conserved sequence among the retroviruses tested, despite variable extents of homology, is present in a small region of the viral genome located between 50 and 400 nucleotides from the 3' end of viral RNA. The cross-hybridization of simian sarcoma virus (SSV) RNA and RPL cDNA (Table 1) clearly demonstrated that a conserved sequence relating mouse viruses and SSV lies near the 3' end. Although cross-reactions between rat viruses and SSV have never been reported, cross-reactions between murine leukemia virus (MuLV) and SSV have been found; from this observation and others, the origin of SSV from a virus related to MuLV has been suggested (6, 23).

Mouse mammary tumor virus (MMTV) is a type B mouse virus distinguished from type C viruses by morphology, immunological specificity, and molecular hybridization (39). Therefore, it was striking that MMTV RNA gave a slight cross-reaction with fractions 3 and 4 of RPL cDNA (Tables 1 and 2). Nonmammalian retroviruses (RSV and AMV) failed to demonstrate any cross-hybridization with the conserved region of mammalian retroviruses under the conditions used in here.

C_t analysis between poly(A)-containing RLV RNA and size-fractionated RPL cDNA. Before extending these studies to a variety of other interviral combinations, C_t analysis was performed to confirm the fidelity of

RPL cDNA₃ transcripts. RPL cDNA₃ (fraction 3) was hybridized with 70S RNAs of mouse RLV with 40% of cross-reaction and C_tt_{1/2} of 0.6 (mol s liter⁻¹), whereas the same probe annealed with the homologous rat RPL 70S RNA with 85% hybridization and C_tt_{1/2} of 0.15 (Fig. 1). The same results were obtained by the reciprocal experiment, that is, the combination of poly(A) (+) RPL RNA and cDNA₃ of RLV (not shown). Since the extent of saturation showed about 40% between RPL cDNA and RLV 70S RNA and between RLV cDNA and RPL 70S RNA, the cross-reacting sequences between these viruses may range from 80 to 170 nucleotides. Together with the results of the thermal denaturation of the hybrids described below, the fourfold-greater C_tt_{1/2} value obtained from the heterologous hybrid may be explained by random base pair mismatching and the known dependence of hybridization reaction rate on the size of DNA fragments (47). It would not result from contamination of 70S viral RNA by extraneous RNAs.

The C_tt_{1/2} value obtained with 10S RLV RNA, which contains poly(A), was much lower than the C_tt_{1/2} value obtained with 70S RNA (Fig. 1). 10S RLV RNA was made by heat denaturing 70S RNA of RLV and centrifuging it through a sucrose gradient. Poly(A) (+) 10S RNA₃ was selected by two successive chromatographic separations on an oligo(dT)-cellulose column. The poly(A) (+) 10S RNA₃ gave a C_tt_{1/2} value 20-fold less than that of 70S RNA and retained the same saturation value as 70S RNA. The lowering of the C_t values with the same extent of

TABLE 2. Homology between size-fractionated cDNA and viral RNAs

Viral RNA	% Hybridization ^a				
	Fraction 1, 2,000-1,100 nucleotides	Fraction 2, 1,100-440 nucleotides	Fraction 3, 440-200 nu- cleotides	Fraction 4, 200-105 nu- cleotides	Fraction 5, 105-56 nu- cleotides
Mouse 58-2T cDNA					
* Mouse 58-2T (type C)	100	100	100	100	100
Rat RPL (type C)	24	37	33	42	20
Mouse MTV (type B)	3.8	5.4	8.1	15	8.3
Cat RD114 cDNA					
Cat RD114 (type C)	100	100	100	100	100
Baboon M7 (type C)	18	27	30	38	30
Mouse RLV (type C)	0.5	0.5	1.2	1.1	0
Baboon M7 cDNA					
Baboon M7 (type C)	100	100	100	100	100
Cat RD114 (type C)	15	22	36	46	42
Mouse RLV (type C)	0.6	0.9	1.6	1.2	1.0
Simian MPMV					
Simian MPMV (type D)	100	100	100	100	100
Baboon M7 (type C)	7.1	9.8	11	12	2.1
Mouse MTV (type B)	1.9	2.6	4.0	1.9	1.4

^a See Table 1.

saturation was also observed by annealing poly(A) (+) RPL RNA with cDNA₃ of RLV or poly(A) (+) M7 RNA with cDNA₃ of MPMV. On the other hand, poly(A) (-) viral RNA showed no hybridization (data not shown). The experiments verified that cDNA₃ was synthesized from the 3' end of the virus RNA when oligo(dT) was used as a primer. Furthermore, it demonstrated that a mouse RLV sequence homologous to a DNA₃ of rat RPL lay near the 3' terminus of the genome RNA. The reciprocal reactions using cDNA₃ of mouse RLV were confirmatory, as described below.

Homology between size-fractionated RLV cDNA and RPL viral RNA. To better ascertain that the RPL viral genome has sequences at the 3' terminus shared by other retroviruses, we performed reciprocal experiments. RLV shared the homologous sequence with RPL in a similar region at the 3' end. The cross-reaction was 30 to 50%. The cDNA₃ of RLV hybridized to RPL most effectively, regardless of which radioactive dXTP was used for cDNA₃ synthesis (data not shown).

When total cDNA of RLV primed with oligonucleotides of calf thymus DNA (37) was incubated with RPL RNA under the same hybrid-

FIG. 1. Hybridization of ³H-labeled RPL cDNA to RPL, RLV 70 S RNA, and poly(A)-containing 10S RNA of RLV. Oligo(dT)₁₀-primed cDNA was prepared from RPL 70S RNA and [³H]dCTP and fractionated into five size classes. cDNA of fraction 3 was used for hybridization. Samples were sealed in 20-μl capillaries containing about 1,000 cpm of cDNA and viral RNA in a buffer containing 0.72 M NaCl, 0.05 M PIPES, NaOH (pH 6.8), 10 mM EDTA, 0.1% SDS, and 50% formamide. After denaturation for 2 to 3 min at 70°C, the capillaries were incubated at 37°C. Radioactivities were measured after S1 nuclease assay. Symbols: (●) RPL 70S RNA; (○ and △) independent preparations of RLV 70S RNA; (▽) poly(A)-containing 10S RNA from RLV.

ization conditions, only 1.7% of RLV cDNA radioactivity hybridized with RPL, in agreement with our previous results (30).

Melting profile of thermal denaturation between RLV cDNA and viral RNA. We determined the extent of sequence divergence within rodent viruses from thermal denaturation profiles of the heteroduplexes. The thermal stability of RLV cDNA₃ (fraction 2) with homologous RLV RNA and heterologous RPL RNA is shown in Fig. 2. Fraction 2 of cDNA₃ was used for the experiments in Fig. 2 because this fraction contains a complete length of the conserved or cross-reacting region between mouse and rat viral genomes. The sequence in the heterologous hybrid was dissociated at approximately 15°C lower than in the homologous hybrid. Therefore, the related sequences of the rodent viruses (mouse and rat) are not strictly preserved but express sequence divergence apparently to the same extent as observed in cellular unique sequences between *Mus* and *Ratus* (8, 22). It must be noted, however, that the homologous region in the hybrid formed between the two rodent viral genomes (RLV and RPL) may range from 80 to 170 nucleotides long, as calculated from the data in Fig. 1 and Table 1. Since larger hybridizable fragments melt at higher temperatures, the thermal stability may be expected 4 to 7°C higher than the apparent thermal profile shown in Fig. 2 (9). All the radioactivity of cDNA incubated with yeast tRNA was eluted from the HA column by a temperature of 65°C.

FIG. 2. Thermal dissociation profile of hybrid molecules formed between RLV cDNA (fraction 2) and RLV and RPL 70S RNA. Symbols: (○) RLV; (●) RPL.

Homology between 58-2T virus cDNA and RNAs of rodent type B and C viruses.

The 58-2T cell is persistently producing a sarcoma virus (Kirsten sarcoma virus), and the viral genome consists of a 30S RNA species in 10-fold excess over 35S RNA (42; N. Tsuchida, personal communication). It is known that the 30S RNAs containing sarcoma viruses are replication defective in that they need helper function of type C viruses that contain a full-sized 35S RNA (1, 19, 41). We found that the replication-defective 58-2T cDNA also contained the conserved region near the 3' end (Table 2). RPL is known to have 35S RNA of rat endogenous virus that is not cross-hybridizable with 30S sarcoma virus-related RNA (28, 40). Since Kirsten sarcoma genome RNA has been shown to have the mouse sequence near the 3' end (35), 42% cross-reaction at the region with RPL suggests that the conserved region in 30S RNA of 58-2T virus is derived from mouse sequences. Another possibility is a related but different sequence derived from rats. Although the exact nature of the region in 58-2T virus remains to be explored in detail, the results of Table 2 indicate that the cross-reacting sequence of mammalian retroviruses is also conserved in the genome of the replication-defective sarcoma virus (58-2T virus) at the 3' end of the 30S RNA.

Homology between size-fractionated RD114 or M7 cDNA and viral RNAs. RD114 was originally isolated from human rhabdomyosarcoma cells grown in a kitten (26). Subsequently, the virus was identified as an endogenous xenotropic cat virus (6, 29). Although the virus is an endogenous virus of domestic cats, immunological and molecular analysis suggested that an ancestral virus endogenous to baboons was transmitted to several species of the cat family and that in the course of evolution the virus has become established in the germ line of these species (4). Table 2 indicates close relatedness between RD114 and M7 at the conserved region near the 3' end of virus RNA. Heteroduplex analysis of the sequence relationship between RD114 and baboon viral RNAs by Hu et al. (18) demonstrated that the regions of high homology lay in the intervals from 1.5 to 2.5 kilobases and from 3.7 to 5.5 kilobases from the 5' end, with no homology near the 3' end. In contrast, by using the enriched cDNA probe, this region in RD114 actually had been more conserved than the total viral genome, which had an average cross-reaction of around 15% with M7 (Tables 2 and 3).

M7 cDNA hybridized to RD114 RNA up to 50% and peaked at fraction 4 (Table 2). Although a certain extent of homology between M7 and

RD114 by using endogenous reaction products has been reported (7, 17, 31), these viruses hybridized far more extensively near the 3' end of the virus genomes of M7 and RD114.

Homology between size-fractionated MPMV cDNA and viral RNAs. Table 2 shows the homology between type D Mason-Pfizer monkey virus (MPMV) cDNA and type C viral RNA. Since the extent of the cross-hybridization between type C and D viruses was low, we attempted to determine the C_{rt} value of the heteroduplex reaction to verify that we were observing the homology of the virus genomes and not other contaminants. Although it was difficult to obtain the $C_{rt1/2}$ values from the low hybridization between MPMV cDNA and M7 RNA, the estimated $C_{rt1/2}$ value (3×10^{-1} mol liter⁻¹) fell into the same range as observed in Fig. 1 (Fig. 3). It is generally known that M7, SSV, and MPMV are quite distinct primate viruses, based on molecular analysis of the viral genomes as well as immunological and virological assays. M7 is an endogenous virus of the baboon, whereas SSV and MPMV are not, but primates are their current hosts. Therefore, it is possible that the homologous sequence found in these distantly related primate viruses may be a conserved sequence among primate viruses or an acquired virus-related host sequence of primates, or both.

Most concentrated region within the conserved sequence of retrovirus RNA. The conserved sequence among the retroviruses tested, despite the variable extents of homology, was located between 50 and 400 nucleotides from the 3' end of viral RNAs (Tables 1-3). The same data also provide information on the location of

FIG. 3. Hybridization of ³H-labeled MPMV cDNA to M7 70S RNA. cDNA of fraction 3 was used for hybridization. The method was as described in Fig. 1.

the most clustered region within the conserved sequence by the following calculation: (percentage of hybridization at a region between fraction N and $N + 1$) = (percentage of hybridization with fraction $N \times$ nucleotide length of fraction N - percentage of hybridization with fraction $N + 1 \times$ nucleotide length of fraction $N + 1$) $\times$ (nucleotide length of fraction N - nucleotide length of fraction $N + 1$)⁻¹.

From this formula, the conserved sequences of RLV, RPL, SSV, M7, and RD114 are particularly concentrated at the region that starts at 80 and ends at 150 nucleotides from the 3' end of retrovirus genomic RNAs.

Comparison of homology between total genome and the conserved region of retroviruses. The homology among retrovirus RNAs at the conserved region exceeded the homology of the total genomes of the viruses under the same stringency of molecular hybridization (Table 3). cDNA₃ prepared from RLV or M7 was enriched at the conserved region. There was little or no cross-reaction of total genomes of the viruses.

DISCUSSION

This is the first report of distinct interval molecular hybridization between rat and mouse type C viruses. The cross-reacting site lay between 50 and 400 nucleotides from the 3' end of genomic RNA in all mammalian retroviruses tested. However, the extent of cross-hybridization of this conserved sequence among the viruses varied according to the relatedness of the viruses within the rodent family, with divergence as shown in Fig. 2. Apparently, the sequence has diverged as much as cellular unique-sequence DNA based on the melting profile of interspecies heteroduplexes (8, 22).

Analysis of cross-reactions of the conserved region may be a method to classify or quantify the phylogenetic relatedness of type C RNA

TABLE 3. Homology of viral RNAs

Viruses	% Homology	
	Total genome ^a	Conserved region ^b
RLV vs PRL	2	40-70
RLV vs MMTV	2	9-12
M7 vs RD114	15	40-50
M7 vs SSV-1	1	7-8
M7 vs MPMV	2	8-23

^a cDNA was synthesized with DNase I-digested oligonucleotides of calf thymus DNA according to Taylor et al. (37). cDNA of RLV was hybridized to RLV, RPL, MMTV, feline leukemia virus, and tRNA. cDNA of M7 was hybridized to M7, RD114, SSV, MPMV, and tRNA. Each radioactivity was determined after S1 nuclease treatment and expressed as percentage of the corresponding homologous hybrid.

^b Results of fractions 2, 3, and 4 of cDNA₃ in Tables 1 and 2 were summarized and expressed by the formula described in the text.

viruses. It has been common to observe no cross-hybridization between rat and mouse viruses, but when the conserved region was analyzed, extensive cross-reaction was obtained.

Cross-hybridization was obtained between MuLV and SSV. Much other evidence confirms that SSV is originally of mouse origin (23). Therefore, RLV, RPL, and SSV are within one group, phylogenetically of rodent origin. Likewise, RD114 and M7 are in one group of baboon origin that has been originally proposed by Todaro's group (6, 23).

The finding that SSV, M7, and MPMV share sequences at this region, in sharp contrast to the lack of cross-hybridization by the total genomes of the viruses, suggests that the conserved regions contain the host sequences which may be the sequences related to the endogenous viruses and be conserved as an important functional unit in the viral life cycle.

The size distribution indicates that the conserved sequence is localized between 50 and 400 nucleotides, particularly clustered between 80 and 150 nucleotides from the 3' terminus of heteropolymeric RNA. However, direct proof of the exact location of the conserved region must await until the sequencing data of the portion in mammalian retroviruses become available.

We can postulate the function of this conserved region. Since the region is retained in all retroviruses regardless of morphological type or immunological differences or defectiveness, the region may be essential for the life cycle of the retroviruses. Several possibilities for the function of this region include: (i) to aid in the integration of the provirus DNA into the host genome; (ii) to transcribe the virus RNA from the provirus DNA; (iii) to terminate the translation of virus mRNA; and (iv) to assemble the virus constituents.

The possible presence of a similar sequence in mammalian cellular DNA has been investigated. Testing for homology at the 100-nucleotide level using cDNA₃ of baboon and mouse retroviruses has provided a method for detecting similar sequences in the genomes of a variety of mammals, including humans (published elsewhere). Besides, the extent and thermal stability of the hybrids between cDNA₃ and cell DNA reflected faithfully the phylogenetic relatedness under less stringent conditions of hybridization (0.72 M NaCl at 67°C) and assays on HA. These observations also confirmed the existence of the conserved region in retroviruses.

ACKNOWLEDGMENTS

We acknowledge the expert technical assistance of Chris Connors and Richard Klein. We are also grateful to Charles

Benton and W. Beard for virus supplies and to R. V. Gilden and H. Okabe for editing the manuscript.

LITERATURE CITED

1. Aaronson, S. A., and W. P. Rowe. 1970. Nonproducer clones of murine sarcoma virus transformed BALB/3T3 cells. *Virology* **42**:9-19.
2. Aviv, H., and P. Leder. 1972. Purification of biologically active globin messenger RNA by chromatography on oligothymidylic acid-cellulose. *Proc. Natl. Acad. Sci. U.S.A.* **69**:1408-1412.
3. Baltimore, D. 1975. Tumor viruses: 1974. Cold Spring Harbor Symp. Quant. Biol. **39**:1187-1200.
4. Benveniste, R. E., M. M. Lieber, D. M. Livingston, C. J. Sherr, G. J. Todaro, and S. S. Kalter. 1974. Infectious C-type virus isolated from a baboon placenta. *Nature (London)* **248**:17-20.
5. Benveniste, R. E., C. J. Sherr, and G. J. Todaro. 1975. Evolution of type-C viral genes: origin of feline leukemia virus. *Science* **190**:886-888.
6. Benveniste, R. E., and G. J. Todaro. 1973. Homology between type-C viruses of various species as determined by molecular hybridization. *Proc. Natl. Acad. Sci. U.S.A.* **70**:3316-3320.
7. Benveniste, R. E., and G. J. Todaro. 1974. Evolution of C-type viral genes: inheritance of exogenously acquired viral genes. *Nature (London)* **252**:456-459.
8. Benveniste, R. E., and G. J. Todaro. 1975. Evolution of type-C viral genes: preservation of ancestral murine type-C viral sequences in pig cellular DNA. *Proc. Natl. Acad. Sci. U.S.A.* **72**:4090-4094.
9. Britten, R. J., D. E. Graham, and B. R. Neufeld. 1974. Analysis of repeating DNA sequences by reassociation. *Methods Enzymol.* **29**:363-418.
10. Coffin, J. M., T. C. Hageman, A. M. Maxam, and W. A. Haseltine. 1978. Structure of the genome of Moloney murine leukemia virus: a terminally redundant sequence. *Cell* **13**:761-773.
11. Fan, H., and D. Baltimore. 1973. RNA metabolism of murine leukemia virus: detection of virus-specific RNA sequences in infected and uninfected cells and identification of virus-specific messenger RNA. *J. Mol. Biol.* **80**:93-117.
12. Friedman, E. Y., and M. Rosbach. 1977. The synthesis of high yields of full-length reverse transcripts of globin mRNA. *Nucleic Acids. Res.* **4**:3455-3471.
13. Haseltine, W. A., and D. G. Kleid. 1978. A method for classification of 5' termini of retroviruses. *Nature (London)* **273**:358-364.
14. Haseltine, W. A., D. G. Kleid, A. Panet, E. Rothenberg, and D. Baltimore. 1976. Ordered transcription of RNA tumor virus genomes. *J. Mol. Biol.* **106**:109-131.
15. Hatanaka, M., R. J. Huebner, and R. V. Gilden. 1971. Specificity of the DNA product of the C-type virus RNA-dependent DNA polymerase. *Proc. Natl. Acad. Sci. U.S.A.* **68**:10-12.
16. Hayward, W. S. 1977. Size and genetic content of viral RNAs in avian oncovirus-infected cells. *J. Virol.* **24**:47-63.
17. Hu, S., N. Davidson, M. O. Nicolson, and R. M. McAllister. 1977. Heteroduplex study of the sequence relations between RD114 and baboon viral RNAs. *J. Virol.* **23**:345-352.
18. Hu, S., N. Davidson, and I. M. Verma. 1977. A heteroduplex study of the sequence relationships between the RNAs of M-MSV and M-MLV. *Cell* **10**:469-477.
19. Huebner, R. J., J. W. Hartley, W. P. Rowe, W. T. Lane, and W. I. Capps. 1966. Rescue of the defective genome of Moloney sarcoma virus from a noninfectious hamster tumor and the production of pseudotype sar-

- coma virus with various murine leukemia viruses. *Proc. Natl. Acad. Sci. U.S.A.* **56**:1164-1169.
20. Joho, R. H., M. A. Billeter, and C. Weissmann. 1975. Mapping of biological functions on RNA of avian tumor virus: location of regions required for transformation and determination of host range. *Proc. Natl. Acad. Sci. U.S.A.* **72**:4772-4776.
 21. Joho, R. H., E. Stoll, R. R. Friis, M. A. Billeter, and C. Weissmann. 1976. A partial genetic map of Rous sarcoma virus RNA: location of polymerase, envelope and transformation markers, p. 127-145. *In* D. Baltimore, A. S. Huang, and C. F. Fox (ed.), *Animal virology*. Academic Press Inc., New York.
 22. Kohn, D. E., J. A. Chiscon, and B. H. Hoyer. 1972. Evolution of primate DNA sequences. *J. Hum. Evol.* **1**: 627-644.
 23. Lieber, M. M., C. J. Sherr, G. J. Todaro, R. E. Benveniste, R. Callahan, and H. G. Coon. 1975. Isolation from the Asian mouse *Mus caroli* of an endogenous type-C virus related to infectious primate type-C viruses. *Proc. Natl. Acad. Sci. U.S.A.* **72**:2315-2319.
 24. Livingston, D. M., and G. J. Todaro. 1973. Endogenous type-C virus from a cat cell clone with properties distinct from previously described feline type-C viruses. *Virology* **53**:141-151.
 25. Loening, U. E. 1969. The determination of the molecular weight of ribonucleic acid by polyacrylamide-gel electrophoresis: the effects of changes in conformation. *Biochem. J.* **113**:131-138.
 26. McAllister, R. M., M. Nicolson, M. B. Gardner, R. W. Rongey, S. Rasheed, P. S. Sarma, R. J. Huebner, M. Hatanaka, S. Oroszian, R. V. Gilden, A. Kabigting, and L. Vernon. 1972. C-type virus released from cultured human rhabdomyosarcoma cells. *Nature (London) New Biol.* **235**:3-6.
 27. Neiman, P. E. 1978. Mapping by competitive hybridization of sequences which differ between endogenous and exogenous chicken leukemia viruses. *Virology* **85**:9-16.
 28. Okabe, H., R. V. Gilden, and M. Hatanaka. 1973. Specificity of the DNA product of RNA-dependent DNA polymerase in type C viruses. II. Quantitative analysis. *Proc. Natl. Acad. Sci. U.S.A.* **70**:3923-3927.
 29. Okabe, H., R. V. Gilden, and M. Hatanaka. 1973b. Extensive homology of RD-114 virus DNA with RNA of feline cell origin. *Nature (London) New Biol.* **244**:54-56.
 30. Okabe, H., R. V. Gilden, and M. Hatanaka. 1974. Specificity of the DNA product of RNA-dependent DNA polymerase in type-C viruses. III. Analysis of viruses derived from Syrian hamsters. *Proc. Natl. Acad. Sci. U.S.A.* **71**:3278-3282.
 31. Okabe, H., R. V. Gilden, M. Hatanaka, J. R. Stephenson, R. E. Gallagher, R. C. Gallo, S. R. Tronick, and S. A. Aaronson. 1976. Immunological and biochemical characterization of type-C viruses isolated from cultured human AML cells. *Nature (London)* **260**: 264-266.
 32. Quintrell, N., H. E. Varmus, and J. M. Bishop. 1974. Homologies among the nucleotide sequences of the genomes of C-type viruses. *Virology* **58**:568-575.
 33. Sanger, F., G. M. Air, B. G. Barrell, N. L. Brown, A. R. Coulson, J. C. Fiddes, C. A. Hutchison III, P. M. Slocombe, and M. Smith. 1977. Nucleotide sequence of bacteriophage ϕ X174 DNA. *Nature (London)* **265**: 687.
 34. Scolnick, E. M., W. Parks, T. Kawakami, D. Kohne, H. Okabe, R. Gilden, and M. Hatanaka. 1974. Primate type C viral nucleic acid kinetics: analysis of model systems and natural tissues. *J. Virol.* **13**:363-369.
 35. Shih, T. Y., H. A. Young, J. M. Coffin, and E. M. Scolnick. 1978. Physical map of the Kirsten sarcoma virus genome as determined by fingerprinting RNase T1-resistant oligonucleotides. *J. Virol.* **25**:238-252.
 36. Tal, J., H.-J. Kung, H. E. Varmus, and J. M. Bishop. 1977. Characterization of DNA complementary to nucleotide sequences adjacent to poly(A) at the 3'-terminus of the avian sarcoma virus genome. *Virology* **79**: 183-197.
 37. Taylor, J. M., R. Illmensee, and J. Summers. 1976. Efficient transcription of RNA into DNA by avian sarcoma virus polymerase. *Biochim. Biophys. Acta* **442**: 324-330.
 38. Todaro, G. J., R. E. Benveniste, S. A. Sherwin, and C. J. Sherr. 1978. MAC-1, a new genetically transmitted type-C virus of primates: "low frequency" activation from stump-tail monkey cell cultures. *Cell* **13**:775-782.
 39. Toozé, J. 1973. The molecular biology of tumour viruses. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
 40. Tsuchida, N., R. V. Gilden, and M. Hatanaka. 1974a. Sarcoma-related RNA sequences in normal cells. *Proc. Natl. Acad. Sci. U.S.A.* **71**:4503-4507.
 41. Tsuchida, N., C. Long, and M. Hatanaka. 1974b. Viral RNA of murine sarcoma virus produced by a hamster mouse somatic cell hybrid. *Virology* **60**:200-205.
 42. Tsuchida, N., M. S. Shih, R. V. Gilden, and M. Hatanaka. 1974. Sarcoma and helper-specific RNA tumor virus subunits in transformed nonproducer mouse cells activated to produce virus by treatment with bromodeoxyuridine. *J. Virol.* **14**:1262-1267.
 43. Wang, L.-H., P. Duesberg, K. Beemon, and P. K. Vogt. 1975. Mapping RNase T₁-resistant oligonucleotides of avian tumor virus RNAs: sarcoma-specific oligonucleotides are near the poly(A) end and oligonucleotides common to sarcoma and transformation-defective viruses are at the poly(A) end. *J. Virol.* **16**:1051-1070.
 44. Wang, L.-H., P. H. Duesberg, S. Kawai, and H. Hanafusa. 1976. Location of envelope-specific and sarcoma-specific oligonucleotides on the RNA of Schmidt-Ruppin Rous sarcoma virus. *Proc. Natl. Acad. Sci. U.S.A.* **73**:447-451.
 45. Wang, L.-H., D. Galehouse, P. Mellon, P. Duesberg, W. S. Mason, and P. K. Vogt. 1976. Mapping oligonucleotides of Rous sarcoma virus RNA that segregate with polymerase and group-specific antigen markers in recombinants. *Proc. Natl. Acad. Sci. U.S.A.* **73**:3952-3956.
 46. Weiss, S. R., H. E. Varmus, and J. M. Bishop. 1977. The size and genetic composition of virus-specific RNAs in the cytoplasm of cells producing avian sarcoma-leukosis viruses. *Cell* **12**:983-992.
 47. Wetmur, J. G., and N. Davidson. 1968. Kinetics of denaturation of DNA. *J. Mol. Biol.* **31**:349-370.