JOURNAL OF VIROLOGY, May 1978, p. 257-264
0022-538X/78/0026-0257$02.00/0

Vol. 26, No. 2
Printed in U.S.A.

# Rescue of Endogenous 30S Retroviral Sequences from Mouse Cells by Baboon Type C Virus

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Received for publication 28 November 1977

*Mus musculus* SC-1 cells were infected with M7 baboon type C virus. The progeny of this infection included viral pseudotypes that contained M7 helper virus and endogenous 30S retrovirus-associated sequences derived from SC-1 cells (RAS). The RAS sequences are unrelated by nucleic acid hybridization criteria to previously described types of murine retroviruses and do not code for known murine viral structural proteins. The RAS genome is present in multiple copies in the DNA of laboratory (*M. musculus*) and Asian (*M. caroli* and *M. cervicolor*) mice, is expressed in the RNA of uninfected mouse cells, and can be efficiently rescued by type C, but not type B, viruses. RAS is closely related to 30S virus-associated RNA in NIH/3T3 and BALB/c JLSV-9 cells and may be analogous to the defective 30S RNA sequences found in rats.

Endogenous, genetically transmitted retroviruses of mice, isolated from laboratory (*Mus musculus*) and Asian (*M. caroli* and *M. cervicolor*) species, can be classified into at least three distinct groups. These include type C viruses isolated from both domestic and Asian mice, type B viruses obtained to date only from *M. musculus*, and "B-like" viruses thus far isolated from *M. cervicolor* and *M. caroli* (4, 14). Type C viruses can be further classified on the basis of host range as either xenotropic, ecotropic, or amphotropic. Although representative members of each of these viral groups have not been isolated from each species, all mouse species contain multiple copies of each set of endogenous viral genes in their normal cellular DNA (4). Moreover, hybridization of DNA viral transcripts frequently detects the transcription of these sequences in mouse cellular RNA, in cases in which transmissible virus is not released (R. Callahan and R. Benveniste, unpublished data). Therefore, it is theoretically possible to recover virus-related sequences by pseudotyping with helper retroviruses.

This paper reports the isolation from mouse cells of a unique set of endogenous retroviral RNA sequences that are possibly associated with a 30S RNA subunit molecule and are unrelated by hybridization criteria to all of the above classes of murine retroviruses. These sequences (hereafter designated "RAS" for "retrovirus-associated sequences") can be rescued from *M. musculus* SC-1 cells by infection with baboon type C virus. A DNA transcript of the resultant viral pseudotype can detect multiple copies (25 to 30/haploid genome) in uninfected mouse DNA, as well as varying degrees of expression in mouse cytoplasmic RNA. The sequences do not code for known retroviral proteins, and passage to nonmouse cell lines results in the loss of the mouse-specific sequences. In several respects, RAS may be analogous to the defective sarcoma virus-associated RNA sequences previously detected in rat cells (26, 29).

## MATERIALS AND METHODS

**Cell lines.** The cell lines used in these experiments included the human A549 lung tumor cell line derived in this laboratory (22); rabbit corneal cells, SIRC (CCL 60), obtained from the American Type Culture Collection, Rockville, Md.; and canine thymus FCf2Th cells, obtained from the Naval Biochemical Research Laboratory, Oakland, Calif. The following mouse cell lines were also used: BALB/3T3 (1), NIH/3T3 (20), JLSV-9 (30), SC-1 (19), C3H/10T1/2 (24), and Mm5mt/$c_1$ (17). Dog kidney cells nonproductively transformed by Moloney mouse sarcoma virus (MSV/DK) were supplied by Paul Peebles (National Cancer Institute). A normal tree shrew cell line obtained from Joseph Melnick (Baylor University) was used to obtain a Kirsten virus-transformed nonproducer clone in this laboratory. All cells were grown in Dulbecco-modified Eagle medium, using 10% calf or fetal calf serum, and were transferred by using 1% trypsin in buffered saline.

**Viruses.** Type C viruses used in these studies included the endogenous type C virus of baboon, M7, isolated from *Papio cynocephalus* (6); ecotropic virus (Rauscher murine leukemia virus [R-MuLV]) and dual host range virus (64 PL-CL11) (U. R. Rapp and G. J. Todaro, Proc. Natl. Acad. Sci. U.S.A., in press) from

*M. musculus*; the xenotropic virus (MOL-X) isolated from *M. musculus molossinus* (21); and the xenotropic viruses from *M. cervicolor* designated CERV C I and CERV C II (4). Other viruses employed included mouse mammary tumor virus (MMTV) and M432, the B-like virus from *M. cervicolor* (13). For synthesis of $^3$H-labeled DNA transcripts and extraction of 70S viral RNA, supernatant medium from virus-producing cultures was harvested at 4- to 12-h intervals and stored at 4°C (9). Viruses were concentrated by centrifugation and banded isopycnically in sucrose to a density of 1.16 g/cm$^3$ before use (28).

**Radiolabeling of viral RNA.** Virus-producing cells were labeled with [$^{32}$P]phosphoric acid, and RNA was extracted from labeled virions and purified on 15 to 30% neutral sucrose gradients according to previously published methods (27). High-molecular-weight RNA was denatured in 0.05 M Tris-borate buffer (pH 8.3) containing 50% formamide for 1 min at 65°C. The denatured RNA was subjected to electrophoresis for 60 min at 4°C in 0.5% agarose–1.5% acrylamide composite cylindrical gels (23) at 5 mA/gel. $^3$H-labeled 28S and 18S RNA standards (Schwarz/Mann, Orangeburg, N.Y.) were tested in the same gels. Gels were fractionated into 1-mm slices, and radioactive material was eluted (3) and counted in a liquid scintillation spectrometer, using appropriate discriminator settings for dual-isotope determination. The data shown have been corrected for spillover (~10%) of $^{32}$P into the $^3$H channel.

**Preparation of [$^3$H]DNA transcripts.** [$^3$H]thymidine-labeled DNA transcripts of viral RNA were prepared by using a 2-h endogenous reverse transcriptase reaction in the presence of 2.8 mM magnesium acetate and 30 μg of actinomycin D per ml (10). [$^3$H]DNA transcripts were then purified and sedimented in alkaline sucrose (5). The specific activity of the [$^3$H]DNA was approximately $1.5 \times 10^7$ cpm/μg.

**Nucleic acid hybridizations.** Viral RNA and cellular RNA and DNA were extracted by previously published methods (5). Nucleic acid hybridization reactions were performed with $^3$H-labeled viral transcripts and different viral or cellular nucleic acids. The hybridization reaction mixtures contained 0.01 M Tris-hydrochloride (pH 7.8), 0.75 M NaCl, 0.001 M EDTA, 0.05% sodium dodecyl sulfate, approximately 25,000 cpm of [$^3$H]DNA per ml, and either 2 to 4 mg of cellular nucleic acid per ml or 0.5 to 1.0 μg of 70S viral RNA per ml. Hybridization reactions were initiated by heating the reaction mixtures for 10 min at 106°C, followed by incubation for various times at 65°C. Hybrids were detected by using the single-strand-specific nuclease $S_1$ (2, 7). $C_0t$ values were calculated by the method of Britten and Kohne and corrected to a monovalent cation concentration of 0.18 M (11, 12). Hybridization reactions were also performed using $^{32}$P-labeled viral RNA and various cellular DNAs. For the latter assays, the reaction mixture described above was modified to include 50% formamide and 100 μg of carrier tRNA per ml. Hybridization reactions using labeled RNA were performed at 41°C instead of 65°C so as to minimize RNA degradation. [$^{32}$P]RNA:DNA hybrids were detected by using RNases A and $T_1$ (Sigma Chemical Co., St. Louis, Mo.).

## RESULTS

**Isolation and characterization of a viral pseudotype containing RAS sequences.** In an attempt to recover mouse virus-associated sequences, *M. musculus* cells were infected with several helper type C viruses that are distantly related to endogenous mouse viruses and grow poorly in cell lines derived from laboratory mice. In preliminary experiments, we determined that the M7 baboon virus replicates at low levels in NIH/3T3 and SC-1 cells (unpublished data). For this reason, human A549 cells producing M7 virus were cocultivated with SC-1 cells for 4 weeks, and the filtered medium from these cultures was used to infect fresh SC-1 cells. After 3 weeks, manganese-dependent viral polymerase activity was detected in the culture supernatants. The virus produced by these cultures will be referred to as RAS (M7) in experiments described below.

To determine whether RAS (M7) contained mouse-specific viral sequences, we conducted a series of experiments to detect the presence of known mouse retroviral proteins. In these experiments, the host range, viral polymerase activity, p30 protein, and phosphoproteins of RAS (M7) were studied and compared with an M7 control. In every experiment, RAS (M7) was indistinguishable from M7 baboon virus (data not shown). There was no evidence for known mouse retroviral proteins in RAS (M7). It was therefore concluded that any mouse-specific viral sequences in RAS (M7) must be defective in terms of coding for identifiable murine viral proteins.

**RAS (M7) virus contains 30S RNA subunits.** To further characterize RAS (M7), $^{32}$P-labeled RNA was extracted from RAS (M7) virus and M7 virus grown on human A549 cells and was purified on 15 to 30% neutral sucrose gradients. M7 viral RNA sedimented as a distinct peak in the 70S region of the gradient. In contrast, RAS (M7) contained high-molecular-weight RNA molecules sedimenting between 50 and 70S without any distinct peak of radioactivity (data not shown). 50 to 70S RNA from RAS (M7) and from M7 virus were then heat denatured and subjected to electrophoresis in 0.5% agarose–1.5% acrylamide composite gels. As shown in Fig. 1, two distinct size classes of subunit RNA molecules were resolved from denatured RAS (M7) 50 to 70S RNA. The larger species migrated as molecules of 35S, probably corresponding to the 35S subunit of the M7 component of RAS (M7). In addition, a smaller 30S molecule (molecular weight, approximately $2.5 \times 10^6$) was detected in RAS (M7) viral RNA.

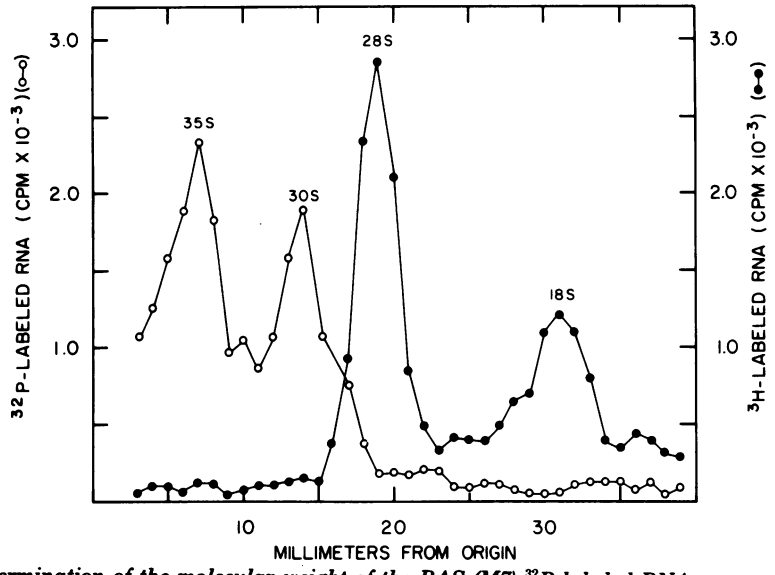


FIG. 1. *Determination of the molecular weight of the RAS (M7) $^{32}$P-labeled RNA genome. 50 to 70S $^{32}$P-labeled RNA from a neutral sucrose gradient was heat denatured and subjected to electrophoresis in an agarose-acrylamide gel. $^{3}$H-labeled markers were tested in the same gel, as described in the text. Symbols: O, RAS (M7); ●, $^{3}$H-labeled marker.*

This 30S RNA molecule was not present in the A549-grown M7 control (data not shown) and was therefore presumed to be derived from the mouse SC-1 cells producing RAS (M7).

**$^{32}$P-labeled RAS (M7) viral RNA hybridizes to baboon and mouse cellular DNA.** $^{32}$P-labeled RAS (M7) viral RNA was hybridized to mouse and baboon cellular DNA in an attempt to determine the origin of 30S RNA sequences in the virions. At a $C_0t$ value of approximately $10^4$ mol × s/liter, pooled 50 to 70S RAS (M7) viral RNA hybridized to a final extent of 55% to baboon cellular DNA and 45% to mouse cellular DNA. This result showed that sequences derived from the SC-1 mouse genome were present in RAS (M7) and might possibly be represented by the 30S RNA subunit molecules. The mouse-specific sequences of RAS (M7) were further characterized by nucleic acid hybridization studies, using [$^{3}$H]DNA transcripts of the viral RNA, as discussed below.

**Detection of mouse sequences in [$^{3}$H]-DNA transcripts of RAS (M7) virus.** A [$^{3}$H]DNA transcript of RAS (M7) virus was prepared by using the endogenous reverse transcriptase reaction. The products of the reaction ranged in size from 8S to 20S as determined by sedimentation in alkaline sucrose gradients. Two separate pools from the sucrose gradient with average transcript sizes of 14S and 20S, respectively, were tested for the presence of baboon and mouse sequences by hybridization to representative cellular RNAs. The results of these experiments are shown in Table 1. The 20S transcript of RAS (M7) contained primarily baboon type C viral sequences, but also a small percentage of mouse-specific sequences. In contrast, the 14S transcript of RAS (M7) contained primarily mouse-specific sequences, with a lower percentage of baboon viral sequences. Comparable results have been obtained by using transcripts prepared over a period of 3 months from cultures of SC-1 cells chronically producing the RAS (M7) virus, suggesting that the proportion of mouse and baboon sequences is stable. When the 14S and 20S transcripts were pooled and hybridized to baboon viral RNA and mouse cellular RNA, 59% hybridization to baboon and 47% hybridization to mouse RNA were detected (Table 1). It is of note that the relative propor-

TABLE 1. *Comparison of the 14S and 20S $^{3}$H-labeled transcripts of RAS (M7) virus*

| Cellular RNA | % Hybridization[a] | | |
|---|---|---|---|
| | 20S transcript | 14S transcript | Pooled transcripts |
| Mouse SC-1 infected with RAS (M7) | 100 | 100 | 100 |
| Human A549 infected with M7 | 82 | 40 | 59 |
| Uninfected SC-1 | 25 | 60 | 47 |
| Rabbit liver | <1 | <1 | <1 |

[a] The percent hybridization is normalized. The actual percentage for homologous hybridizations at a $C_rt$ of 10,000 was >90%.

tion of baboon and mouse sequences in the pooled [$^3$H]DNA transcripts corresponds to the relative proportion of baboon and mouse sequences detected in $^{32}$P-labeled RAS (M7) 50 to 70S RNA. This suggests an equal transcription efficiency for the two sets of sequences in the endogenous reverse transcriptase reaction.

**Mouse-specific sequences of RAS (M7) are lost after passage to a nonmouse cell line.** To further define the nature of the association between the mouse and baboon sequences in the RAS (M7) virus, human A549 cells were infected with the virus, and a [$^3$H]DNA transcript of progeny virions was prepared. As shown in Table 2, a DNA transcript of RAS (M7) produced by human A549 cells contained a full representation of the baboon viral genome, but did not hybridize to SC-1 cellular RNA. Thus, the mouse-specific sequences present in RAS (M7) grown on SC-1 cells were lost when the virus was infected onto a nonmouse cell line. Moreover, when a DNA transcript of M7 baboon virus was hybridized to the RNA of SC-1 cells producing RAS (M7) virus, complete (>95%) copies of the M7 genome were detected. This suggests that a stable mouse-baboon recombinant, in which a major portion of the baboon genome is missing, does not comprise the majority of RAS (M7) virus particles. Taken together, these results indicate that RAS (M7) virus is a pseudotype comprised of M7 helper virus and rescued RAS sequences from mouse cells.

**RAS is present in multiple copies in normal mouse cellular DNA.** To determine whether the mouse-specific sequences of RAS (M7) were present in mouse DNA in single or multiple copies, the 14S RAS (M7) DNA transcript was hybridized to the DNA of various rodent species. The results of these experiments are shown in Fig. 2. The greatest degree of hybridization was seen with *M. musculus* DNA, whether obtained from BALB/c tissue (spleen) or from the NIH/3T3 and SC-1 cell lines. A lower final extent of hybridization was detected with the DNA from either *M. caroli* or *M. cervicolor* tissues, corresponding to the evolutionary divergence of Asian and domestic mice as previously determined for retroviral genes (4). In contrast, RAS (M7) did not hybridize to other rodent species such as rat or hamster, as shown.

The reiteration frequency of RAS (M7) in *M. musculus* DNA, as well as in *M. cervicolor* and *M. caroli* DNA, was calculated from the $C_0t$ analyses shown in Fig. 2 (11). The $C_0t_{1/2}$ for the hybridization of the RAS (M7) viral transcript to various mouse cellular DNAs was 50 to 60 mol × s/liter. Because the $C_0t_{1/2}$ for the self-annealing of nonrepetitive BALB/3T3 cellular

TABLE 2. *Loss of mouse-specific sequences during passage of RAS (M7) to nonmouse lines*

| Cellular RNA | % Hybridization to [$^3$H]DNA transcripts of:[a] | | |
|---|---|---|---|
| | M7 grown on human A549 | RAS (M7) grown on mouse SC-1 | RAS (M7) grown on human A549 |
| Mouse SC-1 infected with RAS (M7) | 96 | 100 | 92 |
| Human A549 infected with M7 | 100 | 40 | 92 |
| Uninfected SC-1 | 3 | 60 | 2 |
| Rabbit liver | <1 | <1 | <1 |

[a] The percent hybridization is normalized. The actual final extent of the homologous hybridization was >90% at a $C_rt$ of 10,000. The DNA transcripts were approximately 14S in size as determined on alkaline sucrose gradients.

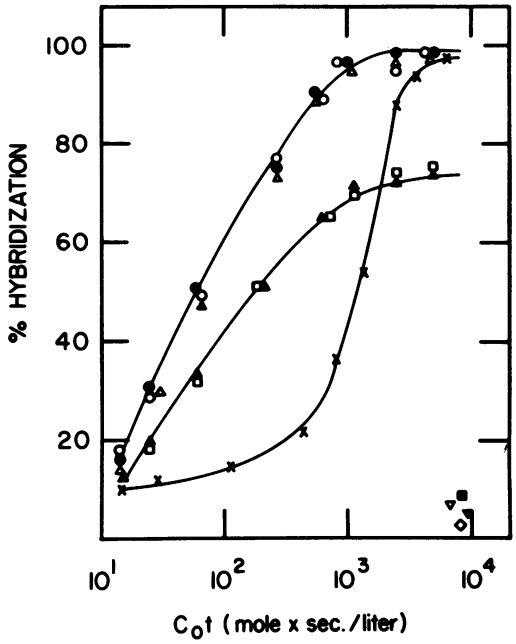


FIG. 2. *Hybridization of the 14S [$^3$H]DNA transcript of RAS (M7) to the DNA of various species. DNA was extracted and tested from M. musculus (BALB/c spleen [●], SC-1 cells [○], and NIH/3T3 cells [△]), M. cervicolor lung (□), M. caroli spleen (▲), rat liver (■), hamster spleen (▽), human A549 cells (▼), and sheep liver (◇). The percent hybridization is expressed as the percentage of available mouse-specific sequences in the RAS (M7) transcript that was saturated by the various cellular DNAs. The 14S RAS (M7) transcript contained approximately 60% mouse-specific sequences and 40% baboon-specific sequences, as shown in Table 1. The self-annealing of unique-sequence BALB/3T3 cell line DNA is shown for reference* (×).

DNA is approximately $1.5 \times 10^3$ mol × s/liter, there are approximately 25 to 30 copies of RAS sequences per haploid genome. Thus, the mouse sequences present in the RAS (M7) viral transcript are present in multiple copies in the DNA of *M. musculus*, and related sequences are present at a similar reiteration frequency in the DNA of the two Asian *Mus* species.

**RAS is unrelated to known murine retroviruses.** To determine the relatedness of RAS to known murine retroviruses, experiments were performed in which a variety of mouse viral [$^3$H]DNA transcripts were hybridized to 50 to 70S RNA extracted from the RAS (M7) virus. The mouse viruses selected for these experiments were representative of the known classes of murine retroviruses. Included were xenotropic, ecotropic, and dual host range type C viruses and type B virus from *M. musculus*, as well as type C I, type C II, and type B-like viruses from *M. cervicolor*. The results of these experiments are shown in Fig. 3. As indicated, there was no hybridization above background between any of these mouse viral transcripts and RAS (M7) 50 to 70S RNA. Negative results were also obtained when the same viral transcripts were hybridized to cytoplasmic RNA from SC-1 cells producing RAS (M7) virus (data not shown). This suggests a lack of homology between RAS and any of the classes of murine retroviruses tested. However, as shown in Fig. 3, RAS (M7) viral RNA did hybridize fully to the [$^3$H]DNA transcripts generated from 30S virus-associated RNA derived from both NIH/3T3 and JLSV-9 cells. Moreover, since the $C_rt_{1/2}$ for the latter two hybridizations approximated that of the homologous reaction with RAS (M7) ($2 \times 10^{-1}$ mol × s/liter), it is likely that all three transcripts represent the same set of sequences. Thus, the RAS sequences rescued from SC-1 cells by M7 virus are unrelated to previously described murine retroviruses, but are very closely related to the 30S virus-associated RNA sequences present in NIH/3T3 and JLSV-9 cells.

Finally, to detect any possible relationship between RAS and known rodent sarcoma viruses, the [$^3$H]DNA RAS (M7) transcript was hybridized to the RNA of two nonmouse cell lines producing Kirsten and Moloney sarcoma viruses, respectively. In each case, there was no hybridization detected between RAS (M7) and the RNA tested at a $C_rt$ of $10^4$ mol × s/liter (data not shown). This demonstrated that RAS is unrelated by these criteria to at least two of the known rodent sarcoma viruses.

**RAS is expressed in mouse cell RNA.** Figure 4 shows the results of experiments in which the [$^3$H]DNA RAS (M7) transcript was hybridized to the cellular RNA of several uninfected

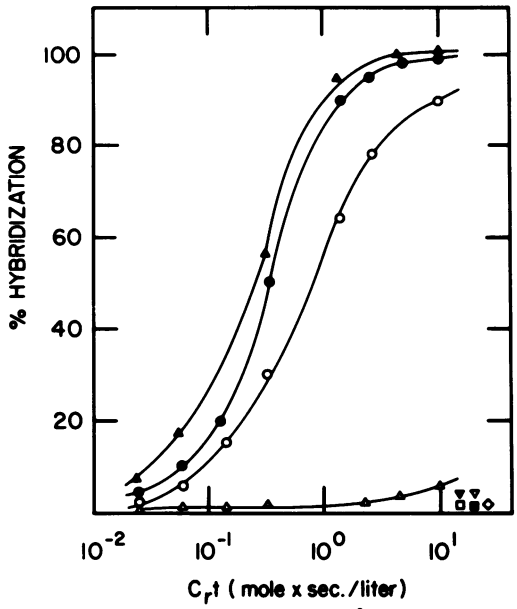


FIG. 3. *Hybridization of various $^3$H-labeled viral DNA transcripts to 50 to 70S RNA extracted from RAS (M7) virus. The DNA transcripts tested were prepared from: RAS (M7) grown on SC-1 cells (●); 30S virus-associated RNA from JLSV-9 cells (○); 30S virus-associated RNA from NIH/3T3 cells (▲); R-MuLV grown on NIH/3T3 cells (△); 64 PL-CL11 grown on NIH/3T3 cells (▼); MOL-X grown on FCf2Th cells (▽); MMTV grown on Mm5mt/$c_1$ cells (□); CERV C I grown on SIRC cells (■); and M432 grown on NIH/3T3 cells (◇). The DNA transcripts of 30S virus-associated RNA from JLSV-9 and NIH/3T3 cells were provided by D. Baltimore and P. Besmer, and E. Scolnick, respectively. The viruses and cell lines are described in the text. The percent hybridization has been normalized. The actual final extents of hybridization for the homologous reactions were >90%.*

mouse cell lines. The cell lines tested included SC-1 (from which RAS was originally obtained), JLSV-9, NIH/3T3, BALB/3T3 (clone A31), Mm5mt/$c_1$ (data not shown), and C3H/10T1/2. All of these lines, with the exception of C3H/10T1/2, were found to express sequences related to RAS in their cytoplasmic RNA. As indicated by the $C_rt_{1/2}$ of these hybridization reactions, there are high levels of expression in SC-1 and JLSV-9 cells, as much as a 10-fold-lower level of expression in NIH/3T3 cells, and even lower levels of expression in BALB/3T3 (clone A31) cells. It is of note that the only completely negative mouse cell line tested, C3H/10T1/2, is a clonal line that displays postconfluency inhibition of division and is nontumorigenic in vivo (24; unpublished data).

**Rescue of RAS by other retroviruses.** In view of the observations that several mouse cell

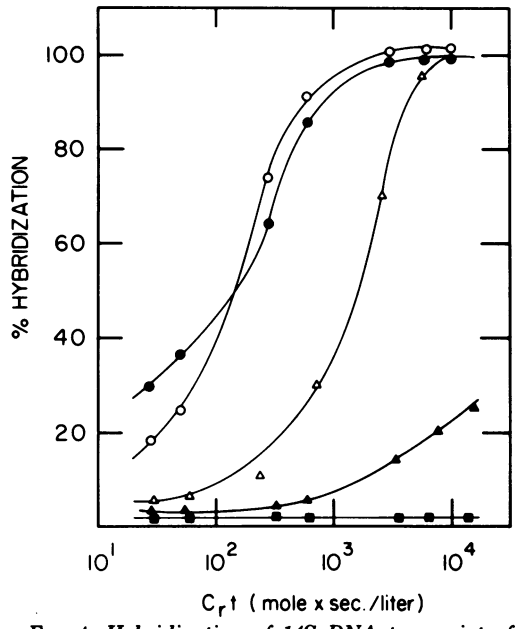


FIG. 4. *Hybridization of 14S DNA transcript of RAS (M7) to cytoplasmic RNA of various M. musculus cell lines. The percent hybridization is expressed as the percentage of available mouse sequences in the RAS (M7) transcript that hybridized to mouse cellular RNA. The 14S RAS (M7) transcript contained 60% mouse-specific sequences and 40% baboon-specific sequences, as shown in Table 1. Symbols: ●, SC-1; ○, JLSV-9; △, NIH/3T3; ▲, BALB/3T3 (clone A31); ■, C3H/10T1/2.*

lines expressed RAS-related sequences in their RNA, experiments were conducted to compare the efficiency of rescue of the RAS genome by M7 and other retroviruses. In preliminary experiments, the [$^3$H]DNA RAS (M7) transcript was shown to detect the presence of closely related sequences in the 50 to 70S RNA of ecotropic murine type C viruses (data not shown). Because RAS had been shown to be unrelated to these same viruses by hybridization criteria (Fig. 3), it was concluded that these viruses were rescuing RAS-related sequences from the mouse cells in which they were grown.

Figure 5 compares the efficiency of rescue of the RAS genome by type C viruses, such as M7 and R-MuLV, and type B or B-like viruses, such as MMTV and M432. These viruses were grown on SC-1, NIH/3T3, or Mm5mt/$c_1$ cells, all shown in separate experiments to contain RAS in their cytoplasmic RNA (see Fig. 4, for example). For each 50 to 70S viral RNA tested in Fig. 5, the hybridization of the RAS (M7) DNA transcript was compared with the hybridization of the homologous helper virus transcript. As shown, the level of RAS-related sequences was high in RAS (M7) and R-MuLV viral RNA, but was low or undetectable in M432 and MMTV viral RNA. Thus, type B-like and type B viruses are distinctly less efficient in rescuing the RAS genome than are type C viruses. Moreover, type C viruses other than M7, such as R-MuLV, can efficiently rescue RAS sequences from mouse cells. However, perhaps because the M7 helper virus grows poorly in mouse cells, the RAS (M7) pseudotype is unique with regard to the high proportion of rescued as compared with helper sequences, particularly as represented in complementary DNA transcripts.

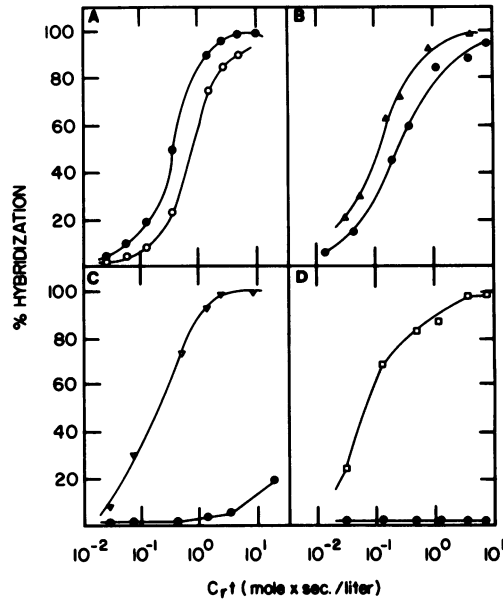


FIG. 5. *Relative efficiencies of rescue of RAS by various retroviruses. For each 50 to 70S viral RNA tested, the hybridization of the RAS (M7) DNA transcript was compared with the hybridization of the homologous helper virus DNA transcript. The percent hybridization is normalized for each homologous hybridization, with the actual final extent of hybridization exceeding 90% in all instances. The percent hybridization to the 14S RAS (M7) DNA transcript is expressed as the percentage of the available mouse sequences (60%) that is saturated. (A) Hybridization of RAS (M7) grown on SC-1 (●) and M7 grown on A549 (○) DNA transcripts to RAS (M7)/SC-1 viral RNA; (B) hybridization of RAS (M7) grown on SC-1 (●) and R-MuLV grown on NIH/3T3 (△) DNA transcripts to R-MuLV/NIH viral RNA; (C) hybridization of RAS (M7) grown on SC-1 (●) and M432 grown on NIH/3T3 (▽) DNA transcripts to M432/NIH viral RNA; (D) hybridization of RAS (M7) grown on SC-1 (●) and MMTV grown on Mm5mt/$c_1$ (□) DNA transcripts to MMTV/Mm5mt/$c_1$ viral RNA. All of the mouse cell lines used to grow these viruses contain RAS in their cellular RNA.*

## DISCUSSION

In this paper we report the rescue of endogenous 30S retroviral sequences from mouse cells by baboon type C virus. These sequences were initially detected in a viral pseudotype designated RAS (M7), which was obtained by infecting mouse SC-1 cells with M7 baboon virus. The mouse-specific RAS sequences are most likely associated with a 30S RNA subunit molecule. The RAS genome is endogenous and present in multiple copies (25 to 30/haploid genome) in the DNA of laboratory and Asian *Mus* species. RAS sequences are widely expressed in uninfected mouse cellular RNA and can be efficiently rescued from those cells by type C viruses but not be type B viruses. Although the RAS genome is unrelated by both antigenic and nucleic acid hybridization criteria to previously described classes of murine retroviruses, RAS appears to be closely related to 30S virus-associated RNA obtained from NIH/3T3 and JLSV-9 cells (16). In fact, it is likely that the RAS sequences are closely related to 30S RNA sequences associated with Kirsten MuLV grown in NIH/3T3 cells and Moloney MuLV grown in mouse TB cells, as recently described (15).

Whether RAS sequences represent a defective mouse viral genome or an as yet undefined class of nonviral cellular RNA sequences has not been definitely established. However, in several respects, the RAS sequences resemble a defective retroviral genome: (i) RAS sequences can be specifically complexed with 50 to 70S viral RNA; (ii) the genome is present in mouse DNA in multiple copies at a reiteration frequency (25 to 30) generally observed for known retroviral genes (8); and (iii) RAS sequences can be efficiently rescued from mouse cells by type C, but not type B, viruses, a property previously attributed to defective type C viral genomes (18).

If the RAS sequences represent a defective retroviral genome, the possibility arises that RAS is analogous to the sarcoma virus-related cellular RNA sequences of rats. Rat cells have been shown to contain 30S RNA sequences that, like RAS, are present in multiple copies in rat DNA, are expressed in uninfected rat cell RNA, and can be efficiently packaged in extracellular viral particles (26, 29). The rat 30S RNA sequences differ from RAS, however, in that only the former are related by nucleic acid hybridization criteria to known rodent sarcoma viruses (25, 26). Nevertheless, the RAS genome may have an association with the transformed phenotype in a not as yet understood manner, since the RAS sequences are expressed at high levels in transformed mouse cells and at low or undetectable levels in nontransformed cells such as BALB/3T3 (clone A31) and C3H/10T1/2.

### ACKNOWLEDGMENTS

We thank E. Scolnick, National Cancer Institute, for providing the [$^3$H]DNA transcript of 30S NIH/3T3 RNA and D. Baltimore and P. Besmer, Massachusetts Institute of Technology, for providing the [$^3$H]DNA transcript of 30S JLSV-9 RNA. MMTV viral RNA and [$^3$H]DNA transcript were kindly supplied by J. Schlom. We also thank C. Sherr and R. Callahan for many helpful discussions. Excellent technical assistance was provided by B. Detwiler, N. Cope, E. Cusick, G. L. Wilson, and R. Heinemann.

This work was supported by the Virus Cancer Program of the National Cancer Institute.

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