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Quantitative Analysis of the Rescue of RNA Sequences by Mammalian Type C Viruses

ROBERT J. GOLDBERG,* REUVEN LEVIN, WADE P. PARKS, AND EDWARD M. SCOLNICK
National Cancer Institute, Bethesda, Maryland, 20014

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The specificity and quantitation of the rescue of RNA sequences by mammalian type C viruses has been investigated. Type C viruses can package with specificity only type C viral RNA. Type C viruses do not encapsidate with comparable efficiency either type B viral or cellular globin mRNA. Conversely, a non-type C mammalian retravirus, MP-MV, cannot encapsidate type C RNA. A revertant of Kirsten sarcoma virus (Ki-SV)-transformed nonproducer cells which fails to rescue biologically active Ki-SV after superinfection with helper virus had no detectable intracellular Ki-SV-specific RNA. The results suggest specific mechanisms by which type C viral proteins can package type C viral RNA and provide an approach to classifying RNA of potentially defective endogenous retraviruses as type C in origin.

Increasing evidence suggests the wide-spread occurrence of genetic recombination between type C viruses and the involvement of this process in the origin of some oncogenic type C viruses. Infection of cells with different avian type C sarcoma viruses results in a high frequency of genetic recombination between either exogenous viruses, or endogenous and exogenous viral information (12, 13, 32, 33). Although recombination in cell culture has not been rigorously proven in mammalian systems (28), the Kirsten and Harvey sarcoma viruses apparently acquired their fibroblast-transforming potential in animals by recombination between mouse type C viruses and rat genetic information (26, 27). Little is known about the specificity or the mechanisms of recombination of type C viral nucleic acids in either avian or mammalian systems. Based on several biological and genetic observations, the first step in the formation of recombinants is presumed to be the heterozygotic packaging of heterologous viral RNA molecules (13, 33).

To study the specificity of packaging of RNA by type C viruses, we have examined several systems in which various mammalian viral and cellular RNAs could be distinguished by molecular hybridization. Quantitative studies of intracellular and extracellular levels of RNAs in cells infected with different reverse transcriptase containing viruses (retraviruses) revealed that mammalian type C viruses specifically encapsidated and purified other type C viral RNAs; the level of incorporation of heterologous type C RNA into extracellular type C particles was proportional to intracellular levels of RNA. Conversely, type C RNA could not be packaged with comparable efficiency by non-type C retraviruses.

MATERIALS AND METHODS

Cells. BALB/c 3T3 and mink lung fibroblast cells transformed but not producing Kirsten (Ki) sarcoma virus (Ki-SV), Ki-BALB, and Ki-Mink have been described (14, 25). A clonal derivative of the continuous feline kidney cell line (Crandell—CCC3a) not producing RD-114 virus was obtained from Peter Fischinger, National Cancer Institute, Bethesda, Md. (8). The mouse erythroleukemia cell line, T-3-C1-2, was obtained from Jeffrey Ross, Madison, Wisc., and induced to make globin mRNA by exposure to culture medium containing 1% dimethyl sulfoxide (ME_2SO) for 3 days at 37 C (15, 16). Characterization of the cell line C_3H-MT C1 12 derived from the murine mammary tumor cell line of Owens and Hackett (19) has recently been reported (E. M. Scolnick, H. A. Young, and W. P. Parks, Virology, in press); this cell line produces type B viral RNA but does not produce detectable levels of extracellular type B virus. Cell lines were propagated in the Dulbecco modification of Eagle medium containing 10% fetal calf serum (Ki-Mink, CCC3a, and C_3H-MT C1 12 cells) or 10% calf serum (Ki-BALB or T-3-C1-2 cells). All cells were monitored by aerobic and anaerobic culture techniques for mycoplasma and were found to be negative. A spontaneous morphologic revertant of a twice-cloned thioguanine-resistant Ki-BALB cell was obtained by modification of the selection procedure in methylcellulose containing bromodeoxyuridine (34). Full details of this selection process will be presented elsewhere. This revertant failed to form colonies at a frequency of $<10^{-4}$ in soft agar and had a 10-fold lower saturation density than Ki-BALB. As noted in

earlier examples of certain Ki-BALB revertants, murine leukemia virus (MuLV) replicated in such cells but failed to rescue transforming Ki-SV from the revertants (11, 17, 20).

Viruses. The Moloney (Mo) and Rauscher strains of MuLV were grown in NIH 3T3 cells as previously described (26). Kirsten strain of murine Leukemia and sarcoma virus (Ki/MuLV/Ki-SV) was propagated in NRK cells as previously described (1). The AT-124 xenotropic murine type C virus (30) was grown in the mink lung cell line. RD-114 and Mason-Pfizer monkey viruses (MP-MV) were propagated in a human rhabdomyosarcoma cell and human lymphocyte cells (NC37), respectively, and obtained from Pfizer Laboratories (Maywood, N.J.). These viruses and avian myeloblastosis virus were obtained through the Office of Program Resources and Logistics of the Virus Cancer Program, National Cancer Institute.

Hybridization. Conditions for hybridization, synthesis of cDNA probes, and analysis of ^{3}H-labeled DNA·RNA hybrids with S1 nuclease have been reported (1). cDNA probes used hybridized to 50 to 80% of the input trichloroacetic acid counts per minute and all hybridizations were normalized to 100%. Specific input trichloroacetic acid counts per minute and conditions for each hybridization reaction are noted in the legends of corresponding figures and tables. C_rt values were corrected to 0.18 M monovalent cation concentration (5) and were calculated according to the procedures of Birnsteil et al. (3).

In those cases where viral RNAs were purified from relatively small volumes of tissue culture fluids, hybridization was analyzed as a function of the relative volume of RNA solution tested and time (V_0t) as recently suggested by Ringold et al. (23). V_0t values were determined at the same time on the same cells from which RNA was extracted for C_rt analysis to be able to relate the intracellular and extracellular RNA ratios on the same culture.

Synthesis of virus-specific ^{3}H-labeled DNA. Single-stranded ^{3}H-labeled DNA transcripts were synthesized in endogenous reactions from sucrose density gradient banded viruses in the presence of actinomycin D as previously described (1; Scolnick et al., Virology, in press). A ^{3}H-labeled DNA transcript was enriched for Ki-SV rat genetic sequences by recycling (25) a cDNA prepared from Ki-MuLV/Ki-SV with total cellular RNA extracted from uninfected NRK cells to a C_rt of 10^4 mol/s per liter. This cell contains an excess of Ki-SV-specific RNA compared to levels of rat type C (RT21C) sequences (25). The S1-resistant ^{3}H-labeled cDNA was recovered by extraction with equal volumes of phenol and chloroform-isoamyl alcohol (24:1) after addition of one-tenth volume of 10% sodium dodecyl sulfate. The RNA was removed by treatment of the extracted material with 0.5 N NaOH for 3 h at 37 C followed by extensive dialysis against distilled water. Approximately 27% of the starting ^{3}H-labeled DNA was recovered after recycling by this procedure and over 95% of this probe specifically hybridized with rat sequences present in either Ki-BALB or Ki-Mink cells. A ^{3}H-labeled DNA transcript of rabbit globin mRNA (Searle Diagnostics, England) was prepared with RNA-directed DNA polymerase from avian myeloblastosis virus as previously described (15, 16). This cDNA probe hybridized to over 95% to rabbit globin mRNA and approximately 40% to mouse globin mRNA and was used to detect mouse globin mRNA in the T-3-C1-2 cells. This percentage of homology between rabbit and mouse globin is similar to that reported by others (16).

Extraction of RNA. Total cellular RNA was extracted by disruption of cells in 0.1 M Tris-hydrochloride (pH 8.0) with 4% sodium-*N*-lauroyl-sarcosinate (K & K Laboratories, Inc., Plainview, N.J.) and centrifugation in cesium chloride as described by Glisin et al. (10). To extract cytoplasmic RNA, cells were washed twice at 4 C with 10 volumes of calcium and magnesium-free phosphate-buffered saline, pelleted by centrifugation at 1,000 × *g* for 10 min, and resuspended in 3 to 5 volumes of 0.01 M Tris-hydrochloride, pH 7.5; 0.01 M NaCl; 0.006 M $MgCl_2$; 0.5% (vol/vol) Triton X-100. After incubation for 10 min at 4 C, the cells were disrupted in a glass Dounce homogenizer with 15 to 20 strokes with a tight pestle. Nuclei were removed by two successive centrifugations at 600 × *g* for 10 min at 4 C; the supernatant was then made 1% with respect to sodium dodecyl sulfate and extracted with equal volumes of buffered aqueous saturated phenol and chloroform:isoamyl alcohol (24:1). One-tenth volume of 20% sodium acetate, pH 5.0, and 2 volumes of absolute ethanol were added to the resultant aqueous phase which was then incubated at −20 C for 16 to 18 h. The precipitated RNA was pelleted by centrifugation at 5,000 × *g* for 20 min at −20 C; the supernatant was discarded and the pelleted RNA was dried by lyophilization.

Poly(A)-containing RNA was purified from cytoplasmic RNA by oligo$(dT)_{12\text{-}18}$ cellulose (Collaborative Research, Waltham, Mass.) chromatography as previously described (16). Extracellular virus was concentrated and purified from tissue culture fluids in excess of 1.3 liters by continuous flow centrifugation in sucrose density gradients. Virus was recovered from smaller volumes of extracellular fluids by centrifugation at 105,000 × *g* for 75 min at 5 C through a 35% glycerol cushion. Concentrated virus was resuspended in 10 ml of 0.01 M Tris-hydrochloride, (pH 7.2), 0.1 M sodium chloride, 10^{-3} M EDTA, and 1% (vol/vol) sodium dodecyl sulfate and extracted with equal volumes of phenol and chloroform:isoamyl alcohol as described previously for the extraction of cytoplasmic RNA. In those cases where viral RNAs were purified from extracellular fluid volumes of 1.3 liters or less, carrier yeast RNA was added prior to phenol extraction at a final concentration of 500 μg per ml.

RESULTS

Rescue of Ki-SV by RD-114. Several biological studies have indicated that the genetic information for transformation can be rescued from sarcoma virus-transformed nonproducer cells by superinfection of such cells with heterologous mammalian type C viruses (24). Therefore, such a system was selected for the initial

comparisons of intracellular and extracellular concentrations of sarcoma virus-specific RNA after superinfection with a heterologous helper virus. Ki-Mink cells superinfected with RD-114 virus provided an ideal system because of the ability to distinguish by molecular hybridization each of these viruses from one another as well as from mink cellular RNA. The RNAs from infected cells and extracellular fluids (300 ml) were hybridized to both RD-114 and Ki-SV-specific cDNA transcripts. Results are shown in Fig. 1. In Fig. 1A, the intracellular levels of each viral RNA can be seen to be highly comparable in that the 0.5 C_rt of the RD-114 RNA is 8.5×10^1 mol/s per liter and the 0.5 C_rt of Ki-SV-specific RNA was 6.5×10^1 mol/s per liter. In Fig. 1B, the extracellular levels of Ki-SV and RD-114 RNA were examined and plotted as a function of V_ot. The pattern of hybridization observed intracellularly was also observed extracellularly. The 0.5 of V_ot value for the hybridization reaction with each viral cDNA probe was approximately 2.0×10^1. Therefore, the ratio of the RD-114 RNA and Ki-SV RNA intracellularly and extracellularly was approximately unity. In other experiments not shown, the levels of intracellular Ki-SV RNA were identical in Ki-SV-uninfected nonproducer cells to those RNA levels shown in Fig. 1A in the RD-114-infected Ki-Mink cells. Additionally, no extracellular Ki-SV RNA was detected in the supernatant of uninfected Ki-Mink nonproducer cultures at V_ot values up to 10^3 ml/h. Thus, the infection of Ki-SV nonproducer cells with a heterologous type C virus, RD-114, results in encapsidation into a sedimentable particle of Ki-SV-specific RNA. Importantly, during the course of the rescue of the Ki-SV-specific RNA, the ratio of intracellular and extracellular sarcoma virus and RD-114 virus RNA remained the same.

Rescue of RD-114 RNA by mouse xenotropic MuLV. The feline embryo cell, CCC3a, has been shown to contain constitutive amounts of RNA homologous to RD-114 virus (18). Thus, these CCC3a cells were infected with a mouse xenotropic virus, AT-124, readily distinguishable from RD-114 by molecular hybridization experiments, and the intracellular and extracellular levels of AT-124 and RD-114 viral RNA were studied (Fig. 2). As shown in Fig. 2A, the 0.5 C_rt for AT-124 RNA was approximately 10^2 and the 0.5 C_rt for RD-114 viral RNA was approximately 5×10^2 in the infected CCC3a cells. As shown in Fig. 2B, no appreciable RD-114 RNA in a sedimentable form was detected by hybridization with a cDNA probe from RD-114 virus in the supernatant of the uninfected CCC3a cells at a V_ot value of up to 10^3. However, after infection of CCC3a cells with AT-124, detectable RD-114 and AT-124 RNAs were present in the extracellular fluids of these cultures. Importantly, no change in the intracellular levels of RD-114 RNA resulted after AT-124 infection and the 0.5 C_rt was again found to be 5×10^2 (data not shown). As can be seen in Fig. 2B, the 0.4 V_ot for the AT-124 RNA in the supernatant from the CCC3a cell was 1.5×10^2 and the 0.4 V_ot for RD-114-specific RNA was approximately 1.2×10^3. Thus, although the levels of RD-114 extracellular RNA were approximately eightfold lower than the levels of AT-124 RNA, this ratio is comparable to the intracellular ratio of AT-124

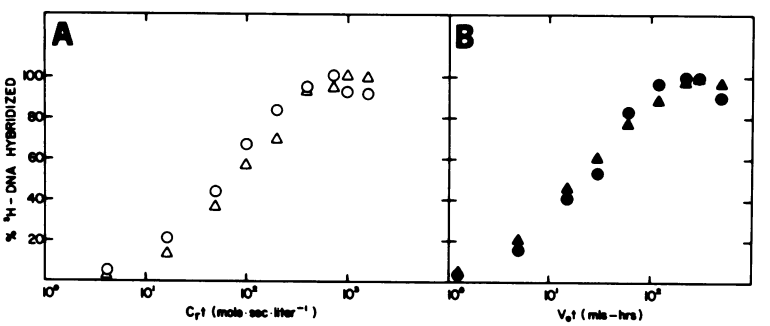

FIG. 1. *Hybridization with Ki-SV ³H-labeled DNA or RD-114 ³H-labeled DNA with RD-114-infected Ki-Mink RNA. Reaction mixtures of 0.05 ml were incubated at 66 C and contained: 0.01 M Tris-hydrochloride, pH 7.2; 0.4 M NaCl; 5 × 10⁻⁵ M EDTA; 0.05% sodium dodecyl sulfate; 20 μg of yeast RNA; 10 μg of calf thymus DNA; and approximately 800 (Ki-SV) or 2,700 (RD-114) trichloroacetic acid-precipitable counts/min of ³H-labeled DNA. Hybridization was monitored with S1 nuclease as previously described (1). Background (S1 nuclease-resistant fraction of each probe in the absence of added cellular or viral RNA) was less than 5% of either ³H-labeled DNA. (A) Total cellular RNA: Ki-SV³H-labeled DNA (○); RD-114 ³H-labeled DNA (△). (B) Extracellular RNA: Ki-SV ³H-labeled DNA (●); RD-114 ³H-labeled DNA (▲).*

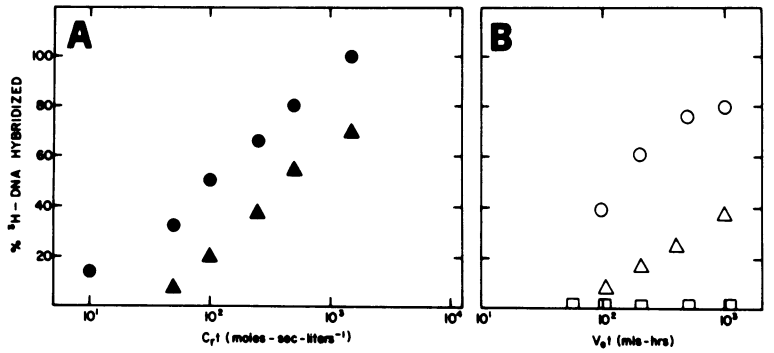

FIG. 2. *Hybridization of extracellular sedimentable RNA with RD-114 or AT-124 ³H-labeled DNA. Six hundred milliliters of supernatant tissue culture fluid from CCC3a infected with AT-124 virus was concentrated and RNA was extracted as described. Hybridization reaction conditions are as in the legend to Fig. 1 except that reaction mixtures of 0.2 ml were used. Each reaction contained approximately 3,300 (RD-114) or 6,000 (AT-124) trichloroacetic acid-precipitable counts/min of ³H-labeled DNA. (A) ●, AT-124 ³H-labeled DNA versus intracellular RNA from CCC3a cells infected with AT-124 virus; ▲, RD-114 ³H-labeled DNA versus intracellular RNA from CCC3a cells infected with AT-124 virus. (B) Extracellular RNA from superinfected cells hybridized with AT-124 ³H-labeled DNA (○), RD-114 ³H-labeled DNA (△), or extracellular RNA from uninfected CCC3a cells hybridized with RD-114 ³H-labeled DNA (□).*

and RD-114 RNA. These results indicate that infection of CCC3a cells with AT-124 virus leads to the appearance of endogenous feline type C viral RNA sequences in the extracellular fluids in direct proportion to the intracellular viral RNA levels prior to and after infection of these cells with an exogenous type C virus. Thus, this observation shares at least two characteristics with the rescue of sarcoma virus RNA after infection with a helper type C virus. First, the efficiency of rescue of endogenous RNA is in direct proportion to the intracellular levels of this genetic information prior to infection of the cells. Secondly, extracellular levels of endogenous viral RNA are significantly increased after exogenous infection of susceptible cells with a heterologous type C virus. Although no extracellular RD-114 p30 antigen was detected by radioimmunoassay after infection of CCC3a cells with AT-124 (sensitivity = 12 ng/assay), the possible presence of some RD-114 proteins in particles released from infected cells cannot be excluded at this time.

Failure of MP-MV to Rescue Ki-SV. Results of the preceding experiments indicate that infection of mammalian cells by heterologous mammalian type C viruses can result in the extracellular appearance of the type C viral RNA which is expressed intracellularly prior to infection. The specificity of this phenomenon was studied by analyzing whether other mammalian retraviruses could rescue type C virus sequences. Ki-Mink cells were infected with MP-MV, another RNA-containing virus with reverse transcriptase. No significant polypeptide homology between MP-MV and mammalian type C viruses has been reported and presently this virus is regarded as a non-type C mammalian retravirus (21, 22). MP-MV and Ki-SV-specific ³H-labeled cDNA were then hybridized to cytoplasmic RNA, to oligo(dT)-cellulose purified cytoplasmic RNA, and to extracellular RNA derived from the same MP-MV-infected Ki-Mink cultures.

The results presented in Fig. 3 show that the cytoplasmic and oligo(dT)-cellulose-purified RNAs from MP-MV-infected Ki-Mink cells annealed to the MP-MV cDNA with 0.5 C_rt values of 2.3×10^2 and 2.4×10^1 mol/s per liter, respectively. A similar 10- to 20-fold purification of the Ki-SV-specific cytoplasmic RNA sequences was achieved after purification by oligo(dT)-cellulose chromatography of the same RNA preparation; the 0.5 C_rt value of the cytoplasmic RNA for Ki-SV-specific RNA was 8.1×10^1 and 3.8×10^0 mol/s per liter for oligo(dT)-cellulose-purified RNA. Most importantly, no appearance of extracellular Ki-SV-specific RNA was detected at a C_rt of 5×10^1 with the supernatant RNA from MP-MV-infected cultures. In contrast, the 0.5 C_rt of MP-MV sequences in extracellular RNA from the same cultures was 7.2×10^0 and positive hybridization was noted at a C_rt of approximately 1.0×10^0. Thus, although Ki-SV sequences were three- to sixfold in excess of MP-MV RNA intracellularly, the MP-MV sequences were at least 50-fold in excess extracellularly. The results of biological assays to detect rescue of Ki-SV were consistent with this bio-

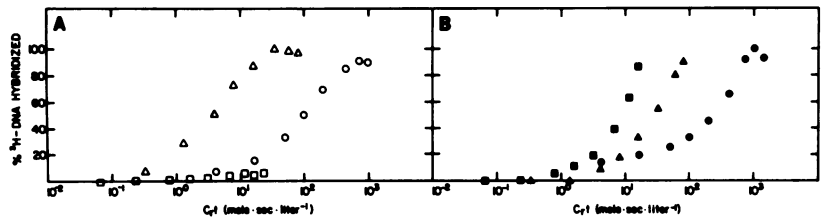

FIG. 3. *Hybridization with Ki-SV ³H-labeled DNA or MP-MV ³H-labeled DNA to various RNAs from MP-MV-infected Ki-Mink cells. Hybridization conditions and analysis by s1 nuclease are exactly as described in Fig. 1. Each hybridization reaction contained approximately 800 (Ki-SV) or 2,000 (MP-MV) trichloroacetic acid-precipitable counts/min of the ³H-labeled DNAs. (A) Hybridization of ³H-labeled Ki-SV-specific DNA to:* (○) *cytoplasmic RNA;* (△) *oligo(dT)-cellulose-purified cytoplasmic RNA;* (□) *extracellular RNA. (B) Hybridization of ³H-labeled MP-MV DNA to:* (●) *cytoplasmic RNA;* (▲) *oligo(dT)-cellulose-purified cytoplasmic RNA;* (■) *extracellular RNA.*

chemical observation; no foci were seen when up to 10^5 MP-MV-infected Ki-Mink cells were plated as infectious centers on normal mink cells. Therefore, MP-MV does not rescue levels of Ki-SV RNA detectable by sensitive biochemical or biological assays.

Attempted rescue of MMTV sequences by murine type C virus. The preceding results indicate a marked degree of specificity in terms of the mechanisms that regulate the rescue of type C virus genetic information after exogenous infection with other RNA-containing retraviruses. This observation was investigated further in the following experiments. A cell line (C_3H-MT C1 12) containing high levels of RNA complementary to type B murine mammary tumor virus (MMTV) was infected with Mo-MuLV. Total cellular RNA and extracellular RNA were purified from Mo-MuLV-infected C_3H-MT C1 12 cultures, and hybridizations were performed with ³H-labeled DNA probes prepared from Mo-MuLV and MMTV. The results are presented in Table 1 as a function of RNA concentration and time intracellularly (C_rt), and volume and time extracellularly (V_ot). Since extracellularly (see below) no detectable MMTV RNA was found, and since we defined this as not even 10% hybridization to the cDNA, the values of 0.1 C_rt or 0.1 V_ot are indicated in Table 1. As expected, cellular RNA from infected cultures contained a high level of Mo-MuLV (0.1 C_rt, 2.1×10^0) and an approximate 35-fold lower level of MMTV RNA (0.1 C_rt, 5.9×10^1).

In experiments not shown, this level of MMTV RNA in Moloney-infected cells was identical to that noted in uninfected cells implying that type C infection had little if any effect on type B RNA expression. In addition, no significant levels of MMTV- or MuLV-specific RNA were detected in extracellular fluids of uninfected C_3H-MT C1 12 cultures at V_ot values of 10^4 ml/h. Even though high levels of Mo-MuLV-specific RNA were released extracellularly from infected cells (0.1 V_ot, 3.2×10^0), no MMTV RNA was detected in these same fluids at V_ot values greater than 1,000-fold higher than those at which MuLV RNA could be detected. Thus, MMTV RNA sequences are not rescued as a consequence of productive infection of cells with a murine type C virus.

Type C viral rescue of globin RNA. Although the preceding experiments indicate little interaction between unrelated viral genomes in encapsidation of viral RNA, the report of 0.001% hemoglobin mRNA in high-molecular-weight type C viral RNA (15) led us to study the interaction further. The mouse cell line (T-3-C1-2) producing Friend type C virus was induced to accumulate large amounts of intracellular mouse globin mRNA by cultivation in medium containing 1% (vol/vol) ME_2SO for 3 days at 37 C (15). RNAs were purified from the cells and from 1.3 liters of extracellular fluid of the same cultures as described above. These RNAs were then tested by hybridization with ³H-labeled DNAs prepared from Mo-MuLV and rabbit globin mRNA (Table 2). Levels of intracellular MuLV-specific RNA (0.1 C_rt of 2.1×10^0) were approximately 30-fold higher than globin mRNA (0.1 C_rt, 6.0×10^1); no hybridization of globin ³H-labeled DNA to extracellular RNA was measured even when this RNA was tested at levels which were 1,300-fold higher than those required for detection of MuLV-specific RNA. Thus, these results suggest that the reported incorporation of globin RNA at levels of 1 part per 1,000 viral RNA molecules is not occurring by the same highly specific and efficient mechanism involved in type C viral rescue of other type C viral RNAs.

Rescue negative Ki-SV revertants. Recently, classes of revertants of Ki-BALB cells have been reported which replicate MuLV but

TABLE 1. *Comparative extracellular and intracellular RNA levels of Moloney virus-infected MMTV cell line*

Source of RNA	^{3}H-labeled DNA probe	0.1 C_rt	0.1 V_ot	Probe[a] hybridized (maximum %)	MuLV/MMTV[b]
Intracellular	Mo-MuLV	2.1×10^0		100	3.5×10^{-2}
	MMTV	5.9×10^1		100	
Extracellular	Mo-MuLV		3.2×10^0	91.9	$<3.5 \times 10^{-4}$
	MMTV		$>9.0 \times 10^3$	<3.0	

[a] Each reaction contained approximately 2,000 counts/min of Mo-MuLV cDNA or 1,300 counts/min of MMTV cDNA.
[b] Based on 0.1 C_rt values intracellularly or 0.1 V_ot values extracellularly.

TABLE 2. *Comparative extracellular and intracellular levels of MuLV and globin RNA from T-3-CL-2 cells*

Source of RNA	^{3}H-labeled DNA probe	0.1 C_rt	0.1 V_ot	Probe[a] hybridized (maximum %)	MuLV/globin[b]
Intracellular	Mo-MuLV	2.1×10^0		100	3.5×10^{-2}
	Globin	6.0×10^1		100	
Extracellular	Mo-MuLV		5.0×10^{-1}	60	$<7.6 \times 10^{-4}$
	Globin		$>6.5 \times 10^2$	<3.0	

[a] Each reaction contained approximately 2,000 counts/min of Moloney cDNA or 3,000 counts/min of globin cDNA.
[b] Based on 0.1 C_rt (values intracellularly) or 0.1 V_ot (values extracellularly).

which fail to rescue infectious transforming Ki-SV (8, 17, 20). To investigate further the specificity of rescue of type C viral sequences by type C viruses, revertants of Ki-BALB cells were selected with the aid of the methylcellulose selection technique (34). One such revertant which did not rescue biological transforming activity was tested for intracellular Ki-SV-specific rat sequences. The results are shown in Fig. 4. As expected, Ki-BALB cells have relatively high levels of Ki-SV sequences; the 0.1 C_rt is 8.0 10^1 mol/s per liter and the 0.5 C_rt is 6 $\times$ 10^2 mol/s per liter. Surprisingly, RNA from the revertant Ki-BALB cells failed to give any (<5%) hybridization with the Ki-SV-specific probe even when tested at a C_rt of 2 $\times$ 10^4. Thus, the failure to rescue Ki-SV from this revertant is a result of the absence of Ki-SV-specific RNA in these cells prior to infection with helper virus.

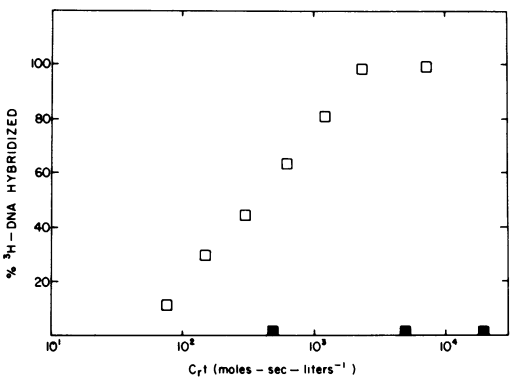

FIG. 4. *Hybridization of Ki-SV-specific ^{3}H-labeled DNA with various cellular RNAs. Hybridization was performed as described in the legend to Fig. 1, except that 0.60 M sodium chloride was used. Each reaction mixture contained approximately 1,400 trichloroacetic acid counts/min of ^{3}H-labeled DNA. Symbols: □, Ki-BALB total cellular RNA; ■, Ki-BALB revertant 625-1 total cellular RNA.*

DISCUSSION

The current studies have investigated the specificity of the rescue of RNA sequences by mammalian type C viruses. All viral and cellular nucleic acid sequences studied could be readily distinguished from each other by molecular hybridization with S1 nuclease and ^{3}H-labeled DNA excess RNA hybridization methods. Thus it was possible to quantitate the intracellular and extracellular ratios of various viral and cellular RNAs in cells infected exogenously with various retraviruses. Since the cells selected for study did not secrete appreciable RNA in particles readily sedimentable at

100,000 × *g* prior to exogenous infection, the intracellular and extracellular levels of RNAs present in the cell before and after infection could be compared.

Type C virus infection of a given cell led to the packaging of heterologous type C sequences which were present in the cell prior to infection. This was true either of the type C sequences of sarcoma viruses in sarcoma virus-transformed nonproducer cells or type C sequences of an endogenous virus (RD-114) present in cat cells prior to exogenous infection. Whether the intracellular level of infecting viral RNA was equal to or greater than the rescued sequences, importantly the ratio of the infecting viral sequences and the rescued sequences remained relatively constant both intracellularly and extracellularly. Interestingly, the example of encapsidation of endogenous viral RNA by an exogenous type C virus is analogous to the postulated first step in recombination after exogenous infection of chf-positive avian cells (13, 33).

In contrast, a type C virus of murine origin failed to rescue either type B viral or globin cellular mRNA sequences from cells expressing these heterologous RNA sequences. The extracellular MuLV was between 100- and 500-fold deficient in either heterologous sequences based on the ratio of the type C to type B or type C to globin sequences intracellularly. In another case, the MP-MV, a distinct class of retravirus, failed to rescue Ki-SV-specific RNA from MP-MV-infected Ki-SV-transformed mink cells even though intracellular levels of MP-MV and Ki-SV RNAs were comparable. Extracellularly the MP-MV was in at least a 50-fold excess of any detectable Ki-SV sequences. Thus, the specificity of rescue of type C viral RNA is remarkably high even though other RNAs can be found in type C viral preparations, as evidenced by the reports of Ikawa et al. (15) and Fidanian et al. (7).

To elucidate further the mechanisms involved in rescue of type C sarcoma viruses by type C helper viruses, a Ki-BALB revertant cell was studied which failed to yield foci after superinfection with MuLV. In this case the reason for the failure of rescue of foci was different from the case of the MP-MV-infected Ki-Mink cell. The Ki-BALB revertant had lost all detectable levels of intracellular Ki-SV-specific RNA and thus the failure of rescue in this case can simply be attributed to the lack of Ki-SV RNA in the cell. Whether the revertant has lost the sarcoma virus or is altered in a step in transcription of Ki-SV-specific RNA is unresolved. Nevertheless these hybridization results are in striking contrast to those reported in other nonrescuable Ki-BALB revertants (11). However, the results are consistent with data on rescue in various mammalian cells transformed by avian sarcoma viruses (4, 6). Whether different classes of Ki-BALB revertants in fact exist can only be resolved by further studies.

Since the mechanism of type C viral recombination presumably requires encapsidation of dissimilar RNAs in single viral particles, the mechanism of both viral assembly and the processes of encapsidation are of direct interest. Generally, little nucleic acid homology has been detected between type C viruses from different mammalian species (2). However, various immunologic cross-reactions have been detected on proteins between all isolates of the known mammalian type C viruses. The major cross-reactions have been detected on the p30 (9, 21) and the reverse transcriptase (22), and, to a lesser extent, on the gp69-71 (29). Since size and poly(A) content of type C, MMTV, and MP-MV RNAs are similar, our results would lead one to speculate that certain proteins of the known class of mammalian type C viruses recognize perhaps small highly conserved sequences on type C viral RNA. The further elucidation of the mechanisms to explain the specificity of type C viral RNA rescue would provide new approaches for further study of cells carrying defective type C viruses of exogenous or endogenous origin since such properties would allow diverse type C viruses to package and purify apparently largely nonhomologous type C RNAs. In addition, the current studies provide an approach for determining if RNAs which are found in type C virus preparations are of type C or cellular origin. This line of experimentation is presently being pursued to determine if the RNA sequences found in normal rat cells which can be packaged by type C viruses (25) and which are homologous to Ki-SV (27, 31) or are of viral or cellular origin.

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