Characterization of C-Type Particles Produced by a Tissue Culture-Adapted Murine Myeloma

LOY E. VOLKMAN AND R. G. KRUEGER

Department of Microbiology, University of Washington, Seattle, Washington 98105, and Department of Microbiology, Mayo Medical School, Rochester, Minnesota 55901

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C-type particles produced by a tissue culture-adapted BALB/c myeloma were characterized. It was determined that although the particles were morphologically and antigenically similar to murine leukemia and sarcoma virus, the size of their RNA was different, they lacked RNA-dependent DNA polymerase, they were unstable in NET buffer, sucrose and citrate but were stable in glycerol and Earle balanced salt solution, and they behaved differently from oncornaviruses when treated with ether and detergent.

Murine myelomas in culture have been reported to produce an RNA-containing virus with antigenicity, morphology, nucleic acid type (RNA), size (74S), and base composition similar to murine leukemia virus (MuLV) (43). The myeloma viruses Watson et al. (43) studied were all from oil-induced tumors except for those from the C-1 cell line, a spontaneous myeloma of a C3H mouse. Aoki et al. (3) showed that the C-type particle produced by MOPC-70A possessed the G (Gross) viral envelope antigen (GVEA) and χ VEA, a viral envelope antigen distinct from GVEA. Recently, Aoki and Todaro (4) demonstrated that clonal lines of spontaneously transformed BALB/3T3 cells released endogenous C-type particles with antigenic properties (χ VEA) in common with the murine myeloma-associated virus in addition to a previously uncharacterized C-type particle.

We induced a myeloma in a BALB/c mouse (FLOPC-1) by the membrane filter-Lucite disk implantation technique (31) and found that it had an unusual early development, i.e., the tumor cells went through a plasma cell maturation cycle (42). We wanted to determine whether tissue culture-adapted FLOPC-1 cells produced particles similar to MuLVs with the following characteristics of oncornaviruses: (i) C-type particle morphology (6, 7); (ii) nucleic acid is RNA; (iii) buoyant density of approximately 1.16 g/ml in sucrose in NET buffer (0.1 M NaCl, 0.01 M Tris-hydrochloride, pH 7.4, and 0.001 M EDTA) (15, 23, 27); (iv) buoyant density shift when treated with mild detergent and/or ether to 1.24 to 1.27 g/ml (11, 37, 38); (v) MuLV-murine sarcoma virus (MuSV) antigenicity (gs₁ and gs₂) (17, 20, 32); (vi) RNA is 60 to 75S, goes to approximately 36S when heated or treated with dimethyl sulfoxide (13, 24, 33); and (vii) RNA-dependent DNA polymerase (5, 39, 41).

All attempts to demonstrate oncogenic activity of the myeloma C-type particles, both in vitro with a variety of tissue culture lines and in vivo with newborn rats and mice, have consistently given negative results (43, unpublished data). Recently, however, Karpas and Cawley (26) described an assay using BALB/c 3T3 cells for detecting biologically active C-type particles in the culture fluid of MOPC-21 myeloma cells co-cultivated with BALB/c 3T3 cells. However, because the role that each of the cell types played in the genesis of the biologically active particles is unknown, strict lineage to the MOPC-21 cells is uncertain.

We found that while FLOPC-1 produced RNA-containing particles which were morphologically similar, had similar antigens to murine leukemia and sarcoma virus (MuLV-MuSV) (G. C. Miller and R. G. Krueger, Abstr. Annu. Meet. Amer. Soc. Microbiol., 73rd, Miami Beach, p. 256, 1973) and were capable of inducing syncytia formation on XC cell monolayers (L. E. Volkman and R. G. Krueger, J. Nat. Cancer Inst., in press), the size of the RNA was different, they lacked RNA-dependent DNA polymerase, they were unstable in NET buffer, sucrose and citrate, and they behaved differently from oncornaviruses when treated with ether and detergent.

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MATERIALS AND METHODS

Materials. All radioisotopes were purchased from

New England Nuclear Corp., Boston, Mass. (except for some ^3H -uridine that was purchased from Amersham/Searle Corp., Arlington Heights, Ill.); uridine-5- ^3H (25.4 to 27.8 Ci/mmol); uridine-2- ^{14}C (52.7 mCi/mmol); thymidine-*methyl*- ^3H 5'-triphosphate (dTTP), tetrasodium salt (18 to 48 Ci/mmol); thymidine-*methyl*- ^3H (20 Ci/mmol); ^3H -adenine (3.3 to 8.4 Ci/mmol).

Rous sarcoma virus (RSV) and Rous associated virus 49 (RAV49) were provided courtesy of Robert Friis; horse serum was from BBL; fetal calf serum was from BBL and GIBCO; Earle balanced salt solution (EBSS) was from GIBCO; dATP, dGTP, and dCTP were from Sigma and Nutritional Biochemical Co.; poly riboadenylate deoxythymidylate was from Miles Laboratories, Inc., and DNase-free bovine pancreatic ribonuclease was from Calbiochem.

Tissue culture. The FLOPC-1 tumor cells, adapted to growth in cell culture as described previously (L. E. Volkman and R. G. Krueger, *J. Nat. Cancer Inst.*, in press), were grown in Dulbecco modified Eagle medium containing 20% fetal calf serum. The cell line has been tested periodically by George Kenney (University of Washington) or Thomas Smith (Mayo Clinic) for mycoplasma contaminants, and none have been detected. Normal hamster kidney cells infected with MuLV or MuSV were obtained from Wade Parks. These cells were grown in minimal essential medium containing 20% heat inactivated fetal calf serum at 37 C.

Labeling and harvesting C-type particles. FLOPC-1 tissue culture cells, at 2 to 4×10^5 cells/ml, were labeled with $5 \mu\text{Ci}$ of ^3H -uridine and $5 \mu\text{Ci}$ of ^3H -adenine per ml unless otherwise indicated. The cells were incubated for 18 h at 37 C in a 15% CO_2 -85% air environment before the C-type particles were harvested.

Two harvesting procedures were used in this study. In the first procedure, the cells were pelleted by centrifugation at $1,000 \times g$ for 10 minutes, the supernatant fluid was centrifuged for 15 min at $10,000 \times g$, and the $10,000 \times g$ supernatant fluid, in turn, was layered on a glycerol cushion (density of 1.27 g/ml) and centrifuged for 60 min at $100,000 \times g$ at 4 C in a Spinco SW27 or SW40 rotor. The material at the interface (density of 1.10 to 1.20 g/ml) was carefully removed by pipetting and treated as the C-type particle containing fraction.

The second procedure was the same as the first, except that the glycerol cushion was eliminated. The $100,000 \times g$ pellet was resuspended by gentle pipetting. MuLV and MuSV were harvested from culture media by this procedure.

Density gradient centrifugation and purification. The C-type particle fraction was layered in linear glycerol gradients in EBSS (density of 1.12 to 1.24 g/ml) and centrifuged for 16 h at $100,000 \times g$ or for 3 h at $235,000 \times g$ at 4 C in a Spinco SW50.1 rotor. The gradients were fractionated with an Isco model 640 gradient fractionator and simultaneously monitored at 254 nm with an Isco UV analyzer (Instrument Specialties Co., Lincoln, Neb.). The density of the gradient fractions was determined by weighing 100- μl samples in tared 100- μl disposable capillary pipettes with a Mettler model H-20 analytical balance.

Trichloroacetic acid precipitation of labeled samples. Two methods of trichloroacetic acid precipitation of labeled samples were used routinely. For 0.1- or 0.2-ml samples, 100 μg of bovine serum albumin (in 0.1 ml) was added as carrier, and 0.15 M NaCl was added to bring the sample volume to 0.5 ml. An equal volume of cold 10% trichloroacetic acid was then added, and the samples were mixed, incubated at 4 C for 30 min, and filtered onto Gelman 25-mm type A glass fiber scintillation filters which were allowed to dry.

For samples of less than 0.1 ml, a batch precipitation method was used. The samples were spotted onto and absorbed by Whatman 3MM 23-mm scintillation pads and placed into a beaker containing ice cold 15% trichloroacetic acid for 15 min. The filters were transferred to a beaker of ice cold 10% trichloroacetic acid for two 15-min washes, the trichloroacetic acid was drained off, and the filters were rinsed briefly with 95% ethanol and allowed to dry. Filters from both procedures were put into scintillation vials containing 10 ml of Liquifluor (New England Nuclear Corp.) and counted in a Beckman LS-100 or LS-355 liquid scintillation counter to 2% error.

Negative staining of C-type particles. The $100,000 \times g$ pellet and gradient-purified 1.18 g/ml particles were negatively stained with phosphotungstate and examined by electron microscopy as described previously (L. E. Volkman and R. G. Krueger, *J. Nat. Cancer Inst.*, in press).

Thin sections of FLOPC-1 in vitro. FLOPC-1 tissue culture cells were grown to 10^6 to 2×10^6 cells/ml in 35×10 mm Falcon tissue culture dishes and fixed in the dish by a modification of the method described by Chambers and Weiser (9). The dishes were trimmed off and sections were cut and stained with uranyl acetate and lead. The sections were examined with an RCA electron microscope.

Assay for RNA-dependent DNA polymerase. The assay procedure of RNA-dependent DNA polymerase was adapted from that described by Green et al. (19) with each assay mixture containing in 200 μl : 40 mmol of Tris-hydrochloride, pH 8.1; 5 mmol of dithiothreitol; 2.46 mmol of MgCl_2 or 1 mmol of manganese acetate; 0.1 mol of each of dATP, dGTP, and dCTP, 25 μCi of ^3H -TTP per ml, 43 mmol of NaCl, 0.1% (vol/vol) of Triton X-100, 0.1 mg of poly dT-poly rA per ml (38), and 5 to 50 μg of viral protein. The reaction mixture was incubated at 37 C, and 50- μl samples were removed at 0, 30, 60, and 90 min and processed by the batch trichloroacetic acid precipitation method described previously. Poly deoxythymidylate poly riboadenylate was added as an additional template because Spiegelman (40) showed it stimulated RNA-dependent DNA polymerase activity better than most other polymers.

Detergent-ether extraction of C-type particles. The C-type particle fraction was treated with a variety of detergents, including Nonidet P-40, Triton X-100, sodium desoxycholate (SDS), and Tween 80 at concentrations from 0.1 to 2% (vol/vol) with or without ether (vol/vol). In a typical experiment, the fraction to be treated was suspended in either EBSS, EBSS plus glycerol, or NET. The appropriate amount and type of detergent was added, and the sample was incubated or vortexed for 3 min to 2 h at 4 C or room

temperature, and then was centrifuged to separate the phases. The aqueous phase was removed and bubbled with N_2 gas to remove residual ether. In some cases, the procedure outlined by Duesberg (14) was followed, in which case the sample was suspended in NET, pH 7.4, 1 mM dithiothreitol, 0.2% Tween 80, plus (vol/vol) ether and vortexed at 4°C for 5 to 20 min. The sample was centrifuged at $2,000 \times g$ for 5 min, the ether phase was pipetted off, and N_2 gas bubbled through the aqueous phase to remove residual ether.

RNA extraction. The RNA extraction method was adapted from a method described by Duesberg (12). Cultures of FLOPC-1 cells, at 5×10^6 cells/ml, were labeled with $5 \mu\text{Ci}$ of ^3H -uridine per ml and incubated for 18 h at 37°C in a 15% CO_2 -85% air environment. The tissue culture medium was centrifuged at $1,000 \times g$ for 15 min, and the supernatant fluid was centrifuged at $100,000 \times g$ for 60 min. The $100,000 \times g$ pellet was resuspended in EBSS, pH 7.0, and banded on glycerol gradients in EBSS by centrifugation at $235,000 \times g$ for 3 h or at $100,000 \times g$ for 16 h in a Spinco SW50.1 rotor. The gradients were fractionated, the tubes containing the 254-nm optical density peak at 1.16 or 1.18 g/ml were pooled, the contents were diluted 1:4 with NET, pH 8.4, and 1% SDS (wt/vol), 1% dithiothreitol (wt/vol) and 1% diethyl oxydiformate were added. The sample was incubated for 5 min at room temperature followed by the addition of a 0.1 volume of redistilled phenol equilibrated with NET, pH 8.5, plus 0.5 M mercaptoethanol, and the mixture was shaken by hand for 15 min and centrifuged at $30,000 \times g$ for 5 min. The aqueous phase was removed and made 0.2 M potassium acetate, and the RNA precipitated with two volumes of cold 95% ethanol at -20°C for 16 h. The precipitate was collected by centrifugation at $1,500 \times g$ for 20 min, resuspended in NET, pH 8.5, made 0.2 M potassium acetate, and reprecipitated with 95% ethanol as before. The RNA was resuspended in NET, pH 7.4, and optical density readings were taken to determine purity. Cold myeloma or mouse liver ribosomal RNA (200 μg) was added as carrier, either before the phenol extraction or before the first ethanol precipitation step.

The RNA extraction procedure for MuLV was identical, barring two changes: monolayers of MuLV-infected normal rat kidney cells were labeled for 18 h with 20 μCi of ^3H -uridine and sucrose in NET gradients were used for viral purification. Murine myeloma and normal mouse liver RNA were isolated as described by Krueger and Kosky (28).

Velocity sedimentation of C-type particle RNA. Samples of labeled C-type RNA and unlabeled myeloma or mouse liver ribosomal RNA in NET, pH 7.4, were layered on linear 5 to 30% sucrose gradients in NET and centrifuged for 2 h at $125,000 \times g$. The gradients were fractionated and monitored at 254 nm, and the trichloroacetic acid-precipitable radioactivity of each fraction was determined.

RESULTS

Electron microscopy of FLOPC-1 cells in vitro. Thin sections of FLOPC-1 tissue culture cells, which were fixed directly in the tissue culture plates, revealed particles with C-type

morphology budding from the plasma membrane (Fig. 1A and B). Groups of particles could be seen external to the cells, but because some of the cells appeared to have degenerated, it is possible that many of the particles were released by a process (cell lysis, for example) other than budding. The majority of the particles appeared to have C-type particle morphology (10, 21), measuring 100 to 135 nm in diameter, being enveloped, and having an electron-dense endocentric nucleoid (Fig. 1C).

Buoyant density of the FLOPC-1 C-type particle. When a ^3H -uridine and ^3H -adenine labeled $100,000 \times g$ pellet was centrifuged on linear 1.10 to 1.24 g of glycerol per ml in EBSS gradient at $100,000 \times g$ for 16 h at 4°C, a major radioactive peak occurred at 1.18 g/ml (Fig. 2A). That the nucleic acid in this peak was RNA and not DNA was indicated by: (i) its presence when ^3H -uridine alone was used; (ii) the absence of the peak when ^3H -thymidine alone was used; and (iii) its sensitivity to RNase treatment but not to DNase. The smaller radioactive peak at the top of the gradient (density of 1.10 g/ml) is presumably material that did not band on the gradient.

An electron micrograph of particles in the $100,000 \times g$ pellet sample used to layer on the gradients is shown in Fig. 1D and 1E. The particles demonstrate the spherical shape and spikes characteristic of oncornaviruses (2, 7, 10).

Electron microscopy of the structures in the 1.18 g/ml peak revealed spiked structures similar to those seen in the $100,000 \times g$ pellet (Fig. 1D), although some of the integrity of the structures had been lost.

When an isotopically labeled C-type particle preparation was centrifuged for 3 h at $235,000 \times g$ instead of $100,000 \times g$ for 16 h, the major radioactive peak appeared at 1.16 g/ml rather than at 1.18 g/ml (Fig. 3, control). To determine whether or not the two peaks were analogous, a 1.16 g/ml peak was isolated, pelleted by centrifugation at $100,000 \times g$ for 3 h, and centrifuged on glycerol in EBSS gradient for 16 h at $100,000 \times g$. The major peak of radioactivity occurred at 1.18 g/ml. Thus, the buoyant density of the C-type particle varied as a function of centrifugal velocity and time.

Stability of FLOPC-1 C-type particles. The C-type particles appeared to be unstable in sucrose and in potassium citrate, two of the most commonly used materials in density gradient purification of viruses, as multiple peaks of radioactivity and/or optical density were seen at different buoyant density positions, depending on the gradient material (Table 1). Of all the materials tested, a single major repro-

FIG. 1. Electron micrograph of FLOPC-1 C-type particles budding from FLOPC-1 cells in tissue culture (arrows). Approximate magnification (A) $\times 9,150$ and (B) $\times 12,250$; (C) typical C-type morphology is clearly demonstrated by the FLOPC-1 extracellular particles. Approximate magnification $\times 68,000$; (D) and (E) are negatively stained FLOPC-1 extracellular particles in a partially purified $100,000 \times g$ pellet from tissue culture fluid. Approximate magnification (D) $\times 156,000$ and (E) $\times 52,000$.

FIG. 2. The effects of SDS and ether $\pm$ RNase on the FLOPC-1 C-type particles. (A) FLOPC-1 cells in Dulbecco modified Eagle medium containing 10% were labeled with $5 \mu\text{Ci}$ of ^3H -uridine and $5 \mu\text{Ci}$ of ^3H -adenine per ml for 18 h, and the C-type particles were isolated as described in Materials and Methods. Two samples were made 0.1% sodium desoxycholate and (vol/vol) ether, shaken for 2 h at 21 C, and centrifuged for 5 min at $2,000 \times g$ to separate the phases. The ether phase was removed, and the aqueous phase was bubbled with N_2 to remove any residual ether. One sample was further treated with $10 \mu\text{g}$ of RNase per ml at 4 C for 30 min. These samples were then layered on glycerol in EBSS + 0.5% bovine serum albumin gradients and centrifuged for 16 h at $100,000 \times g$ at 4 C. The gradients were fractionated and processed as described in Materials and Methods. Symbols: , control; , detergent treated; , detergent + RNase treated. (B) The $100,000 \times g$ fraction was prepared as in (A) except the cells were labeled for 24 h. A sample of $100,000 \times g$ fraction was treated with $10 \mu\text{g}$ of RNase per ml for 30 min at 4 C, then was layered on glycerol in EBSS, pH 5.6, + 1% dimethyl sulfoxide gradient, and was centrifuged for 16 h at $100,000 \times g$ at 4 C. Symbols: , control; , RNase treated.

FIG. 3. The effects of Tween 80 and ether (Duesberg) on FLOPC-1 C-type particles. FLOPC-1 tissue culture cells in Dulbecco modified Eagle medium containing 20% fetal calf serum, labeled for 18 h with 5 μ Ci of 3 H-adenine and 5 μ Ci of 3 H-uridine per ml, were pelleted by centrifugation at $1,000 \times g$ for 10 min. The supernatant fluid was further centrifuged at $100,000 \times g$ for 60 min, and the $100,000 \times g$ pellet was treated by the procedure described by Duesberg (14). The samples were layered in sucrose in NET gradients and centrifuged at $235,000 \times g$ for 3 h at 4°C. The gradients were fractionated and monitored for absorbance at 254 nm, density, and trichloroacetic acid-precipitable radioactivity as described in Materials and Methods. Symbols: —, FLOPC control; ■■■, FLOPC treated.

ducible peak with FLOPC-1 C-type particles was only observed with glycerol in EBSS gradients. The buoyant density of MuLV, MuSV, and RSV in glycerol, sucrose, and citrate gradients was determined; as reported previously (13, 23), these virions were stable in sucrose and citrate gradients buffered with NET or EBSS and had buoyant densities of 1.16 to 1.17 g/ml. It is interesting, however, that only RSV was stable in glycerol in EBSS gradients with a density of 1.19 g/ml whether centrifuged for 3 h at $235,000 \times g$ or for 16 h at $100,000 \times g$, whereas MuLV and MuSV had densities of 1.17 to 1.24 g/ml.

Effects of detergent and ether and RNase treatment. Because FLOPC-1 C-type particles were enveloped, they were expected to be sensitive to mild detergent-ether treatment, which would either release the internal nucleoid, as

described for the oncornaviruses (11, 37, 38), or would destroy the structure. When a cushioned $100,000 \times g$ fraction was treated with 0.1 to 2% Tween 80, Nonidet P40, Triton X-100, or SDS plus an equal volume of ether and was vortexed for as long as 2 h at 22°C, the 1.18 g/ml peak was not lost and had approximately the same amount of radioactivity as the control (Fig. 2A). If the detergent-ether treated sample was exposed to RNase, however, the peak disappeared (Fig. 2A), as compared with an RNase-treated sample which had not been first exposed to detergent and ether (Fig. 2B).

Because the detergent-ether extraction procedure had been modified from that described as being effective for oncornaviruses due to the instability of the FLOPC-1 C-type particles in sucrose and NET, the same procedure was tried on an RSV control. Approximately one-third of the RSV retained its density of 1.19 g/ml on glycerol in EBSS gradients after being vortexed with 0.1% SDS plus (vol/vol) ether for 2 h at 22°C. Because of these results, FLOPC-1 C-type particles and RSV were disrupted by the procedure described by Duesberg (14) and analyzed on sucrose in NET gradients. Figure 3 shows that a portion of the FLOPC-1 C-type particle structures in the less dense peaks and all of the structures in the 1.26 to 1.28 g/ml peak were removed by the detergent-ether treatment; as expected, RSV was completely converted (data not shown) to a structure with a density of 1.26 g/ml (14). Thus, many of the FLOPC-1 C-type particle structures that survived the more vigorous detergent-ether treatment in the presence of EBSS, pH 7.0, were destroyed by a less stringent treatment in the presence of NET and dithiothreitol, and the 1.26 g/ml peak, perhaps analogous to the oncornavirus 1.26 g/ml peak containing the nucleoid core (14), was particularly sensitive.

RNA-dependent DNA polymerase. Because

TABLE 1. Buoyant densities of FLOPC-1 C-type particles in different gradient materials^a

Gradient type	FLOPC-1 C-type particles (g/ml) ^b
Glycerol in EBSS, pH 7.0	1.16
Glycerol in NET, pH 7.4	1.25 or none
Sucrose in NET, pH 7.4	1.15, 1.17, 1.18, 1.26
Sucrose in EBSS, pH 7.0	1.19, 1.25
Potassium citrate in NET, pH 7.4	1.13, 1.16, 1.22

^a Samples layered on the gradients were the partially purified $100,000 \times g$ pellets. The gradients were centrifuged for 3 h at $235,000 \times g$ and 4°C in an SW50.1 rotor of a Beckman L2-65B ultracentrifuge.

^b The major gradient peaks.

the presence of RNA-dependent DNA polymerase is a specific characteristic of most of the murine C-type particles (1, 36, 39), we tested for its presence in the FLOPC-1 C-type particles. The assay was performed as described in Materials and Methods by using gradient-purified MuLV as a control. When samples from gradient-purified C-type particles were tested, no activity was detected (Table 2). Furthermore, other experiments (data not shown) revealed no indication of activity when ^3H -TTP with higher specific activity was used, and Mn^{2+} replaced Mg^{2+} in the assay.

To determine whether enzyme activity in the FLOPC-1 C-type particles was masked by an inhibitor or endogenous RNase, an assay was carried out in which MuLV and C-type particle samples were mixed (Table 2). The MuLV activity was reduced by one-half when the MuLV sample was halved, but there was no evidence of inhibition.

A partially purified $100,000 \times g$ pellet was also tested for activity to rule out the remote possibility that the enzyme might have been lost during the purification of the FLOPC-1 C-type particles. The $100,000 \times g$ pellet, however, did not possess activity (Table 2).

Characterization of the FLOPC-1 C-type particle RNA. RNA extracted from gradient-

purified 1.16 and 1.18 g/ml C-type particles (glycerol in EBSS gradients) was analyzed by velocity sedimentation in sucrose gradients (Fig. 4). Mouse myeloma RNA was added to the 1.16 g/ml particles as a carrier and an optical density marker prior to RNA extraction, whereas mouse liver ribosomal RNA was added after extraction to the 1.18 g/ml sample. Two species of RNA were prominent in both C-type particle samples, an 18S species and a 28S species which co-sedimented with the 18S and 28S ribosomal RNA markers. It should be noted that there was no evidence of significant RNase degradation for the ribosomal RNA appeared to be intact.

RNA from gradient-purified MuLV was analyzed on a similar gradient as a control (data not shown). The characteristic high-molecular-weight species (54S), which was broken down to smaller units (29S and 15S) upon heating, was observed. It was determined that the buoyant density of the FLOPC-1 1.18 g/ml C-type particle RNA in CsSO_4 was 1.668 g/ml, the same as cellular ribosomal RNA.

DISCUSSION

The FLOPC-1 C-type particles were unstable in gradients of potassium citrate or sucrose in NET, unlike other C-type particles, whereas they appeared to be stable in glycerol in EBSS gradients, again unlike many other C-type particles. This stability, however, was not complete, for electron micrographs of particles purified on glycerol gradients showed many fragmented and pleomorphic structures. The observation of a shift in the buoyant density of the C-type particle with centrifugation time suggests inclusion of gradient material in the particles through membrane breaks (James McSherry, personal communication).

Centrifugation of FLOPC-1 C-type particle in sucrose in NET gradients resulted in multiple peaks, the major ones being at 1.15, 1.17, and 1.24 to 1.27 g/ml. The 1.24 to 1.27 g/ml peak was particularly sensitive to Tween 80-ether treatment when done according to Duesberg (14). It is interesting that nucleoid cores of oncornaviruses, which are obtained by Tween 80-ether treatment of whole particles but which are destroyed by excessive treatment (45), also band at 1.24 to 1.27 g/ml. Watson et al. (43) reported that the P3 myeloma C-type particles had a density of 1.20 to 1.22 g/ml on sodium tartrate in NET gradients and were very sensitive to treatment with SDS, ether, or Tween 80. Thus, the P3 and FLOPC-1 myeloma C-type particles are different from oncornaviruses; the latter (FLOPC) by their behavior and buoyant

TABLE 2. RNA-dependent DNA polymerase assay of gradient-purified FLOPC-1 C-type particles and MuLV^a

Expt	Virus	Time of incubation (counts/min)			
		0	30	60	90
I	MuLV (8 μg)	2,148	5,006	6,830	8,675
	FLOPC, 1.16 (10 μg)	3,487	3,557	3,105	3,760
	FLOPC, 1.18 (42 μg)	3,451	3,591	3,358	4,018
	FLOPC, 1.26 (27 μg)	2,956	3,291	3,416	3,690
	Control	2,850	4,100	3,658	4,350
II	MuLV (16 μg)	1,514	50,568	90,302	133,500
	MuLV (8 μg) + FLOPC, 1.16 (15 μg)	1,355	25,208	42,821	73,500
	MuLV (8 μg) + FLOPC, 1.18 (15 μg)	1,654	20,981	41,471	62,500
	MuLV (8 μg) + FLOPC 100,000 $\times$ g pellet (27 μg)	1,574	20,497	43,244	67,200
	FLOPC 100,000 $\times$ g pellet (55 μg)	1,668	1,961	1,873	2,160
	Control	1,382	1,765	1,379	1,440

^a Gradient-purified MuLV and FLOPC-1 C-type particles and the $100,000 \times g$ pellet were prepared as described in Materials and Methods. The assay was carried out as described in Materials and Methods, and the amount of viral protein per reaction mixture is indicated in parentheses. The control sample is a reaction mixture without viral protein.

FIG. 4. RNA from FLOPC-1 C-type particles. C-type particles labeled with ^3H -uridine were banded on glycerol in EBSS gradients, extracted with phenol, and analyzed by velocity sedimentation in sucrose gradients. Symbols: (A) —, mouse myeloma ribosomal RNA optical density marker; ---, RNA from 1.16 g/ml FLOPC-1 C-type particles. (B) —, mouse liver ribosomal RNA optical density marker; ---, RNA from 1.18 g/ml FLOPC-1 C-type particles.

density on sucrose in NET, and their extreme sensitivity to the Duesberg Tween 80-ether treatment (14), and the former (P3) by their buoyant density on tartrate and their sensitivity to Tween 80-ether.

The observation that SDS and other detergents in the presence of ether had little effect on the density of the FLOPC-1 C-type particle or RSV when they were treated in culture medium or EBSS and centrifuged on glycerol gradients indicates that either the absence of cations (+EDTA) or the prevention of sulfhydryl bond formation (+DTT) is necessary for solubilization of the membranes. Alternatively it may be that glycerol interacts with the particles in such a way that the nucleoid core and the whole particle appear to have the same buoyant density. Glycerol does seem to affect C-type particles in different ways and of all the particles tested, only RSV and FLOPC-1 C-type particles were stable in it.

The search for an RNA-dependent DNA polymerase associated with FLOPC-1 C-type particles yielded negative results. The possibility that a nonspecific inhibitor was present in the FLOPC preparation was ruled out by mixing FLOPC C-type particles with MuLV and demonstrating activity. However, the possibility that polymerase activity was masked by a specific inhibitor still exists. Other possibilities are that: (i) a different polymerase is present which cannot be detected with the assay conditions used to detect oncornavirus RNA-depend-

ent DNA polymerase (the myeloma A-type particles, for example, have a polymerase activity different from that of oncornaviruses [44]); and (ii) the particle has no polymerase of its own and depends on the cellular polymerase for its replication.

The RNA contained in the 1.18 and 1.16 g/ml FLOPC-1 C-type particles was determined to be 18S and 28S, the same as the ribosomal marker, with barely a hint of a larger species. Also, the buoyant density of the 1.18 g/ml RNA in CsSO_4 was identical to the ribosomal marker, 1.668 g/ml. These results suggest ribosomal contamination in the purified C-type particle gradient fractions. It is possible that because all of the direct evidence (electron microscopy) for the presence of C-type particles in the 1.16 or 1.18 g/ml regions of the glycerol gradients was with unlabeled C-type preparations, the labeled preparations may have been different; possibly by a preferential labeling of ribosomal RNA and ribosomes being co-purified with the particles or ribosomes or ribosomal RNA being incorporated into the particles. If this were true, one would have expected some evidence of 70S RNA if C-type particles were present at all. There is one group of RNA viruses, the arenaviruses, which incorporates host ribosomal RNA, but these virions also contain other species of RNA (8).

Another possibility is that the 18S and 28S species are truly viral RNA species. The buoyant density of C-type virus RNAs in CsSO_4 is in fact the same as the ribosomal marker RNA

(22, 34). It has also been shown that the 60 to 70S oncornavirus genome is composed of smaller subunits of about 3×10^6 daltons, and it is estimated that 3 to 4 are contained within each virion (16). Green has reported that cells infected with MuSV contain viral RNA sequences in 35s and 20s molecules (18). Furthermore, McCain et al. (30) have shown that the 69S RNA of murine sarcoma-leukemia virus can be dissociated into 37S, 18S, and 4S species. Kuff (29) has also reported that myeloma intracisternal A-type particles contain one major species, 29S RNA. It is possible, therefore, that the 18S and 28S species of RNA are subunits of a defective C-type particle genome.

The FLOPC-1 C-type particles do possess properties in common with MuLV, including C-type morphology and similar antigenicity. G. C. Miller and R. G. Krueger (Abstr. Annu. Meet. Amer. Soc. Microbiol., 73rd, Miami Beach, p. 256, 1973) have shown by complement fixation that gradient purified FLOPC-1 C-type particles react with rabbit anti-MuLV serum and Fischer rat anti-MuLV-MuSV serum and that rabbit anti-FLOPC-1 C-type particle sera react with MuLV and MuSV. Furthermore, the capacity of gradient-purified FLOPC-1 C-type particles to induce syncytium formation in the XC cell cytopathogenicity test is also characteristic of MuLV (25). In addition, MuLV gs antigens were detected on FLOPC-1 C-type particles and FLOPC-1 tumor cells by radioimmunoassay (Wade Parks, personal communication (35). Thus, the FLOPC-1 C-type particle appears to be either a MuLV subtype, a defective MuLV virion, or perhaps a new murine virus. The latter conclusion is in agreement with the observations of Aoki and co-workers (3, 4) on other murine myeloma associated viruses.

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