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RNA Associated with Murine Intracisternal Type A Particles Codes for the Main Particle Protein

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Intracisternal type A particles were isolated from MOPC-104E myeloma grown subcutaneously and from N4 neuroblastoma cells in culture. Polyadenylated RNA was prepared from the particles and tested in a cell-free translation system derived from rabbit reticulocytes. RNA from the two sources directed the synthesis of multiple polypeptides with similar distributions of electrophoretic mobilities in sodium dodecyl sulfate-containing polyacrylamide gels, including one component of the same size as the major A-particle structural protein (73,000 daltons). Analysis of the RNAs by electrophoresis in methyl mercury-containing agarose gels revealed a 35S component common to A-particles from both cell types. This was a major component of the N4 preparations, whereas a 28S species predominated in the case of MOPC-104E. These two RNAs (35S from N4 cells and 28S from MOPC-104E), when isolated on isokinetic sucrose gradients, each directed the synthesis of a 73,000-molecular-weight polypeptide that comigrated on gels with authentic A-particle structural protein. Identity of the cell-free product was confirmed by two-dimensional analysis of the [^{35}S]methionine-labeled tryptic peptides. The N4 RNA preparations also contained a major 32S component which did not code effectively for the A-particle structural protein.

Intracisternal type A particles appear regularly in the early stages of mouse embryogenesis (2-4) and often in mouse tumor cells (for references, see 14). Their expression is not correlated with the formation of type B and C RNA tumor viruses (10, 13), from which they differ in morphology, intracellular localization, and the properties of their component proteins (5, 14, 35, 36). The particles may be isolated from various tumor cells (14, 15, 17); however, infectivity studies with the isolated preparations have not yet succeeded, and therefore it has not been possible to identify an A-particle genome on a functional basis.

High-molecular-weight polyadenylated RNA has been found in intracisternal A-particles isolated from several types of tumor cells (12, 19, 29, 37, 38). Conflicting results have been obtained in studies of possible homologies between A-particle-associated RNA and the RNAs of type C tumor viruses; some investigators have suggested extensive homologies (12, 30), whereas others have found little or none (19, 37, 39). We recently identified a set of RNA sequences which were specifically concentrated in A-particles prepared from three different types of mouse tumor cells (19). These sequences are multiply represented in the DNA of both normal and tumor cells from several mouse strains (18) are not related to the set of RNA sequences which is common to a variety of murine type C viruses (19). The findings support the concept that intracisternal A-particles contain a distinctive type of RNA, without, however, defining its specific informational content.

In the present study, A-particle RNA prepared from two types of transformed mouse cells was found to direct the cell-free synthesis of the major A-particle structural protein. The results indicate that the high-molecular-weight polyadenylated RNA associated with A-particles is indeed the genomic RNA for an intracellular virus-like entity.

MATERIALS AND METHODS

Sources and isolation of intracisternal A-particles. Particles were prepared from MOPC-104E plasma cell tumors maintained by subcutaneous or intraperitoneal transplantation in BALB/c mice and from the N4 tissue culture line derived from a neuroblastoma of A/Jax origin as previously described (19). Gradient-purified particles were isolated by a slight modification (19) of a previously published procedure (15).

Preparation of polyadenylated RNA. RNA was extracted from the A-particles with phenol-chloroform-isoamyl alcohol after digestion of the particle protein with Pronase in the presence of sodium dodecyl sulfate (SDS). The polyadenylated fraction was selected by binding to oligodeoxythymidylic acid [oligo(dT)]-cellulose. Details of the procedures have been described previously (19). Polyadenylated RNAs

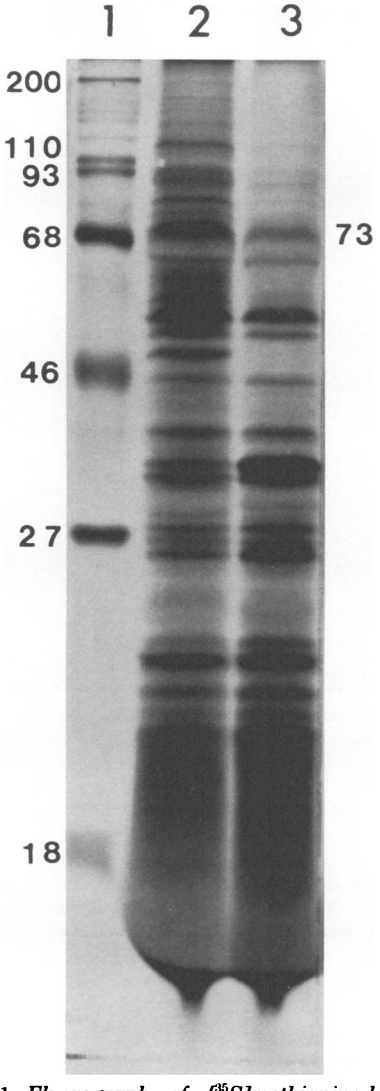


FIG. 1. *Fluorograph of [^{35}S]methionine-labeled polypeptides synthesized in an mRNA-dependent rabbit reticulocyte lysate cell-free system in response to 0.5 to 2.0 μg of oligo(dT)-cellulose-purified A-particle-associated RNA. Polypeptides were fractionated on an SDS-12% polyacrylamide gel. The dried gel was fluorographed at −90°C for 15 h on Kodak XR-5 film. Approximately 60,000 cpm were loaded in each slot: (slot 1) [^{14}C]formaldehyde-labeled marker proteins—myosin (200,000 molecular weight [200K]), beta-galactosidase (110K), phosphorylase a (93K), bovine serum albumin (68K), ovalbumin (46K), chymotrypsin (27K), and lysozyme (18K); (slot 2) polypeptides synthesized in response to RNA prepared from A-particles isolated from N4 neuroblastoma cells; (slot 3) polypeptides synthesized in response to RNA prepared from A-particles isolated from MOPC-104E tumors. Under the conditions of electrophoresis, the 73K polypeptide migrated just behind an endogenous unlabeled protein present in the lysate in large amounts, resulting in a slight displacement of the 73K labeled polypeptide. Endogenous synthesis is shown in Fig. 3, slot 1.*

were further purified by recycling on oligo(dT)-cellulose columns after being heated in low-ionic-strength buffer (10 mM HEPES [*N*-2-hydroxyethyl piperazine-*N'*-2-ethanesulfonic acid]–1 mM EDTA [pH 7.4], 10 min at 65°C). The heated RNAs were rebound after addition of sodium chloride to 0.5 M, and the polyadenylated fraction was eluted in low-salt buffer as in the first cycle (19). About 60% of the original polyadenylated RNA was recovered in the bound fraction after this purification step.

Fractionation of RNA on sucrose gradients. Polyadenylated A-particle RNA selected by two cycles of binding to oligo(dT)-cellulose was further fractionated by centrifugation on 12-ml isokinetic sucrose density gradients (25) containing 10 mM Tris-hydrochloride–1 mM EDTA–0.2% SDS, pH 7.4. The parameters of the gradients (25) were: C_t, 15% sucrose (wt/vol); C_r, 33.2% sucrose (wt/vol); V_m, 9.0 ml. The RNA samples were heated for 5 min at 65°C in 10 mM HEPES–1 mM EDTA (pH 7.4), cooled, applied to the gradients, and centrifuged for 14 h at 205,000 × *g* (average) in a Beckman SW41 rotor operated at 20°C. rRNA was centrifuged simultaneously on separate gradients to provide standards of sedimentation rate. The gradients were scanned for optical density at 260 nm, and RNA was recovered from the peak fractions by precipitation with ethanol in the presence of carrier *Escherichia coli* tRNA.

Agarose gel electrophoresis. RNA samples (0.5 to 2 μg) were analyzed by electrophoresis in 1.5% agarose gels (Sigma type II agarose) containing 6 mM methylmercury hydroxide as described by Bailey and Davidson (1). RNA was visualized by staining with ethidium bromide (1 μg/ml in 0.5 M ammonium acetate). Size markers consisted of 28S and 18S mouse rRNA's (5.2 and 2.1 kilobases, respectively) prepared from neuroblastoma cells and the 35S avian myeloblastosis virus RNA extracted from viral pellets as previously described (31).

Cell-free translation of A-particle RNA and identification products. A-particle polyadenylated RNAs were translated in the mRNA-dependent reticulocyte lysate (28) as described previously (26). Generally, 0.5 to 2.0 μg of RNA was translated in a final reaction volume of 25 μl. [^{35}S]methionine-labeled cell-free products were analyzed on SDS-polyacrylamide slab gels (16), together with authentic A-particle proteins prepared from N4 or MOPC-104E ascites tumor cells that had been incubated for 4 h in methionine-deficient medium supplemented with [^{35}S]methionine (Amersham/Searle; 335 Ci/mmol) at 45 μCi/ml. Labeled polypeptides were extracted for tryptic peptide analysis as described previously (26). Small portions of the digested peptides were applied to precoated thin-layer silica gel plates (Merck) in water or 10% isopropanol and subjected to electrophoresis at pH 3.5. Electrophoresis was for 4 h at 200 V, using a water-cooled (5°C), Desaga flat-plate electrophoresis unit. Ascending chromatography in the second dimension, using *n*-butanol–acetic acid–water–pyridine (30:6:24:20 or 30:6:24:24), was continued until the sol-

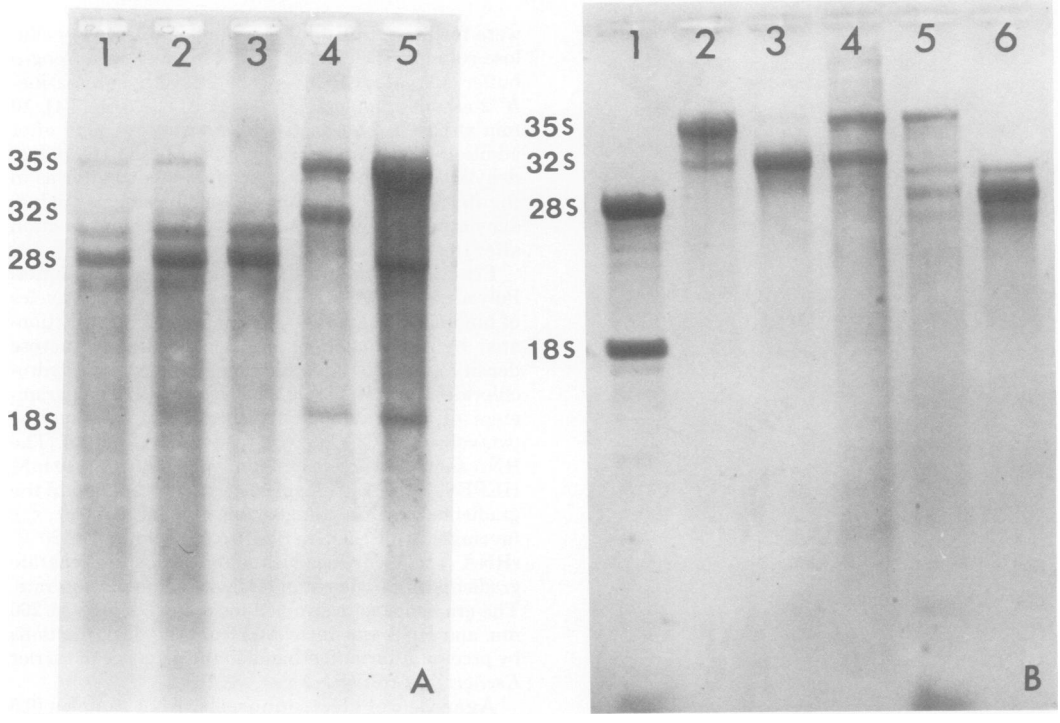


FIG. 2. *Analysis of oligo(dT)-cellulose-purified A-particle-associated RNAs on 1.5% agarose gels containing 6 mM methylmercury hydroxide. (A) (Slot 1) total MOPC-104E A-particle RNA, (slot 2) same as (slot 1) except that deoxycholate-extracted A-particle cores (36) were used as the starting material for the RNA preparations, (slot 3) MOPC-104E A-particle 28S RNA prepared by isokinetic sucrose gradient fractionation, (slot 4) total N4 A-particle RNA, (slot 5) total avian myeloblastosis viral RNA [not oligo(dT)-cellulose purified]; (B) (slot 1) rRNA markers from N4 cells, (slot 2) N4 A-particle 35S RNA prepared by isokinetic sucrose gradient fractionation, (slot 3) N4 A-particle 32S RNA prepared by isokinetic sucrose gradient fractionation, (slot 4) total N4 A-particle RNA, (slot 5) MOPC-104E A-particle 35S RNA prepared by isokinetic sucrose gradient fractionation, (slot 6) MOPC-104E A-particle 28S RNA prepared by isokinetic sucrose gradient fractionation. The RNAs in (B), slots 2 to 6, were heated to 65°C before oligo(dT)-cellulose fractionation.*

vent front reached within 1 cm of the top of the plate (4 to 6 h). The plate was fluorographed at −90°C by the method of Bonner and Stedman (Anal. Biochem., in press).

RESULTS

Cell-free translation of total polyadenylated A-particle RNA. Polyadenylated RNA was prepared from A-particles derived from two different tumor cell types (neuroblastoma and myeloma) and translated in an mRNA-dependent reticulocyte cell-free system. Both RNA preparations directed the synthesis of a similar spectrum of polypeptides, ranging in molecular weight from about 20,000 to 100,000. The major structural protein of the A-particle has a molecular weight of 73,000 (23), and as shown in Fig. 1, a polypeptide of about this size was among the labeled products in each case. This polypeptide was not detected in the translation products of the total cellular poly(A) RNA from the same A-particle-containing cells (data not shown), suggesting that preparations of A-particles were enriched for the mRNA species that codes for the 73,000-dalton species.

Size distribution of A-particle-associated polyadenylated RNAs. The polyadenylated RNA preparations were examined by electrophoresis in 1.5% agarose gels containing methyl mercury hydroxide (1) to resolve the components under fully denaturing conditions. As shown in Fig. 2A, both preparations contained an RNA species which migrated slightly more slowly than the 35S avian myeloblastosis viral RNA marker, which has a size of 8.1 kilobases (24). The 35S A-particle RNA component was greatly enriched in preparations from neuroblastoma cells as compared with those obtained from the myeloma solid tumors. A 32S species was also prominent in the neuroblastoma prepara-

tion but barely detectable in the myeloma material. The predominant component of the myeloma RNA was a 28S species that did not appear in the neuroblastoma preparation. Both preparations contained other discrete components as well as considerable amounts of heterogeneous unresolved RNA. The results are consistent with previous studies showing the presence of high-molecular-weight RNAs associated with isolated intracisternal A-particles (12, 19, 29, 37, 38), but provide a more precise comparison of the various RNA species.

Cell-free translation of various size classes of A-particle RNA. The polyadenylated RNA fractions described above were denatured by heating in low-ionic-strength buffer and passed again over oligo(dT)-cellulose as described in Materials and Methods. In this way, much of the heterogeneous material was removed, as well as some rRNA that had persisted through the first selection procedure (compare the unfractionated N4 RNAs in Fig. 2A and B). The samples were then fractionated by centrifugation on isokinetic sucrose gradients, and the RNAs corresponding to the major optical density peaks were recovered by alcohol precipitation. The gradient-purified fractions were examined by electrophoresis in methyl mercury agarose gels (Fig. 2B). Although none of the preparations was homogeneous, a single electrophoretic component was strongly predominant in the N4 35S and 32S and the MOPC-104E 28S fractions. The MOPC 35S fraction showed considerable enrichment of this species with respect to the original polyadenylated RNA, but other components, including 28S RNA, still composed the bulk of the preparation. Attempts to further purify the individual components by recycling on sucrose gradients resulted in prohibitive losses without significantly greater enrichment.

When individual RNA fractions were tested in the cell-free translation system (Fig. 3), we observed a marked simplification in the electrophoretic patterns of the product polypeptides compared with those given by the unfractionated RNAs. Both the 35S RNA fraction from neuroblastoma and the 28S fraction from myeloma directed the synthesis of a 73,000-dalton polypeptide that comigrated with authentic [^{35}S]methionine-labeled A-particle structural protein prepared from N4 cells. The 32S species from neuroblastoma A-particles consistently yielded smaller amounts of product in this size range and a higher proportion of material larger than 90,000 daltons. An increased proportion of higher-molecular-weight products compared with the 73,000-dalton polypeptide was seen with one preparation of neuroblastoma 35S RNA. The 35S RNA fraction from myeloma was also found to code for synthesis of a 73,000-dalton product (data not shown); however, because the fraction was contaminated with 28S RNA (Fig. 2B), it was not possible to ascribe this activity to the 35S molecules.

The broad band formed by the cell-free product of the myeloma 28S RNA indicates the presence of more than a single electrophoretic

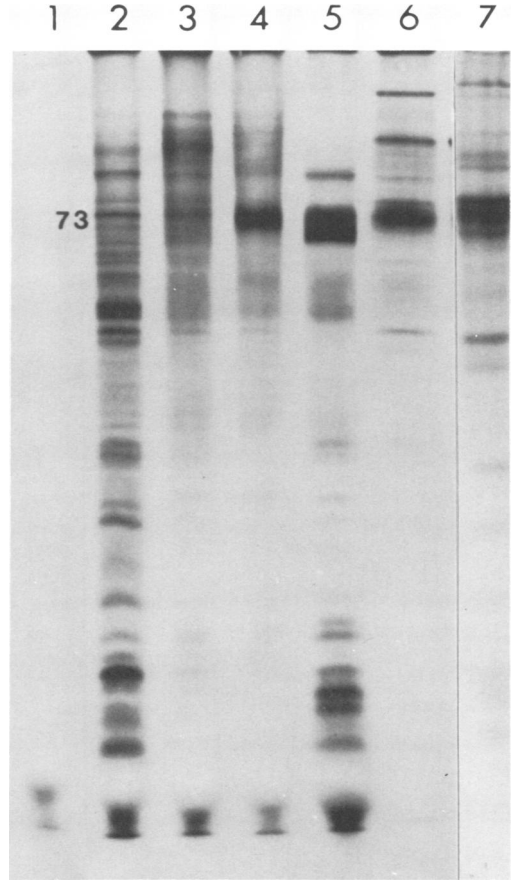


FIG. 3. *Fluorograph of [^{35}S]methionine-labeled polypeptides synthesized in an mRNA-dependent rabbit reticulocyte lysate. RNA samples were heated to 90°C for 30 s before translation, and 0.5 μg of the different size classes of A-particle-associated polyadenylated RNA prepared by isokinetic sucrose gradient fractionation were translated in a reaction volume of 25 μl. Polypeptides (5 μl of the reaction) were fractionated on an SDS–11% polyacrylamide gel. (Slot 1) No added RNA; (slot 2) total polyadenylated RNA from N4 A-particles; (slot 3) 32S RNA from N4 A-particles; (slot 4) 35S RNA from N4 A-particles; (slot 5) 28S RNA from MOPC-104E A-particles; (slot 6) authentic [^{35}S]methionine-labeled, 73,000-dalton, A-particle structural protein prepared from N4 cells; (slot 7) authentic [^{35}S]methionine-labeled, 73,000-dalton protein from MOPC-104E ascites tumor cells.*

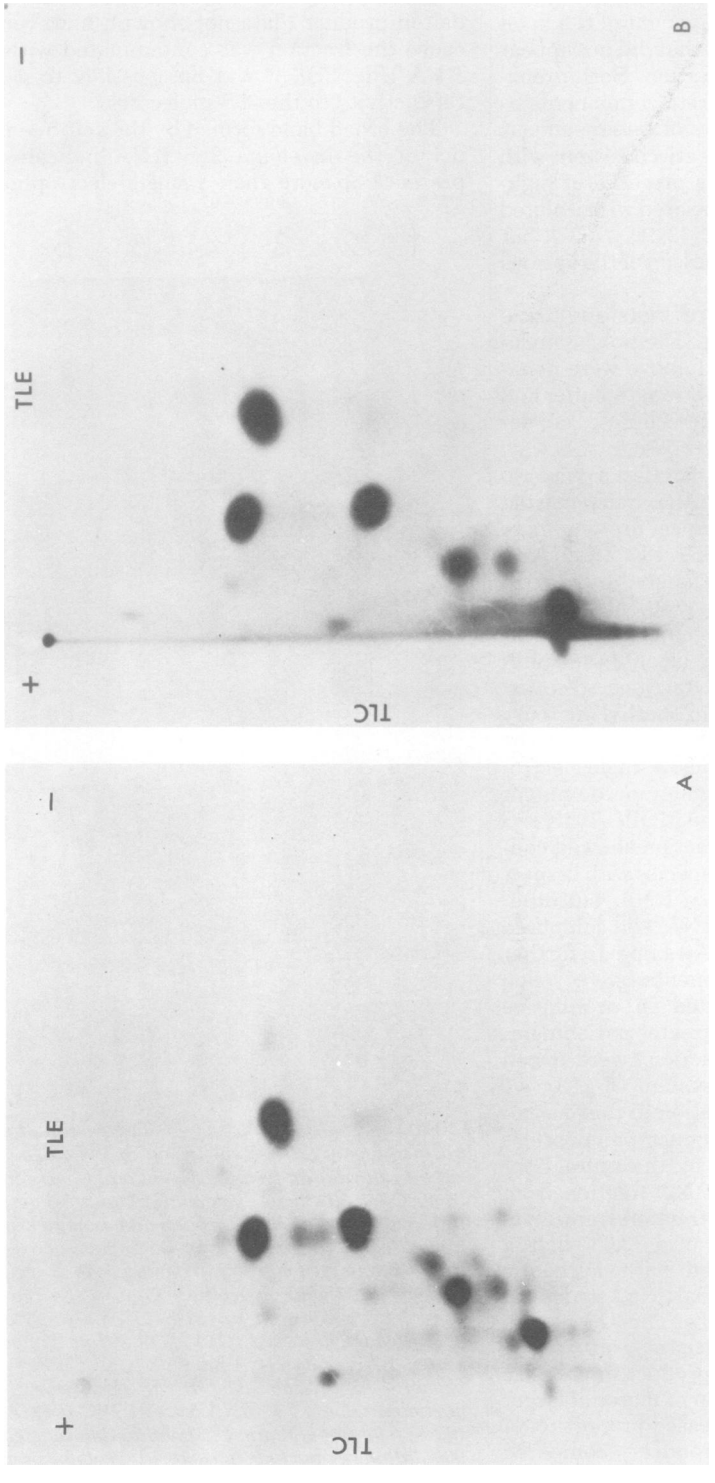
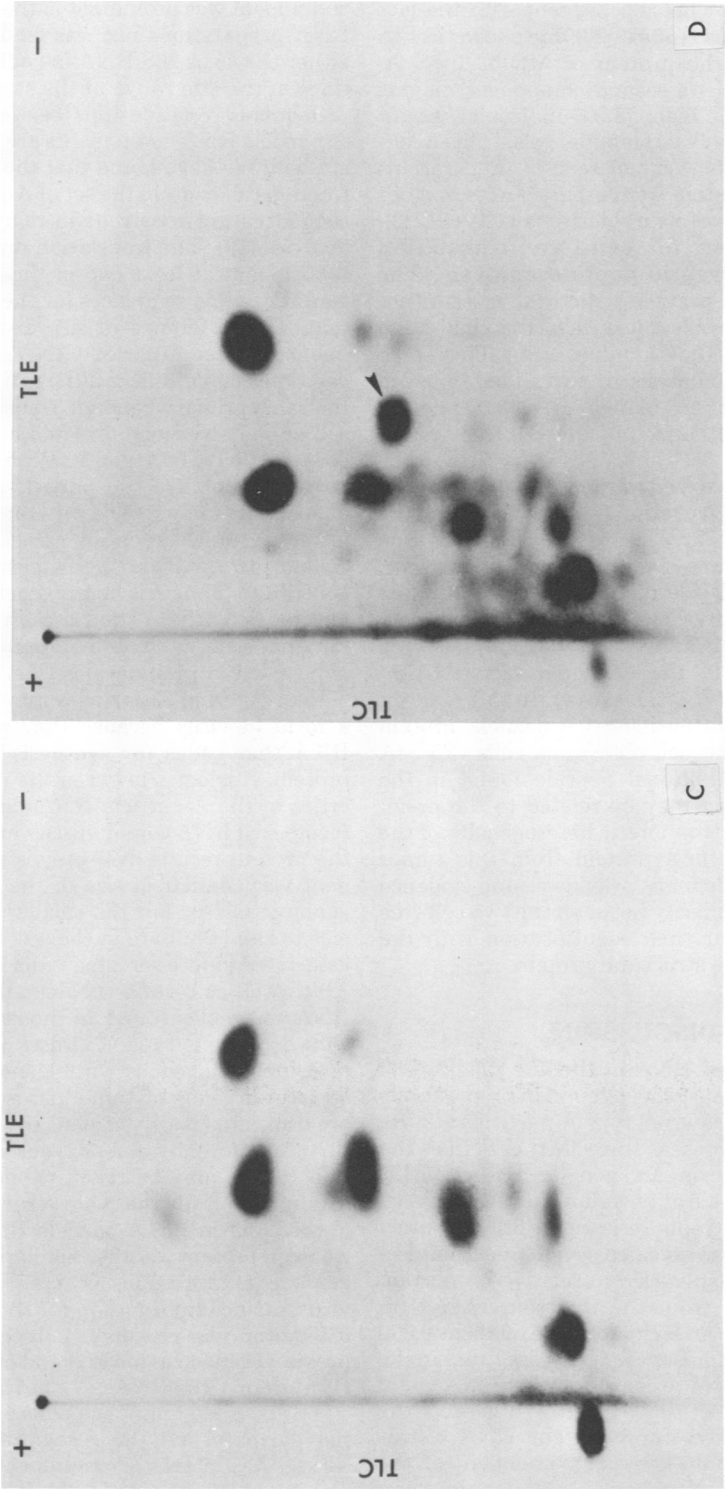


FIG. 4. *Fluorograph of the [^{35}S]methionine-containing tryptic peptides of the 73,000-dalton polypeptides synthesized in the mRNA-dependent rabbit reticulocyte lysate in response to the various size classes of A-particle-associated RNA and of authentic 73,000-dalton structural protein of A-particles prepared from N4 cells. (A and C) Peptides of authentic 73,000-dalton protein prepared from A-particles isolated from N4 cells. The samples appeared as shown in Fig. 3, slot 6. (B) Peptides of 73,000-dalton polypeptide synthesized in the cell-free system in response to 35S RNA prepared from N4 A-particles. Sample is shown in Fig. 3, slot 4. (D) Peptides of 73,000-dalton polypeptide synthesized in the cell-free system in response to 28S RNA associated with MOPC-104E A-particles. Sample is shown in Fig. 3, slot 5. Tryptic peptides were subjected to electrophoresis on silica gel plates in pH 3.5 buffer; ascending chromatography was carried out with n-butanol–acetic acid–water–pyridine mixtures (30:6:24:20) in (A) and (B) and 30:6:24:24 in (C) and (D). Plates were exposed for 48 h at −90°C.*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species (Fig. 3). This is consistent with the fact noted in previous studies (23) and shown in Fig. 3, slot 7, that the protein of MOPC-104E A-particles contains a second component moving just ahead of the main 73,000-molecular-weight band in SDS-polyacrylamide gels. This additional component was not seen in preparations of A-particle protein from other sources, including several lines of neuroblastoma cells (17, 23).

Identification of cell-free translation products by tryptic peptide analysis. The 73,000-dalton A-particle structural protein, isolated from neuroblastoma cells that had been labeled with [^{35}S]methionine, was subjected to tryptic peptide analysis to two dimensions on thin-layer silica gel plates. The 73,000-dalton polypeptide synthesized in the cell-free system in response to 35S and 28S A-particle RNA fractions was analyzed in the same fashion. The tryptic peptide patterns are shown in Fig. 4A through D. All three preparations shared five major methionine-containing peptides, with very similar relative mobilities in both dimensions. The cell-free product coded by the neuroblastoma 35S RNA was lacking a major tryptic peptide found in the myeloma 28S RNA-directed product (Fig. 4D, arrow). In this respect, it resembled the authentic A-particle protein isolated from the neuroblastoma cells (Fig. 4A and C). The additional peptide found in the myeloma product may be related to the previously noted electrophoretic heterogeneity of the A-particle structural protein from this tumor (23). The peptide maps provide strong evidence for the basic similarity between the two cell-free products and for their identification with the major A-particle structural protein.

DISCUSSION

Polyadenylated RNA in the size range of 30 to 35S has previously been found in preparations of murine intracisternal type A particles (12, 19, 29, 37, 38). The present study further defines the molecular properties and genetic content of this RNA in particles from myeloma and neuroblastoma cells. Electrophoresis under fully denaturing conditions has revealed several macromolecular species which varied in relative proportion in preparations from the two sources. A 35S component with an estimated size of about 9,000 nucleotides was present in both cases, and in the instance of the N4 preparation this RNA could be shown to direct the cell-free synthesis of the major particle structural protein. RNA of this size class was only a minor component of the myeloma preparation, however, and in this case, coding activity was associated with a predominant 28S RNA (about 5,200 nucleotides). A 32S component was prominent in the neuroblastoma RNA preparations but was far less active than either the 35 or 28S RNA in coding for polypeptides in the size range of the structural protein.

Sequence relationships between the various A-particle RNA components are currently being studied. We have found that the 35, 32, and 28S fractions all contain the set of A-particle-specific sequences previously defined by hybridization analysis (19). The translation results further establish that at least two of these RNA species contain coding sequences for the structural protein. The difference in size distribution of sequence-related A-particle RNA species from two cell types could reflect differential processing of the same primary genomic transcription in each cell or transcription of related but different sets of A-particle DNA genes. With respect to the latter possibility, A-particle-associated sequences are known to be reiterated in the mouse genome (18). Degradation associated with intracellular turnover of the particles (17) could also contribute to the size heterogeneity of the RNAs and particularly to the unresolved background material seen in electropherograms of the total poly(A) RNA preparations (Fig. 2).

Intracisternal A-particles may be regarded as a form of virus because they contain specific RNA that codes for synthesis of the particle protein. Analogies between the molecular properties of the A-particle RNAs and those of the recognized RNA tumor viruses are reinforced by the present results. Not only is the 35S component very similar in size to the leukemia virus genomic RNAs, but the smaller A-particle species (32 and 28S) are in the size range of virion-associated mouse sarcoma virus RNAs (6, 20–22, 32) as well as certain subgenomic virus-specific RNA molecules found in mouse cells infected with exogenous type C viruses (7, 9, 33) or expressing endogenous viral genes (8). Internal structural genes of tumor virus genomic RNA are translated poorly, if at all, in cell-free systems (27); therefore, by analogy, our present translation results may be taken to indicate that the coding region for the main structural protein is 5′ proximal in the A-particle RNAs. If so, this would represent another similarity in the basic genome organizations of type A and type C viruses, since information for the 75,000- to 80,000-dalton *gag* precursor polypeptide is the 5′-proximal gene in avian (27) and probably murine (11) tumor virus RNAs.

The possible relationship between intracisternal particles and the recognized RNA tumor viruses has interested a number of investigators (10, 12–14, 19, 30, 34, 37, 38). It should be noted that, in spite of the similarities mentioned above, there are clear-cut differences in sequence con-

tent (19, 37, 39) and protein structure (14, 23, 35, 36) which suggest that intracisternal type A particles are distinct as a class from the other viruses. It will be important to search for additional protein products of the A-particle genome and also to determine whether A-particle RNA can be encapsulated in type C virions or whether recombinational events can result in the transfer of A-particle-related information into RNA of infectious tumor viruses.

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ADDENDUM IN PROOF

Recently, we have found that A-particle 35S RNA has partial sequence homology with the RNA of a transmissible extracellular retrovirus derived from the Asian mouse *Mus cervicolor* and that A-particle-related sequences are represented in the genomic DNA of that species (E. L. Kuff, K. K. Lueders, and E. M. Scolnick, submitted for publication).

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