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Characterization of DNA Polymerase and RNA Associated With A-Type Particles from Murine Myeloma Cells

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The RNA-dependent DNA polymerase present in intracisternal A-type particles from mouse myeloma tumor cells has been studied. This polymerase can use either endogenous A particle RNA or an exogenous synthetic polynucleotide [poly(rA)] as a template. The DNA reaction product is small (4*S*–10*S*) and over 90% of it hybridizes to A particle RNA, whereas up to 50% of it hybridizes to murine sarcoma-leukemia virus RNAs. The RNA isolated from purified A particles is generally of low molecular weight (5*S*–15*S*) but contains small amounts of 70*S* and 35*S* components. These results suggest that A-type particles may be related to C-type oncornaviruses.

Intracisternal A-type particles are commonly found in a variety of transformed cells, although they have occasionally been observed in normal cells as well (3, 7, 17). Thus, while these particles resemble morphologically the cores of B-type and immature C-type particles (8), their relationship to bona fide tumor viruses remains uncertain. The resolution of this problem is made difficult by the fact that the intracisternal particles apparently do not ordinarily leave the cell, and when they are purified from disrupted cells they are not infectious (7). Our approach to this question of the viral relatedness of A particles has been to look for virus-specific macromolecules in purified particles. In particular, we felt that if a true reverse transcriptase activity could be found in these particles it would strongly imply a close relationship to the two other classes of oncornaviruses. This approach has also been followed by several other groups of investigators, with varied results. Some workers have reported the presence of a DNA polymerase in A particles which is different from viral reverse transcriptase in that it will transcribe only exogenous synthetic homopolymer templates (15, 16). Others have found that while reverse transcriptase activities exist in myeloma cells they are not associated with A- or C-type particles (5).

In the present communication, experiments are described which show that A-type particles do in fact contain a reverse transcriptase which is similar to those found in true oncornaviruses. While this work was in progress, Yang and Wivel (19) also reported that an endogenous reverse transcriptase activity can be detected in A particle preparations. A preliminary report of some of our data has been presented elsewhere (12).

MATERIALS AND METHODS

Reagents. Triton X-100, sucrose (RNase free), actinomycin D, pancreatic RNase A (type I-A), DNase I, deoxynucleoside triphosphates (dGTP, dATP, dCTP, and dTTP), and bovine serum albumin were all purchased from Sigma Chemical Co.; pronase (B grade) was from Calbiochem; cesium sulfate (ultrapure) was from Alfa Inorganics; and [^{3}H]thymidine triphosphate and [^{3}H]deoxyguanosine triphosphate were from New England Nuclear.

Preparation of A particles. Intracisternal A particles were prepared and purified according to the method of Wilson et al. (15, 16) from MOPC 460 solid tumor plasmacytoma (myeloma) cells, with the exception that the initial homogenization buffer contained 0.5 mg of heparin per ml. The membrane fraction (mitochondria, heavy microsomes, and lysosomes) was prepared by spinning the post-nuclear supernatant 20,000 $\times$ *g* for 20 min. The pellet was resuspended to a final volume equal to one-half the original volume of tissue in solution A (0.25 M sucrose, 0.05 M Tris-hydrochloride, pH 7.6, 0.025 M KCl, 0.005 M $MgCl_2$). The membranes were made 0.2% in Triton X-100 and 5 μg/ml in DNase and sheared at least five times each through 21-, 23-, 25-, and 27-gauge syringe needles. The sheared membranes were incubated for 15 to 20 min at room temperature and then diluted 7 to 9 times in 0.15 M potassium citrate, pH 7.2, and centrifuged for 30 min at 35,000 rpm in a Spinco type 42 rotor. The supernatant was discarded and the pellet was resuspended in solution B (25% sucrose in 0.05 M potassium citrate, pH 7.2) and sheared five times through a 25-gauge needle. The resuspended particles were clarified by centrifugation at 10,000 $\times$ *g* for 10 min, layered on top of a 48% sucrose cushion containing 0.05 M potassium citrate, pH 7.2, and centrifuged overnight in a Spinco SW-27 rotor at 25,000 rpm. The pelleted particles were resuspended in 0.05 M potassium citrate, pH 7.2, and then banded by centrifugation for 18 h in a 33 to 68% sucrose gradient containing 0.05 M potassium citrate, pH 7.2, in a SW-27 rotor at 25,000 rpm. The gradients were fractionated and analyzed for UV absorption at 280 nm. Peak fractions were made 3 mM β-mercaptoethanol, aportioned, and stored in liquid nitrogen.

Polymerase assay. Endogenous polymerase activity was assayed using the following reaction mixture in a final volume of 50 μliters: 50 mM Tris-hydrochloride, pH 8.3, 12 mM $Mg(OAc)_2$, 0.2 M KCl, 6 mM β-mercaptoethanol, 350 μg of bovine serum albumin per ml, 0.2% Triton X-100, 19% (vol/vol) glycerol, 0.3 mM [^{3}H]dTTP (500 counts/min per pmole), 0.5 mM each of dATP, dGTP, dCTP, and A particles (300 μg of protein per ml). This same reaction mixture was used to assay exogenous polymerase activity, except that dCTP, dGTP, and dATP were omitted and 0.460 mg of poly(rA)·oligo$(dT)_{9,10}$ per ml (1:1) was added to the mixture. Actinomycin D and oligo$(dT)_{9,10}$ when used in the endogenous assay were at 50 μg/ml and 0.18 μg/ml, respectively. The above assay mixtures were pre-incubated without triphosphates for 10 min on ice and then the reaction was initiated by addition of triphosphates. Incubation was for 90 min at 37 C. Incorporation of [^{3}H]dTTP was determined either by precipitation with cold trichloroacetic acid or by adsorption on DEAE filters. The trichloroacetic acid method consisted of adding 100 μg of tRNA carrier and 1.0 ml of 12.5% trichloroacetic acid containing saturated Na_3PO_4-$Na_4P_2O_7$ (15), followed by filtration of the precipitate with HAWP membrane filters (Millipore Corp.). The filters were then washed with 15 ml of 5% trichloroacetic acid (containing no phosphate). In the DEAE filter method, the 50-μliter reaction mixture was pipetted directly onto a numbered filter, and the filters were washed batchwise six times in 0.5 M Na_2HPO_4 (10 ml per filter and 5 min per wash), rinsed twice with distilled water, and once each with 95% ethanol and ethyl ether. Both the membrane filters (Millipore Corp.) and DEAE filters were dried and counted in a toluene base scintillation fluid. The DEAE filter method proved to be more reliable and gave lower background values.

Preparation of [^{3}H]DNA reaction product. The reaction mixture (usually 1 to 2 ml) was made either 1% in sodium dodecyl sulfate (SDS) and incubated for an additional 10 min at 37 C, or 0.5% in SDS and 500 μg/ml in pronase and incubated for 30 to 45 min at 37 C. The mixture was then extracted two times with an equal volume of phenol and washed three times with ether. Carrier tRNA (200 μg/ml) and one-tenth volume of 20% NaOAc, pH 5.4, were added, followed by 2.5 volumes of absolute ethanol. After precipitation overnight at −20 C and centrifugation for 20 min at 20,000 × g, the pellet was dissolved and passed over a Sephadex G-50 column (40 cm by 0.9 cm) in 0.1 M NH_4HCO_3, pH 8.0, and the void volume was then ethanol precipitated as above. For those experiments requiring removal of template (and carrier) RNA, the preparation was hydrolyzed by boiling for 10 min in 0.3 N NaOH and neutralized with HCl before G-50 chromatography.

Cs_2SO_4 equilibrium banding of [^{3}H]DNA reaction product. The reaction products were prepared as described above, dissolved in 10 mM Tris-hydrochloride, pH 7.2, and 1 mM EDTA, and added along with a dsDNA marker (S1 nuclease-treated MOPC 460 cellular DNA) and a ssRNA marker (*Escherichia coli* K-12 tRNA) or a dsRNA marker (encephalomyocarditis virus replicative form) and enough buffer (10 mM Tris-hydrochloride, pH 7.2, 1 mM EDTA) to give a 3.1-ml volume. Solid Cs_2SO_4 (2.5 g) was added to give a final density of 1.55 g/cm^3. Centrifugation was for 60 h at 4 C in a Spinco SW-56 rotor at 33,000 rpm. Fractions were collected and made 5% in trichloroacetic acid, and precipitates were collected by filtration and counted.

Rate zonal sedimentation of nucleic acids. For detection of high-molecular-weight components in A particle RNA and RNA:DNA hybrids, samples were layered on a 5 to 30% sucrose gradient containing TNE Buffer (10 mM Tris-hydrochloride, pH 7.2, 0.1 M NaCl and 1 mM EDTA) plus 0.5% SDS and centrifugation was for 60 min at 53,000 rpm at 20 C in a Spinco SW-56 rotor. Fractions were collected and analyzed by measuring absorption at 260 nm (A_{260}) or by precipitation in 5% trichloroacetic acid and counting.

Extraction of RNA from A particles. Purified A particles were made 1% in SDS and extracted twice with an equal volume of phenol. The aqueous phase was made 3 mM in $Mg(OAc)_2$, 4 volumes of absolute ethanol was added, and RNA was precipitated at −20 C overnight and collected by centrifugation in a Spinco SW-40 rotor at 40,000 rpm and 0 C. The RNA pellet was dissolved in sterile TNE Buffer and kept frozen at −20 C. Murine C type RNA used in hybridization studies was a generous gift from Maurice Green (13). The mRNA and rRNAs used in hybridizations were prepared (by Charles Lawrence) from an S-30 extract from Krebs II_a ascites tumor cells by SDS-phenol extraction, oligo(dT)-cellulose chromatography, and sucrose gradient sedimentation. The mRNA was that material which adhered to the column in high salt but was eluted in low salt buffer. The 18*S* and 28*S* rRNAs were peak fractions from a sucrose gradient sedimentation of the material which did not adhere to the oligo(dT)-cellulose column in high salt.

Hybridizations. Alkali-treated [^{3}H]DNA prepared from actinomycin D containing reactions was used in all hybridization reactions. These were performed at 68 C in 0.6 M NaCl, 25 mM Tris-hydrochloride, pH 7.4, and 2 mM EDTA with 10 μg of RNA per ml. Either 50- or 100-μliter volumes were used and were overlaid with mineral oil. To assay for hybridized DNA the mixture was diluted with 1.0 ml S1 nuclease buffer (30 mM NaOAc, pH 4.5, 0.2 M NaCl, 0.3 mM $ZnSO_4$), and 30 μg of denatured cold DNA per ml and 800 U of S1 nuclease per ml were added. After a 30-min incubation at 48 C, 150 μg of carrier tRNA was added, followed by an equal volume 20% trichloroacetic acid, and the precipitate was collected by filtration and counted. S1 nuclease was prepared according to the method of Sutton (9) and kept frozen in 25% glycerol in liquid nitrogen.

Electron microscopy. A particles were adsorbed to thin carbon films (supported by thick reticulated carbon films mounted on copper grids), positively stained with 2% uranyl acetate in 30% ethanol, rinsed with 30% ethanol, dried, and examined in a Philips EM-300 electron microscope.

RESULTS

Initial studies with crude particle preparations revealed extensive physical heterogeneity within the A particle population, as well as the presence of contaminating polymerase activities. Figure 1 shows an equilibrium sucrose density gradient profile of the membrane fraction (mitochondria, heavy microsomes, and lysosomes) from MOPC 460 myeloma cells which has been sheared in the absence of detergent through a 27-gauge needle before centrifugation through a linear 10 to 68% sucrose gradient. Two major exogenous polymerase activities [defined by the poly(rA)·oligo(dT)-dependent polymerization of dTTP; see Materials and Methods for details] which band at approximately 1.22 g/cm^3 and 1.11 g/cm^3 can be seen. In addition, there is endogenous polymerase activity (defined as the polymerization of all 4 dNTPs with no added template) spread throughout most of the gradient, with a peak around 1.09 g/cm^3. Electron microscope analysis of the gradient fraction revealed high concentrations of A particles in the pellet and at 1.22 g/cm^3, as well as smaller numbers of particles scattered throughout the entire gradient (relative amounts of A particles are indicated by "plus" signs in Fig. 1). The reason for the scatter is apparent in the electron microscope: all but the densest particle (in the pellet) are attached to varying amounts of membranous material. This phenomenon is shown in Fig. 2a, which is an electron micrograph of the material in gradient fraction 5. It is important to note that the data in Fig. 1 show no indication of contamination by C-type particles. Had they been present in significant quantities, they would have given rise to peaks of exogenous and endogenous activity banding at 1.16 g/cm^3. They also would have been detected by electron microscope analysis.

The higher density region of the gradient (ρ = 1.20 to 1.25 g/cm^3) appears to contain relatively little endogenous polymerase activity. However, when the material in this region was further purified by shearing through a 27-gauge needle in the presence of 0.1% Triton X-100 and then rebanded in a sucrose gradient, a distinct peak of endogenous polymerase activity could be seen (data not shown). Thus the lack of obvious endogenous activity in the crude high density fractions (Fig. 1) is due to the high background level. This background is apparently ascribable to the endogenous polymerase activity which is maximal in the 1.08 to 1.12 g/cm^3 region of the gradient. This activity is distinguishable from that in A particles by the fact that it was only slightly inhibited by the inclusion of 50 μg of actinomycin D per ml in the standard assay, whereas the purified A particle activity showed marked inhibition under the same conditions, as will be discussed further below. Moreover, most of the DNA synthesized by the polymerase(s) in fraction 19 could not be hybridized to A particle RNA.

To obtain a more purified A particle preparation, we followed the purification scheme developed by Wilson et al. (15, 16). Figure 3 shows the equilibrium banding profile from a 33 to 68% sucrose density gradient. The A_{280} peak at 1.22 to 1.24 g/cm^3 contains both endogenous and exogenous polymerase activities, as well as the majority of the A particles (scored by electron microscopy; data not shown). There is also a peak of polymerase activity at 1.19 g/cm^3, which may represent particles still bound to excess membrane. (The size of this second peak

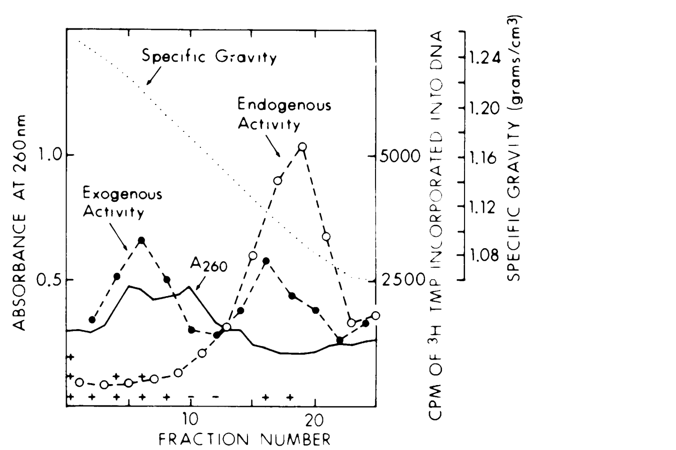

FIG. 1. *Banding profile of crude A-type particles in a sucrose density gradient. A particles were prepared by shearing a MOPC 460 tumor cell membranes fraction through a 27-gauge needle without detergent and in the presence of DNase. This preparation was then layered on a 10 to 68% sucrose density gradient containing 0.05 M potassium citrate, pH 7.2, and centrifuged for 8 h at 40,000 rpm in a Spinco SW-40 rotor. Fractions from the gradient were analyzed for A_{260}, specific gravity, presence of A-type particles (relative amounts indicated by plus or minus signs; fractions lacking signs were not examined), and exogenous and endogenous polymerase activity as described in Materials and Methods, except that cold dNTPs were used at one-third the normal concentrations.*

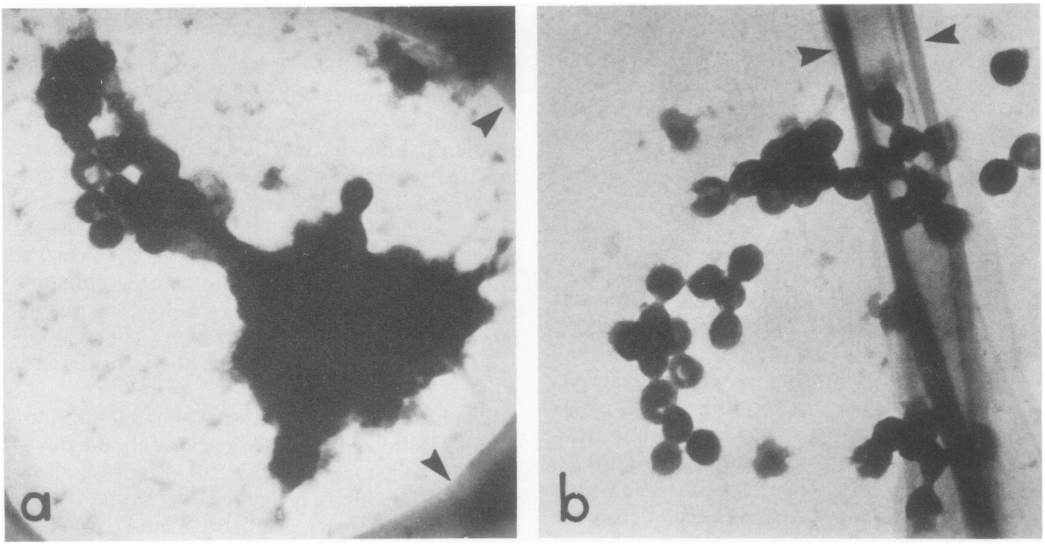

FIG. 2. *Electron micrograph of A-type particles at different stages of purification. Typical samples of particles banding at approximately 1.23 g/cm³ from Fig. 1 and Fig. 4 are shown in panels a and b, respectively. Total magnification as printed is ×82,000. Arrows indicate portions of thick carbon reticulum.*

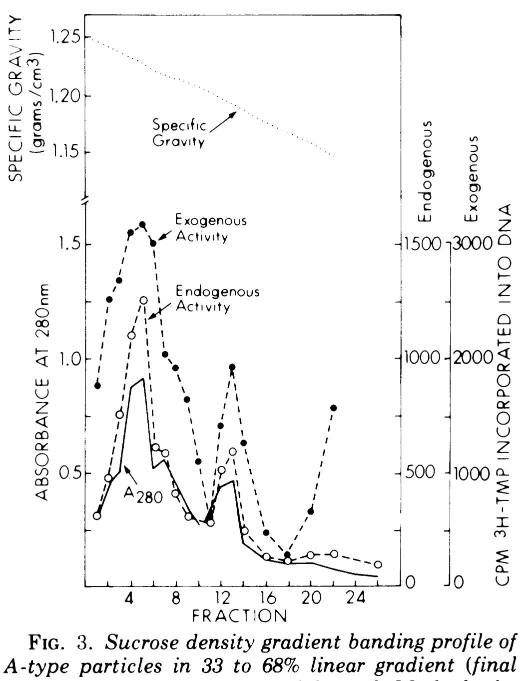

FIG. 3. *Sucrose density gradient banding profile of A-type particles in 33 to 68% linear gradient (final purification step; see Materials and Methods for details). The gradient was fractionated and analyzed as in Fig. 1, except that cold dNTPs were used at normal concentrations.*

is variable, and it has been absent in our more recent preparations.) The purity of the A particles prepared in this manner can be seen in Fig. 2b.

The coincident banding of both exogenous and endogenous polymerase activities with the bulk of the A particles suggests that the polymerase is contained within the particles. Further evidence for this was obtained by performing a sedimentation rate analysis of the particles and the two polymerase activities. The purified A particle preparation was sheared through a 27-gauge needle to break up aggregates and layered on a 15 to 30% sucrose gradient on a 68% sucrose cushion. The gradient was centrifuged for 15 min at 15,000 rpm, and analyzed as before. The results of this experiment are seen in Fig. 4, which shows A_{280}, endogenous and exogenous polymerase activities, and presence of A particles. The A particles were found in two peaks. The first peak contained those particles which sedimented through the gradient and banded on the 68% cushion; these were observed in the electron microscope to be highly aggregated. The second peak contains those particles which were still sedimenting through the lighter region of the gradient when centrifugation was stopped; these were observed to be either single or in small clusters. The fact that particles and the polymerase activities sediment at the same rate implies that both of these enzymatic activities are physically associated with A particles, and renders unlikely the possibility that they might simply have been unrelated contaminants (such as membrane-bound polymerases) which happened to band in sucrose at the same bouyant density as A particles.

Some of the requirements for the A particle-

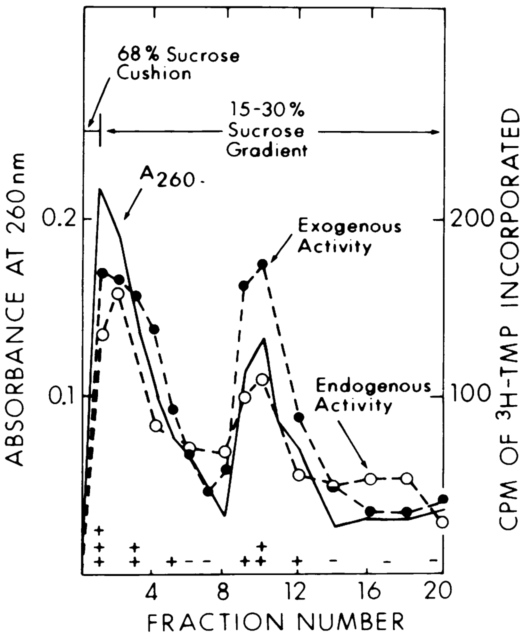

FIG. 4. *Sedimentation rate profile of A-type particles in a sucrose density gradient. Particles from the peak fraction of Fig. 3 were pelleted and resuspended in TNE buffer sheared, and centrifuged through a 15 to 30% linear sucrose density gradient containing TNE buffer (on a 68% sucrose cushion) in a Spinco SW-56 rotor. Centrifugation was for 15 min at 15,000 rpm. The gradient was analyzed as in Fig. 1.*

associated endogenous polymerase reaction are indicated in Table 1. This enzymatic reaction shares many of the general characteristics common to the oncornavirus reverse transcriptases (11). There is a pronounced requirement for dGTP, dATP, and dCTP for the incorporation of [^{3}H]dTTP. The optimum dNTP concentrations are all rather high, with maximum activity not attained at the highest concentration tested, 0.4 mM (data not shown). This result might explain the difficulty of detecting the endogenous reaction reported by some workers (15).

A non-ionic detergent such as Triton X-100 is required for optimal DNA synthesis in the endogenous reaction, as are both mono- and divalent cations (Table 1). The divalent cation requirement can be met either by Mg^{2+} at 12 mM or Mn^{2+} at 2 mM. The latter conditions give about 1.5-fold greater incorporation than the former (data not shown). The endogenous reaction is stimulated by the addition of oligo(dT), which may act by complexing with poly(A) sequences in the endogenous template and serving as a primer. The reaction is partially inhibited by actinomycin D and RNase, consistent with results reported for the RNA tumor virus enzymes (11).

The partial inhibition of the reaction by actinomycin D was examined further. Time courses for reactions with or without the drug are shown in Fig. 5. During the first 15 min the amount of DNA synthesis in the two reactions is about the same. However, at later times, there is a total cessation of synthesis in the presence of actinomycin D, whereas in its absence synthesis continues at a near linear rate up to 2 h. This effect is similar to that observed with oncornavirus polymerases and suggests the presence of a DNA:RNA hybrid as a reaction intermediate.

To seek further evidence for such an intermediate the [^{3}H]DNA product from a 15-min reaction containing actinomycin D was prepared as in Materials and Methods and then banded in a Cs_2SO_4 equilibrium density gradient. In Fig. 6 it can be seen that a significant amount of the newly synthesized DNA bands in the DNA:RNA hybrid region (ρ = 1.54 g/cm^3). That this hybrid is an intermediate in the reaction is suggested by the fact that it is only seen in the presence of actinomycin D. A small amount of the DNA product bands in the RNA region of the gradient (ρ = 1.66 g/cm^3). This material is eliminated by prior RNase treatment; it may represent short pieces of newly synthesized DNA bound to high-molecular-weight RNA template.

Since the DNA:RNA hybrids appear to be

TABLE 1. *Requirements for endogenous DNA polymerase activity*

Reaction mixture	Incorporation of [^{3}H]dTTP (Percent of complete reaction)
Complete[a]	100
− β-mercaptoethanol	17
− KCl	62
− $Mg(OAc)_2$	14
− dGTP, dCTP, dATP	14
+ Oligo$(dT)_{9,10}$	173
− Triton X-100	21
+ Actinomycin D	29
+ RNase	48
− A particles	5
+ Boiled A particles	11

[a] Complete endogenous assay was as described in Materials and Methods. RNase treatment (10 μg/ml) was for 30 min at 37 C before initiation of polymerase reaction by addition of triphosphates; remaining RNase-resistant activity is expressed relative to a similarly incubated minus RNase control. All incubation times were for 90 min at 37 C.

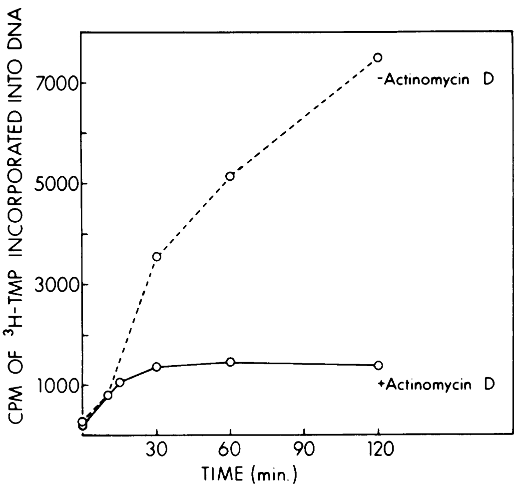

FIG. 5. *Time course of DNA synthesis in presence or absence of 50 µg/ml actinomycin D. Reaction conditions and assay were as described in Methods.*

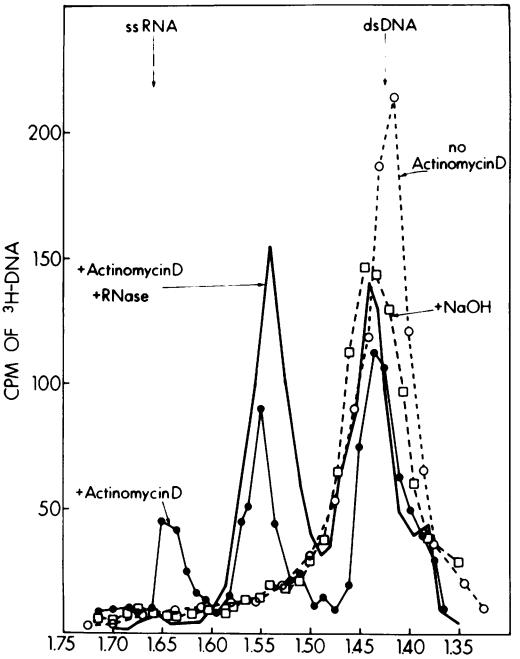

FIG. 6. *Cs_2SO_4 density gradient banding profile for [³H]DNA reaction products. DNA samples were prepared as in Materials and Methods and consisted of the following: total nucleic acid prepared from a 15-min reaction containing actinomycin D (●); the same, but RNase treated (10 µg/ml for 30 min at 37 C in 0.1 M NaCl, pH 7.2) (——); total nucleic acid prepared from a reaction lacking actinomycin D (30-min reaction) (O); the same, but treated with NaOH to remove RNA (□). Fractions were collected and analyzed as in Materials and Methods.*

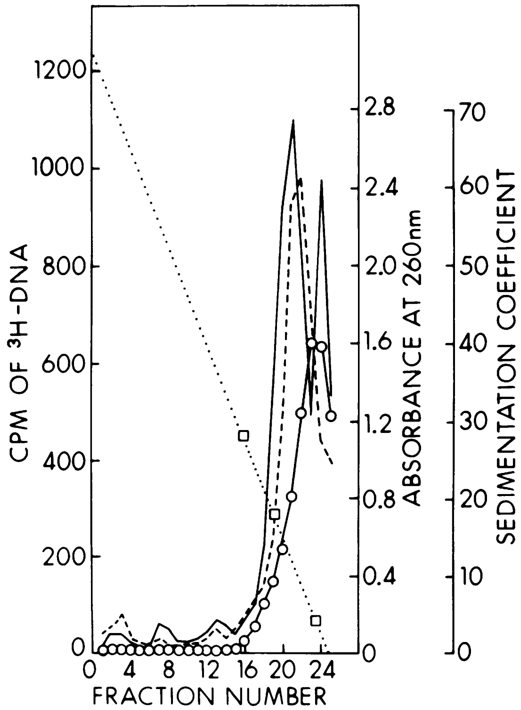

FIG. 7. *Sedimentation rate profile of A particle RNA and [³H]DNA:RNA complexes. RNA was extracted from purified A particles and analyzed by sedimentation as described in Methods (- - -). [³H]DNA:RNA was extracted from a 15-min actinomycin D-containing reaction, purified, and run on a similar gradient (——); a portion of this complex was treated with alkali before sedimentation (O). Ribosomal RNA markers (□) were used to calculate sedimentation coefficient.*

stable and to accumulate in reactions containing actinomycin D, it should be possible to determine the size of the template-product complex by sedimentation analysis. The total nucleic acid prepared from a 15-min actinomycin D-containing reaction was layered on a 5 to 30% sucrose gradient containing SDS, centrifuged, and analyzed as described in Materials and Methods. The sedimentation profile is shown in Fig. 7. It is interesting to note that a small amount of [³H]DNA sediments in the 70*S* region of the gradient (about 2% of the total radioactivity). There are also two other high-molecular-weight peaks at approximately 55*S* and 35*S* (each peak represents about 3 to 4% of the total radioactivity applied to the gradient). However, the majority of the [³H]DNA sediments in the 10*S* to 20*S* region, whereas there is a small peak at 4*S*. The [³H]DNA which sediments at 70*S* probably does so because it is bound to 70*S* RNA present in the A particles, since the DNA itself is quite small. This is shown by sedimentation analysis of the DNA after removal of RNA by boiling in alkali (Fig. 7).

RNA associated with A-type particles. The above experiments indicate that the A particle-associated DNA polymerase is similar to the reverse transcriptase found in true oncornaviruses. To provide further evidence for this we wanted to know whether the DNA product of the reaction is complementary to an endogenous RNA template. For this purpose the RNA from A particles was prepared by phenol-SDS extraction and analyzed. A sucrose gradient sedimentation profile is shown in Fig. 7. The bulk of the RNA sediments in a broad peak from 5*S* to 15*S*, with minor peaks at approximately 35*S*, 55*S*, and 70*S*. The significance of the latter three peaks is uncertain at present. It is possible that they represent vestiges of the original, undegraded A particle RNA, whereas the 5*S* to 15*S* peak consists of degraded fragments of these forms. This view is supported by the work of Yang and Wivel (18). However, the small quantities of these high-molecular-weight components raises the suspicion that they might be contaminants which are unrelated to A particles. Further work is necessary to clarify this question.

The RNA extracted from A particles hybridizes to the DNA reaction product (Fig. 8). As much as 94% of the DNA hybridizes to A particle RNA, suggesting that this DNA was synthesized using A particle RNA as template. A $C_rt_{1/2}$ value of approximately 3×10^{-2} mol-s/liter for this hybridization reaction was estimated from the data shown in Fig. 8 and 9 and other similar experiments. This number is similar to those obtained with RNA and DNA from other oncornaviruses (1, 10). This suggests that the sequence complexity of A particle RNA is comparable to that of the RNA in C-type particles, even though the average sizes of the two RNAs are very different. This in turn is consistent with the view expressed above that A particle RNA as prepared might be highly degraded, and may have originally consisted of much larger molecules.

Most of our preparations of A particle RNA contain traces of discrete components sedimenting at 18*S* and 27*S*. These are not resolved in Fig. 7 because of the short sedimentation time that was used in order to reveal the 55*S* and 70*S* components; however, in longer sedimentation runs they show up clearly (12). The presence of 18*S* and 27*S* species in the A particle RNA suggests the possibility of contamination by rRNA. To determine whether this material is ribosomal or viral, and whether it is transcribed into DNA by the polymerase, the 27*S* A particle RNA was purified and compared to 28*S* rRNA with regard to its ability to hybridize to the labeled DNA reaction product. A small amount

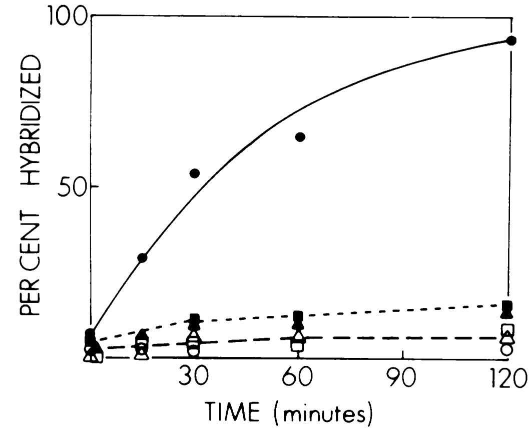

FIG. 8. *Time course of the hybridization of [³H]DNA reaction product to A particle RNA. Alkali-treated DNA from actinomycin D-containing reactions was used in all hybridizations. DNA (5,000 counts/min per ml) was hybridized as described in Materials and Methods for the indicated times. RNAs (10 µg/ml) used were: A particle RNA (●); 27S A particle RNA fraction (▲); 28S rRNA (■); 18S rRNA (□); cell mRNA (△); no RNA (○). See Materials and Methods for preparation of 28S and 18S rRNA and cell mRNA.*

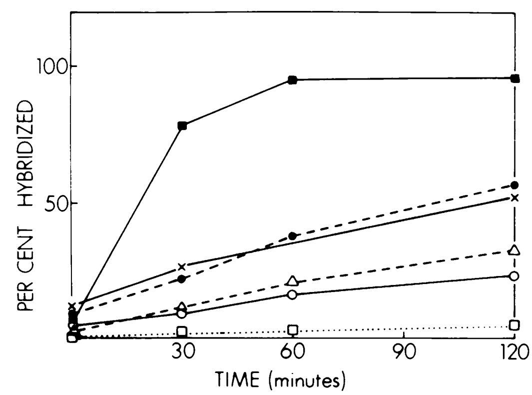

FIG. 9. *Time course for hybridization of [³H]DNA to A particle RNA or to murine C-type particle 70S RNA. RNAs used were as follows: A particle RNA (■); Moloney Murine Sarcoma Virus-Murine Leukemia Virus (MuSV-MuLV) 70S RNA (●); Harvey MuSV-MuLV 70S RNA (×); Rauscher MuLV 70S RNA (○); AKR MuLV 70S RNA (△); no RNA (□). Hybridization details were as in Materials and Methods.*

of the DNA does hybridize to both RNA species (Fig. 8). This observation has been repeated with a number of different A particle preparations, although the amount of the DNA that hybridized to the two RNA species has been variable. Although these results suggest that the small amount of 27*S* RNA present in the A particle preparation is ribosomal, and can be at least partially transcribed, the significance of these findings is unclear. In any event, this situation is not unprecedented since rRNA is frequently found in varying amounts in C-type particle preparations (2, 6, 14).

It is worth noting that bulk Krebs cell mRNA does not hybridize at all with the DNA probe (Fig. 8). This result, plus the low $C_rt_{1/2}$ value noted above, plus the hybridization studies with rRNA indicate that the A particle RNA is not a random collection of degraded cellular RNA.

To determine whether A particles are genetically related to known murine oncornaviruses, we performed hybridization experiments with RNA from several C-type viruses. These results are shown in Fig. 9. Of the RNAs used, those from the sarcoma-leukemia virus complexes share more homology with A particle RNA than do those from the leukemia viruses. Although these results are only preliminary, and can be interpreted in several different ways, they suggest that A particles are indeed genetically related to certain sequence components within C type RNA viruses.

DISCUSSION

Our results demonstrate the presence of an RNA-dependent DNA polymerase associated with the intracisternal A-type particles from mouse plasma cytoma cells, in agreement with the recent report of Yang and Wivel (19). Whether or not this enzyme is actually present inside of the particles (as opposed to being present in the form of a tightly bound external contaminant) is a moot question, as it is also for the oncornaviruses. Experiments designed to test the external contaminant interpretation failed to detect polymerase activity in the 1.20 to 1.23 g/cm^3 density fraction of membranes from Krebs II cells, thus implying that reverse transcriptase activity is not generally associated with mouse tumor cell membranes (data not shown).

The possibility that our results might be artifacts arising from contamination of A particles by C-type virus cores must be considered. Myeloma tumor cells have occasionally been observed to produce C- as well as A-type particles, especially when grown in tissue culture (7). If some of these C particles had been present as contaminants in our original microsomes plus mitochondria fraction, their cores could possibly have copurified with the A particles throughout subsequent steps, and therefore they could conceivably be the source of our DNA polymerase activities. However, other workers have investigated this possibility using a variety of methods and have failed to detect such contamination (4, 15, 18). In addition, several observations of our own indicate that if contamination of this kind occurs at all in our preparations it is not a significant source of polymerase activity in A particles. First, as noted previously, no evidence for contaminating C particles can be seen in Fig. 1. Had they been present in significant amounts, a peak of activity should have appeared at approximately 1.16 g/cm^3, and they would also have been detected by electron microscopy. Second, studies on the C-type particles produced by a MOPC 460 tissue culture line recently characterized in our laboratory have shown that the specific activities (both exogenous and endogenous) of the C particle polymerase (assayed in disrupted virions) are no higher than those of the A particle enzyme. This result indicates that the activity associated with the latter is unlikely to be due simply to contamination by the former. It should be noted that none of our results rule out the possibility that A particles may be identical or very closely related to the C particle cores. This possibility is currently being investigated.

The RNA isolated from A particles is generally small (5*S* to 15*S*) but minor components can be detected which sediment at 70*S*, 55*S*, and 35*S* in sucrose gradients. The reason for such small quantities of high-molecular-weight RNA is currently under investigation. In any event, the presence of the reverse transcriptase and the possible existence of 70*S* and 35*S* RNA suggest a relationship between A-type particles and true RNA tumor viruses. The extent of hybridization of A particle complementary DNA with sarcoma and leukemia virus RNAs (Fig. 9) provides additional evidence that these particles may be related to the other oncornaviruses. These results are consistent with the view that A particles are simply defective sarcoma-like viruses which mistakenly bud into the cisternae of the rough endoplasmic reticulum, instead of into the extracellular medium. Whether the A-type particles can under certain circumstances bud through the plasma membrane remains an open question.

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