Cellular Origin of a Mouse Leukemia Viral Ribonucleic Acid

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Mouse erythroblastosis virus, a member of the mouse leukemia virus group, was obtained from chronically infected C₃H mouse embryo cells and purified on sucrose gradients. The ribonucleic acid (RNA) extracted from ribonuclease-treated virus consisted of a rapidly sedimenting (72S) species and a more slowly sedimenting component (4 to 30S). The 72S RNA did not contain base sequences homologous to deoxyribonucleic acid (DNA) from infected cells as determined by hybridization studies. In contrast, the slowly sedimenting RNA enclosed within the virus had base sequences homologous to DNA from infected and uninfected C₃H mouse embryo cells.

Previous reports have dealt extensively with the isolation and purification of ribonucleic acid (RNA) tumor viruses from avian and mammalian sources (4, 5, 7-9, 12, 18-21). These RNA viruses fit broadly into the myxovirus group because of their large size, outer lipid-containing membrane, and maturation by budding from the cell surface. All RNA tumor viruses thus far studied appear to contain single-stranded RNA molecules sedimenting at an approximate *S*₂₀ value of 60 to 70. However, profiles of RNA extracted from purified viruses also reveal a component of more slowly sedimenting RNA which has not been extensively investigated. The present report deals with the location of this slowly sedimenting RNA within intact virus particles and presents evidence that it is of host-cell origin.

Since RNA tumor virus replication is sensitive to inhibition by actinomycin D (2, 3, 23) and requires a short period of deoxyribonucleic acid (DNA) synthesis after infection (1, 2, 24), it has been postulated that these viruses synthesize a DNA replica by using input viral RNA as a template. In turn, the DNA replica acts as the template for progeny viral RNA synthesis (24). We were unable to detect such a DNA template by sensitive hybridization techniques in mouse embryo cells chronically infected with a murine leukemia virus.

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MATERIALS AND METHODS

Virus growth. Murine erythroblastosis virus (MEV; 13, 14) was obtained from supernatant fluids of an erythroblastotic mouse spleen line which continually releases infectious MEV (22). A C₃H mouse embryo culture (C₃H-MEC) was established in this laboratory and infected with MEV in September 1967 by methods previously described (22). Periodic bioassays in newborn C₃H mice and in primary C₃H mouse embryo cultures have shown release of infectious MEV progeny from C₃H-MEC cells. Uninfected C₃H-MEC cells were used as control cultures. They were repeatedly checked for contaminating virus by electron microscopy and bioassays and were found to be negative. The media for all cell lines consisted of Medium 199 with 10% fetal calf serum (Grand Island Biological Co., Grand Island, N.Y.). Cells were incubated at 37 C in 5% CO₂-95% air atmosphere.

Radioactive labeling of virus. Confluent monolayers of chronically infected C₃H-MEC cells were incubated at 37 C with fresh culture medium containing 10 μ C of ³H-uridine per ml (Schwarz Bio Research, Inc., Orangeburg, N.Y.; specific activity, 20 c/mmole) for 24 hr. Uninfected cells were labeled with 2 μ C of ¹⁴C-uridine per ml (New England Nuclear Corp., Boston, Mass.; specific activity, 30 c/mmole) for 24 hr. At the end of the incubation period, the spent medium was centrifuged at 9,000 $\times$ *g* for 10 min to remove cell debris and stored at -70 C.

Concentration and purification of virus. Labeled MEV stocks were rapidly thawed at 37 C and layered onto 1-ml cushions of 15% sucrose in tris(hydroxymethyl)aminomethane (Tris) buffer (0.01 M Tris, pH 7.2, 0.1 M NaCl, 0.001 M ethylenediaminetetraacetic acid) in 12-ml ultracentrifuge tubes. They were centrifuged for 90 min at 130,000 $\times$ *g* at 4 C. The pellets resulting from this centrifugation were drained,

resuspended in 3 ml of Tris buffer, layered onto 25-ml preformed sucrose gradients (30 to 60%, w/v, in Tris buffer), and centrifuged to equilibrium in a Spinco SW25.1 rotor at $63,000 \times g$ for 20 hr at 2°C. Gradients were collected by using an ISCO model D density gradient fractionator and model UA-2 ultraviolet analyzer (Instrumentation Specialties Co., Lincoln, Neb.). Fractions were collected for determination of radioactivity and refractive index as well as for isolation of viral RNA.

Radioactivity measurements of virus preparations. Samples (0.1 ml) were taken from each fraction of the sucrose equilibrium gradient, precipitated with an equal volume of cold 10% trichloroacetic acid, and filtered through HA filters (0.45- μ m pore size; Millipore Corp., Bedford, Mass.). The filters were washed with 25 ml of cold 5% trichloroacetic acid, dried, and dissolved in 10 ml of Bray's (6) scintillation fluid for counting in a Packard Tricarb scintillation counter.

Buoyant density determinations. Specific gravities of fractions from sucrose gradients were determined from measurements of the refractive indices of one-drop samples by using a Zeiss refractometer.

Viral RNA extraction. The material in the fractions from sucrose equilibrium gradients containing the peak of absorbance at 258 nm (OD_{258}) was made 0.5% with sodium dodecyl sulfate (SDS), and yeast-soluble RNA (Schwarz Bio Research) was added as carrier. It was then extracted at least three times with chloroform-isoamyl alcohol (25:1) at room temperature. The final aqueous layer was precipitated with two volumes of cold ethyl alcohol. The precipitate was collected from the ethyl alcohol by centrifugation at $8,000 \times g$ at -20°C for 20 min, washed once with 80% ethyl alcohol, and recentrifuged. The pellet resulting from the second centrifugation was dissolved in buffer (0.01 M Tris, 0.1 M NaCl) and stored at -20°C for future use.

Ribosomal RNA extractions. The cell sheets from C₃H-MEC which had been used to prepare radioactive virus were removed from the glass surface with glass beads into 0.05 M Tris, pH 7.2. The cells were pelleted at $8,000 \times g$ for 5 min and resuspended in 0.05 M Tris. The suspension was solubilized in 1% SDS and extracted with phenol (equilibrated with 0.05 M Tris, pH 7.2) three times for 15 min at room temperature. The final aqueous phase was made 0.1 M with NaCl and precipitated with two volumes of cold ethyl alcohol. The precipitate was collected after centrifugation at $8,000 \times g$ for 20 min, washed twice with 80% ethyl alcohol, dissolved in 0.05 M Tris, 0.001 M MgCl₂, and 50 μ g of electrophoretically purified deoxyribonuclease per ml (Worthington Biochemical Corp., Freehold, N.J.), and incubated at 37°C for 30 min. After incubation, it was extracted with chloroform-isoamyl alcohol at least three times, or until no material could be found at the interphase. The aqueous phase was made 0.1 M with NaCl and precipitated with two volumes of cold ethyl alcohol. The precipitate was collected as before, washed with 80% ethyl alcohol, dissolved in buffer (0.01 M Tris, pH 7.2, 0.1 M NaCl), and stored at -20°C .

Determination of sedimentation coefficient of RNA. Samples of RNA preparations were layered onto 5-ml

preformed sucrose gradients (5 to 20%, w/v, sucrose in 0.01 M Tris, pH 7.2, 0.1 M NaCl) and centrifuged at $165,000 \times g$ in a Spinco SW50 rotor at 2°C for 90 min for preparation of viral RNA or 150 min for cellular RNA preparations. Previously prepared ribosomal ³H-RNA was added as marker for determination of sedimentation coefficients. After centrifugation, the tube was punctured at the bottom and 25-drop fractions were collected. The fractions were precipitated with cold 10% trichloroacetic acid and 100 μ g of yeast RNA was added to each fraction as carrier. They were collected on Millipore HA filters (0.45- μ m pore size), washed with 25 ml of cold 5% trichloroacetic acid, and counted in 10 ml of Bray's scintillation fluid. Sedimentation coefficients for viral RNA were determined by comparing the distance traveled by the RNA species in question to added 28S ribosomal RNA (16).

Preparation of viral 72S and slowly sedimenting RNA. Viral RNA was separated on sucrose gradients as described for determination of sedimentation coefficients. The fractions containing known peaks of radioactivity were pooled and used for various hybridization experiments. RNA concentrations were determined by measurements of its absorbance at 260 nm.

DNA extractions were performed according to the method of Marmur (15).

DNA-RNA hybridizations. The method of Gillespie and Spiegelman (10) was used. Filters were washed and hybridized in $6 \times \text{SSC}$ (0.15 M NaCl plus 0.015 M sodium citrate). Hybridizations were carried out at 66°C for 16 hr. All filters were loaded with 100 μ g of DNA per filter as measured by the optical density of the native DNA preparations at 260 nm (E at $A_{\text{max}} = 260 \text{ nm}$; 1 mg/ml = 20).

Hybridization competition. RNA used for hybridization competition experiments was extracted from uninfected C₃H-MEC cells. The concentration of the RNA preparation was determined by measurement of its absorbance at 260 nm (E at $A_{\text{max}} = 260 \text{ nm}$; 1 mg/ml = 25). Dilutions of a preparation of unlabeled cellular RNA were added to samples of the same preparation of viral slowly sedimenting ³H-RNA, and the mixtures were hybridized as previously described. To control for nonspecific interference, the experiment was repeated by using Torula RNA (Sigma Chemical Co., St. Louis, Mo., extracted three times with phenol) in place of mouse-cell RNA.

RESULTS

Equilibrium sedimentation of virus. When media from MEV-infected C₃H-MEC cells was concentrated by ultracentrifugation and sedimented on equilibrium sucrose density gradients as described, a fairly sharp band of ultraviolet absorbance was found in the gradient at a buoyant density of 1.175 g/cc. When MEV-infected cells were labeled with ³H-uridine, the peak of radioactivity in the gradient coincided with the peak of ultraviolet absorbance (Fig. 1). That this ultraviolet-absorbing band is indeed

virus was demonstrated by the induction of erythroblastosis in newborn mice only with material from this region of the gradients. When media from uninfected C₃H-MEC cells was concentrated by ultracentrifugation in the same way as described for infected cells, no pellets could be obtained.

To determine whether the radioactive material in the peak at 1.175 g/cc was integrated into the virus itself or into cell debris that became associated with the virus during ultracentrifugation, the following experiment was done. Uninfected C₃H-MEC cells were labeled for 24-hr periods with 2 μ C of ¹⁴C-uridine per ml as described. The media from these cells was mixed with an equal volume of 24-hr medium from unlabeled MEV-infected cells. These pooled media were concentrated by ultracentrifugation, and the resulting pellets were resuspended and sedimented on a sucrose equilibrium gradient as described. The results of this experiment are shown in Fig. 2. Although a band of ultraviolet-absorbing material was produced in this experiment, no ¹⁴C-containing material cosedimented specifically with this peak.

After treatment of culture supernatant fluids from infected C₃H-MEC cells with ribonuclease (Worthington pancreatic RASE, 10 μ g/ml for 30 min at 25 C) or deoxyribonuclease (Worthington, electrophoretically purified, 10 μ g/ml for 30 min at 25 C), the peaks of ultraviolet absorbance after sucrose gradient centrifugation were found at the same position as untreated preparations. However, all ultraviolet absorbance and radioactivity were found at the top of the gradient when supernatant fluids were pretreated with 0.1% SDS or pronase (Calbiochem, Los Angeles, Calif., 10 μ g/ml for 30 min at 25 C).

When infected culture supernatant fluids were concentrated by precipitation with an equal volume of cold, saturated ammonium sulfate, and the precipitate was redissolved in Tris buffer and sedimented on sucrose gradient, two peaks of ultraviolet absorbance and radioactivity were consistently obtained. This appears to be an artifact of high salt concentration, since concentration of virus by ultracentrifugation never resulted in more than one virus band in sucrose gradients.

Viral RNA extraction. RNA was extracted

FIG. 1. Equilibrium sedimentation of MEV. Infected C₃H-MEC cells were labeled with 10 μ C of ³H-uridine per ml for 24-hr periods. Culture supernatant fluids were collected, concentrated by ultracentrifugation as described, and layered onto a 30 to 60% sucrose gradient. The gradient was centrifuged for 20 hr at 63,000 $\times$ g, and collected by using an ISCO density gradient fractionator and ultraviolet analyzer (—, OD₂₅₅). Samples (0.1 ml) from each fraction were precipitated with trichloroacetic acid and filtered through HA filters. The filters were counted in a liquid scintillation counter (●, trichloroacetic acid-precipitable, ³H counts per minute). The average specific gravity of each fraction was determined by measuring the refractive index with a Zeiss refractometer; (▲, specific gravity, g/cc).

FIG. 2. Equilibrium sedimentation of MEV supernatant fluids mixed with uninfected cell supernatant fluids. Uninfected C₃H-MEC cells were labeled with 2 μ C of ¹⁴C-uridine per ml for 24-hr periods. Culture supernatant fluids were collected and mixed with an equal volume of 24-hr-old supernatant fluids from unlabeled infected cells. The pooled material was concentrated by ultracentrifugation and layered onto a 30 to 60% sucrose gradient. The gradient was centrifuged for 20 hr at 63,000 $\times$ g and collected as in Fig. 1. —, OD₂₅₈; ●, trichloroacetic acid-precipitable, ¹⁴C counts per minute; ▲, specific gravity, g/cc.

from the ultraviolet-absorbing peak at 1.175 g/cc as already described. The extracted RNA contained a peak of rapidly sedimenting RNA which had a sedimentation coefficient of 72S in 0.1 M NaCl as compared to the 28S ribosomal RNA marker (Fig. 3). This RNA was single-stranded, as suggested by a shift of the peak in a sucrose gradient in 0.01 M NaCl to a position corresponding to 55S RNA. In addition, all the RNA in the 72S peak was completely degraded, within 30 min at room temperature in 0.3 M NaCl, by 10 μ g of pancreatic ribonuclease (preheated to 90 C for 10 min) per ml, to material which was soluble in cold 10% trichloroacetic acid.

RNA extraction of material from the top of the equilibrium gradient yielded no RNA sedimenting faster than 4S.

DNA-RNA hybridization of rapidly sedimenting RNA. Filters were loaded with 100 μ g of DNA extracted from whole C₃H mouse embryos, from uninfected C₃H-MEC cultures, or from C₃H-MEC chronically infected with MEV. To determine whether the amount of DNA used contained an excess of binding sites, RNA binding capacities were determined for 28S ribosomal

RNA from MEV-infected cells and slowly sedimenting viral RNA (Fig. 4). It was found that 100 μ g of DNA was in excess of that amount necessary for maximal annealing of the RNA concentrations employed in all experiments.

The results of the hybridization experiments for the 28S ribosomal and 72S viral RNA are presented in Table 1. Binding of homologous RNA was demonstrated by the annealing of 4 to 5.5% of input 28S ribosomal RNA extracted from infected cells. Yet there was no significant binding of RNA to the filters when the hybridization solution contained material from the 72S viral RNA peak.

The 72S RNA used for hybridization was stable under the conditions used (6 $\times$ SSC, 66 C for 16 hr). This was demonstrated by the fact that over 90% of the trichloroacetic acid-precipitable counts added to the annealing mixture were recoverable after hybridization. This RNA remained completely susceptible to ribonuclease, after the SSC concentration was lowered to 2 $\times$.

In the case of the 28S RNA and slowly sedimenting viral RNA, over 90% of the trichloroacetic acid-precipitable counts added to the mix-

FIG. 3. Sedimentation profiles of RNA extracted from MEV. RNA was extracted from virus bands obtained on sucrose equilibrium gradients and sedimented through 5 ml of 5 to 20% sucrose gradients for 90 min at $165,000 \times g$. Fractions were precipitated with trichloroacetic acid, collected on HA filters, and counted in Bray's scintillation fluid. Dashed line, trichloroacetic acid-precipitable counts per minute of RNA extracted from a sample of a virus preparation treated with ribonuclease ($10 \mu\text{g/ml}$ at 25°C for 30 min) prior to RNA extraction; solid line, trichloroacetic acid-precipitable counts of RNA from an untreated sample of the same virus preparation.

ture were recoverable from both the filter and the solution remaining when the filter was removed. However, of the material remaining, 30 to 40% of the counts were ribonuclease-resistant at $2 \times \text{SSC}$ when the filter contained DNA, whereas less than 10% of the counts were ribonuclease-resistant when blank filters were added. This indicates that some of the DNA-RNA hybrids formed were loosened from the filter during annealing.

Slowly sedimenting viral RNA. The profile of RNA extracted from MEV purified on sucrose density equilibrium gradients (Fig. 3), in addition to the 72S RNA, contained RNA which sedimented in a range from 30S to 4S after centrifugation for 150 min rather than 90 min. To determine whether this material was contained within intact virus particles or derived from co-sedimenting cell degradation products, the virus peak from an equilibrium gradient was treated for 30 min at 25°C with $10 \mu\text{g}$ of pancreatic ribo-

FIG. 4. DNA binding capacity. Filters were loaded with $100 \mu\text{g}$ of DNA. (A) Ribosomal RNA hybridized with uninfected cell DNA; (B) ribosomal RNA hybridized with infected cell DNA; (C) slowly sedimenting viral RNA hybridized with uninfected cell DNA; (D) slowly sedimenting viral RNA hybridized with infected cell DNA. In A and B, specified dilutions of a single preparation of $28\text{S } ^3\text{H-RNA}$ from MEV-infected cells was used (specific activity, 3.1×10^4 counts per min per μg). In C and D, the dilutions were made from a single separation of slowly sedimenting RNA extracted from virus produced by the same cells from which the ribosomal RNA was extracted (specific activity, 2.7×10^4 counts per min per μg). The value for each point represents the mean result and standard deviation of three or more identical mixtures. Solid lines, ribonuclease-resistant counts bound to filters after hybridization; broken lines, average percentage of ribonuclease-resistant counts bound for each dilution, as compared with trichloroacetic acid-precipitable counts remaining in the hybridization solution after filters were removed.

nuclease per ml prior to RNA extractions. Ribonuclease treatment of intact virus repeatedly failed to alter the proportion of 72S to slowly sedimenting RNA subsequently extracted from the preparation (Fig. 3).

As an additional control, the material from the ultraviolet-absorbing peak of Fig. 2 (produced by mixing ^{14}C -uridine-labeled uninfected cell medium with unlabeled infected cell medium) was pooled with material from the peak of Fig. 1 (produced from ^3H -uridine-labeled infected-cell medium). The pooled material was treated with

TABLE 1. DNA-RNA hybridization

RNA source	Source of DNA on filters ^a					
	Uninfected C ₃ H cultures		Whole normal C ₃ H embryos		Infected C ₃ H cultures	
	Mean ratio ^c	Hybridization efficiency (%) ^d	Mean ratio ^c	Hybridization efficiency (%) ^d	Mean ratio ^c	Hybridization efficiency (%) ^d
28S ribosomal ^b						
Batch 1	217/5516	4.0 ± .3			(C) 274/5593	4.8 ± .4
2	35/580	5.5 ± .4			(C) 30/642	4.2 ± .3
3			90/1632	5.3 ± 0.8	(D) 88/2062	4.3 ± .8
72S viral ^e						
Batch 1	6/1792	0.2 ± .2			(C) 4/1772	0.1 ± .1
2			4/844	0.5 ± 0.2	(D) 5/736	0.6 ± .1
3			6/757	0.5 ± 0.1	(D) 5/732	0.6 ± .2
Slowly sedimenting viral RNA ^f						
Batch 1	272/2588	6.6 ± .3			(C) 179/2303	7.6 ± .6
2			121/1360	8.3 ± 0.9	(D) 177/1991	5.8 ± .5

^a All filters were loaded with 100 μ g of DNA. One preparation of uninfected cell DNA and one of embryo DNA was used throughout. Two different preparations of DNA from infected cells are designated (C) and (D).

^b 28S ribosomal RNA was derived from the same C₃H-MEC cells used in the production of virus. Batches 1 and 2 were from the same RNA preparation, except that batch 2 was a 1/10 dilution of batch 1. Batch 3 was from another RNA preparation. The specific activity of batch 1 was 3.1×10^4 counts per min per μ g, and of batch 3 was 2.8×10^4 counts per min per μ g.

^c Each value is the mean of at least three separate experiments with the same RNA and DNA preparations. The ratio represents the ribonuclease-resistant counts bound to the filter/trichloroacetic acid-precipitable counts remaining in the hybridization solution after the filters were removed.

^d Hybridization efficiency was calculated from the mean per cent of counts bound to the filter after subtracting the per cent of ribonuclease-resistant counts bound to empty filters (0 to 0.2%). A few stray counts above background remaining on filters significantly alters the hybridization efficiency when the radioactivity in the entire system is low, leading to false high values.

^e 72S viral RNA was obtained from fractions 3 to 6 of viral RNA prepared as in Fig. 3. Specific activity was 4.1×10^4 counts per min per μ g for batches 1 and 3 and 4.0×10^4 counts per min per μ g for batch 2.

^f Slowly sedimenting viral RNA was prepared from the same gradients used in the preparation of the 72S RNA (fractions 9–14 of Fig. 3). Batches 1 (slow RNA) and 1 (72S RNA) were from a single gradient, as were batches 2 (slow RNA) and 2 (72S RNA). The specific activity of slowly sedimenting RNA from batch 1 was 2.7×10^4 counts per min per μ g; batch 2 was 2.5×10^4 counts per min per μ g.

ribonuclease as before, and the RNA was extracted. The sedimentation profile of this RNA showed no ¹⁴C contaminating either the 72S or the slowly sedimenting material.

The slowly sedimenting viral RNA was hybridized as described for the 72S viral RNA. As shown in Table 1, the slowly sedimenting RNA extracted from virus hybridized to cellular DNA with a slightly greater efficiency (6.6 to 8.3% of input) than did ribosomal RNA. The efficiency of hybridization appeared not to be significantly dependent on the type of DNA used, since DNA from normal embryos and uninfected cultures had about the same ability to hybridize viral slow component as did DNA extracted from virus-releasing cultures.

In the experiment shown in Fig. 5, unlabeled RNA was extracted from confluent monolayers of uninfected C₃H-MEC, layered on 5 to 20% sucrose gradients, and sedimented under the conditions used for the preparation of viral RNA. Fractions equivalent to those used for hybridization of the viral slow component were collected for hybridization competition experiments with the viral slow component. This experiment showed that RNA extracted from uninfected cells will compete with slowly sedimenting viral ³H-RNA for the binding sites on DNA. This competition for homologous binding sites specifically depended on the use of mouse cell RNA, since the addition of the same amounts of yeast RNA had little effect on the binding of

FIG. 5. Hybridization competition. RNA extracted from uninfected C₃H-MEC cells was added to the hybridization mixtures of A and B in the specified amounts. Yeast RNA was used in C. All hybridization mixtures contained the same quantity of a preparation of slowly sedimenting viral RNA (fractions 9-14 of Fig. 3; specific activity, 2.7×10^4 counts per min per μ g). In A and C, the filters contained 100 μ g of uninfected C₃H-MEC DNA; in B, filters contained 100 μ g of infected C₃H-MEC DNA. Each value represents the mean result and standard deviation of at least three experiments with identical hybridization mixtures. Solid lines, ribonuclease-resistant counts bound to filters after hybridization; broken lines, average per cent of ribonuclease-resistant counts bound to filter at each dilution, as compared with trichloroacetic acid-precipitable counts remaining in the hybridization solution after filters were removed.

slowly sedimenting viral RNA. It was concluded that the slowly sedimenting material extracted from intact virions consisted of cellular RNA not specified by the virus.

DISCUSSION

The results presented here suggest that intact virions of MEV, a member of the RNA tumor virus group, contain cell RNA in addition to the high molecular weight viral RNA. The cellular RNA is slowly sedimenting in relation to viral RNA and could be obtained from purified virions that were treated with ribonuclease prior to RNA extraction. That the synthesis of slowly sedimenting RNA extracted from virions is specified by the host cell is illustrated by the following evidence. The slowly sedimenting RNA from virions hybridizes with DNA extracted from uninfected culture cells and from whole mouse embryos, as well as from chronically infected culture cells. Furthermore, RNA extracted from uninfected cells competes with this RNA from viruses for DNA binding sites. The slowly sedimenting RNA has been isolated from MEV recovered from infected cells at various in vitro passage levels and, therefore, appears to be an

integral component of the virions. It is of interest that purified avian myeloblastosis virus of the BAI strain A derived from fibroblast cultures and plasma of infected chickens contains cellular RNA (4, 5). The significance of the entrapped cellular RNA remains to be determined.

When considering the manner in which RNA tumor viruses mature by budding from the cell membrane, it is not surprising to find, within intact virions, RNA molecules coded for by cellular DNA. It is conceivable that some cytoplasm becomes entrapped during the process in which the virion acquires its outer lipoprotein coat. Although this interpretation seems most plausible at the present time, other alternatives should be considered. Our data do not preclude the existence of "pseudovirions," i.e., a population of virions composed of either viral or cellular RNA analogous to polyoma pseudovirions (17). However, the following consideration would argue against this interpretation. RNA tumor viruses tend to band at two or more buoyant densities when concentrated by high salt solutions or centrifuged to equilibrium in gradients of high salt concentrations (19). The phenomenon appears to be an artifact of centrifugation or high salt concentration, or both. In the case of MEV, a difference of 0.1 g/cc in densities between the two viral peaks was repeatedly found when virus was concentrated by ammonium sulfate precipitation before equilibrium gradient centrifugation. However, the same RNA-sedimentation profiles were obtained from RNA extracted separately from each virus peak. This finding makes the existence of significant numbers of pseudovirions unlikely, since each peak would be expected to yield either cellular or viral RNA profiles. Interpretations other than accidental inclusion of cellular RNA in virions should also be considered, particularly the role of the included cytoplasm during the early phases of virus replication. We are currently studying the location of the cellular RNA in relation to the viral nucleoid. Although the attachment of the cellular RNA to the outer virion coat has been excluded, it is not known whether cell RNA is contained within the nucleoid or elsewhere in the internal virus structure.

We have shown that the 72S viral RNA contained no significantly detectable sequences homologous to DNA from infected C₃H-MEC cells. This result is in disagreement with findings of homology between cellular DNA from transformed cells and viral RNA of Rous sarcoma virus (25) and Rauscher leukemia virus (11). Our findings of cellular RNA contained within a murine leukemia virus, as well as those of Bauer

(4) and Bonar et al. (5) for avian myeloblastosis virus, would explain the reported viral RNA-DNA homology.

Since the virus used in this study does not transform mouse embryo fibroblasts in vitro, the virus-cell relationship may differ from that of the Rous sarcoma virus. With the availability of established mouse cell lines which can be transformed by murine sarcoma viruses, a system comparable to Rous virus infection in chicken cells, we are now investigating whether sequence homology exists between the DNA of transformed cells and the high molecular weight RNA of virus produced by these cells.

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