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Characterization and Translation of Transmissible Gastroenteritis Virus mRNAs

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Three protein species were identified in purified transmissible gastroenteritis virus particles (strain Purdue). They are thought to represent constituents of the peplomer (E2; molecular weights of 280,000 and 240,000), the envelope (E1; molecular weights of 28,000, 31,500, and 33,000), and the nucleocapsid (N; molecular weight of 48,000). In infected cells, proteins with molecular weights of 195,000 (E2), 48,000 (N), and 28,000 (E1) were detected. Tunicamycin, an inhibitor of N glycosylation, prevented the appearance of polypeptides with molecular weights of 195,000 and 28,000 in infected cells; instead, proteins with molecular weights of 160,000 and 25,000 were observed. One minor and five major mRNA species were detected in porcine cells after infection. Their size was determined to be 23.6 kilobases (kb) (RNA1), 8.4 kb (RNA3), 3.8 kb (RNA4), 3.0 kb (RNA5), 2.6 kb (RNA6), and 1.9 kb (RNA7). The RNAs were translated in vitro. RNA7 was shown to code for the N protein. Although complete separation of RNA6 could not be achieved, it was shown to encode an unglycosylated (molecular weight of 25,000) precursor of E1 (molecular weight of 28,000). RNA4 was translated into a nonstructural protein with a molecular weight of 24,000. Translation of RNA3 resulted in proteins with molecular weights of 250,000 and 130,000 and smaller molecules which could be precipitated with a monoclonal antibody directed against E2.

Transmissible gastroenteritis virus (TGEV) is a member of the family *Coronaviridae* as evidenced by virion morphology (34), demonstration of a positive-stranded RNA genome (3), and antigenic relatedness to other members of the group (18). The protein composition of the virion has been known since 1975. There are three main structural proteins: a glycosylated peplomer polypeptide, an envelope glycoprotein, and an unglycosylated nucleocapsid protein (9). Recently, coronaviruses have attracted much attention because of their unusual replication strategy, which involves the synthesis of a nested set of mRNAs with common 3′-terminal sequences. In each RNA only the 5′-terminal sequence is translated; this sequence is absent in the next smaller RNA (22, 32). The mouse hepatitis and infectious bronchitis virus subgenomic mRNAs contain leader and body sequences that are not contiguous on the genome but that are joined by discontinuous transcription (4, 13, 25, 26).

Transmissible gastroenteritis of swine causes high mortality in young piglets. In spite of its economic importance, TGEV is one of the least studied coronaviruses. Data on virion proteins (7, 9, 10, 12) and intracellular RNAs (6, 7, 9; summarized in Table 1) have been published; however, neither the virus specificity of the RNAs nor the proteins they encode are known. To complete the picture we present here a new analysis of the RNA, including the translation of individual TGEV mRNAs in a cell-free system to determine the proteins that they encode.

MATERIALS AND METHODS

Cells and virus. The PD5 pig kidney cell line (obtained from Duphar B. V., Weesp, The Netherlands) was grown in Dulbecco modified Eagle medium (DMEM) supplemented with 10% newborn calf serum and containing penicillin (100 U/ml) and streptomycin (100 μg/ml). The Purdue strain of TGEV was plaque cloned twice, and cultures were infected with a mutiplicity of infection of about 15. Virus adsorption was allowed to proceed for 1 h at 37°C, and virus was harvested 20 h postinfection (p.i.). Titers approaching 10^8 PFU/ml were routinely obtained. For plaque titration on PD5 cells, an overlay of 0.6% agarose in DMEM supplemented with 1% fetal calf serum was used. After about 40 h of incubation, the cells were fixed with 10% formaldehyde in phosphate-buffered saline, the overlay was removed, and the cells were stained with crystal violet.

Antisera. An ascites fluid sample from a field case of feline infectious peritonitis was used; it recognized the structural proteins of TGEV in a radioimmunoprecipitation assay (12). In addition, a monoclonal antibody (ascites fluid) directed against the peplomer protein of TGEV was used which neutralized 100 times the 50% tissue culture infective dose of TGEV at a dilution of 1:1,000.

Radioactive labeling of virion proteins. Virus was labeled with [^{35}S]methionine by incubating 4×10^6 cells in a 25-cm^2 tissue culture flask from 7 to 12 h p.i. in 2 ml of DMEM containing 2% fetal calf serum and 50 μCi of [^{35}S]methionine per ml. All isotopes were obtained from the Radiochemical Centre, Amersham, England. The virus was precipitated from the clarified culture medium by the addition of 100 g of polyethylene glycol 6000 (BDH, Poole, England) per liter and 23.3 g of NaCl per liter. After overnight incubation at 4°C the virus suspension was centrifuged at $10,000 \times g$ for 15 min at 4°C. The pellet was suspended in TES buffer (20 mM Tris hydrochloride, 1 mM EDTA, 100 mM NaCl [pH 7.4]). The preparation was layered on top of a 12-ml linear 20 to 50% (wt/wt) sucrose gradient and centrifuged for 8 h at 20,000 rpm in an SW 27.1 rotor. The sucrose gradient was fractionated, and the virus was located by measuring the radioactivity.

Radioactive labeling of intracellular virus-specific proteins. PD5 cells grown in 35-mm-diameter plastic dishes were infected with TGEV. Labeling was performed with [^{35}S]methionine by incubating the cells from 9 to 9.5 h p.i. in 1 ml of DMEM without methionine, supplemented with 2% dialyzed calf serum and 100 μCi of [^{35}S]methionine per ml.

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After incubation the medium was removed, and the cells were washed three times with phosphate-buffered saline and lysed with 0.4 ml of lysis buffer (2% Triton X-100, 0.5% 1,5-naphthalene disulfonate sodium in TES buffer). The cell lysate was clarified by centrifugation (2 min at 10,000 × *g*), and the supernatant was stored at −20°C.

Immunoprecipitation and electrophoresis of proteins. A portion (5 μl) of the translation mixture or 50 μl of lysate of infected cells was mixed with 5 μl of anti-feline infectious peritonitis virus (FIPV) ascites fluid or 1 μl of monoclonal antibody. After overnight incubation at 4°C, a one-sixth volume of 3 M KCl was added, followed by an equal volume of a 10% suspension of Formalin-treated *Staphylococcus aureus* cells in TES buffer containing 0.1% Triton X-100. Incubation was continued for 30 min before the immune complexes were collected by centrifugation for 2 min at 10,000 × *g*, washed three times with 0.2 ml of TES buffer containing 0.1% Triton X-100, and dissolved in electrophoresis sample buffer (10 mM Tris hydrochloride [pH 8.0], 1 mM EDTA, 10% glycerol, 2% sodium dodecyl sulfate [SDS], 5% 2-mercaptoethanol, 0.001% bromophenol blue). SDS-polyacrylamide gel electrophoresis was performed on unheated samples in slab gels containing 15% acrylamide, 0.1% bisacrylamide, 375 mM Tris hydrochloride (pH 8.8), and 0.1% SDS. A stacking gel (1 cm deep) containing 4% acrylamide, 0.1% bisacrylamide, 125 mM Tris hydrochloride (pH 6.8), and 0.1% SDS was used. The reservoir buffer contained 50 mM Tris base, 384 mM glycine, and 0.1% SDS. Electrophoresis was carried out at 100 V for about 5 h. The translation products of RNA3 (8.4 kilobases [kb]) were analyzed in 12.5% polyacrylamide gels.

^{32}P labeling and isolation of virus-specific intracellular RNAs and genome RNA. Monolayer cultures of PD5 cells in 75-cm^2 tissue culture flasks were infected with TGEV. Incubation was in 6 ml of phosphate-free DMEM buffered with 20 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2 ethanesulfonic acid)–KOH (pH 7.2) containing 2% dialyzed fetal calf serum. At 7 and 8 h p.i., actinomycin D (1 μg/ml) and 50 μCi of [^{32}P]phosphate, respectively, were added. The cells were lysed at 10 h p.i., and RNA was extracted as described by Spaan et al. (27). The final RNA precipitate was dissolved in high-salt binding buffer (10 mM Tris hydrochloride) [pH 7.5], 0.05% SDS, 500 mM NaCl) and subjected to oligo(dT)-cellulose chromatography to select the poly(A)-containing RNAs. The material eluted with 10 mM Tris hydrochloride buffer (pH 7.5) was concentrated by ethanol precipitation. TGEV was precipitated from the culture medium with polyethylene glycol as described above. The pellet was suspended in lysis buffer, and RNA was extracted as described by Spaan et al. (27).

Preparative isolation of virus-specific mRNAs. Cytoplasmic RNA was isolated from 9 × 10^7 TGEV-infected PD5 cells grown in roller bottles. About 560 μg of poly(A)-containing material that was recovered after ethanol precipitation was fractionated by isokinetic sucrose gradient centrifugation (5% [wt/wt] top concentration) as described by van der Zeijst and Bloemers (35). Centrifugation was carried out in an sw40Ti rotor for 6 h at 40,000 rpm. Sucrose solutions were made in gradient buffer (50 mM Tris hydrochloride [pH 7.4], 100 mM LiCl, 1 mM EDTA, 0.1% SDS). Before centrifugation the RNA was dissolved in sample buffer (50 mM Tris hydrochloride [pH 7.4], 10 mM EDTA, 0.4 mM LiCl, 1% SDS) and heated for 1 min at 56°C. The gradient was fractionated, and the RNAs were recovered by ethanol precipitation.

Agarose gel electrophoresis. RNA samples were denatured with glyoxal-dimethyl sulfoxide and analyzed by electrophoresis in a 1% agarose–6 M urea horizontal slab gel as described by Spaan et al. (27). The molecular weights of the RNAs were calculated by using an *Eco*RI digest of phage lambda as markers.

Kinetics of labeling viral RNAs. PD5 cells grown on cover slips (18 by 18 mm) in 35-mm-diameter tissue culture dishes were infected or mock infected. After adsorption, 1 μg of actinomycin D per ml was added. Two hours p.i. 10 μCi of [^{3}H]uridine was added, and incubation at 37°C was continued. At intervals, isotope incorporation was measured.

In vitro translation of mRNAs in a rabbit reticulocyte cell-free system. Volumes (1 μl) containing about 1 μg of RNA were incubated with 0.5 μl of [^{35}S]methionine (10 μCi) and 4 μl of rabbit reticulocyte lysate (N90; Radiochemical Centre, Amersham, England) for 60 min at 30°C. The lysates were analyzed by SDS-polyacrylamide gel electrophoresis either directly or after immunoprecipitation.

Northern blot hybridization. Total RNA extracted from TGEV-infected cells was denatured by incubation for 30 min at 56°C in 50% deionized formamide–2.2 M formaldehyde–0.5 mM EDTA. The samples were cooled on ice, and after the addition of 0.6 volume of sample buffer containing 0.5% SDS, 0.025% bromophenol blue, 25% glycerol, and 25 mM EDTA, the samples were electrophoresed (50 V for 7 h) in a horizontal submarine gel containing 1.1 M formaldehyde and 1% agarose in 10 mM phosphate buffer (pH 7.0). RNA was blotted from the gel to a membrane filter (Gene Screen; New England Nuclear Corp., Boston, Mass.). After prehybridization in 50% formamide, 5× SSC (20× SSC is 3 M sodium chloride plus 0.3 M sodium citrate), 10× Denhardt solution (0.2% Ficoll, 0.2% polyvinylpyrrolidone, 0.2% bovine serum albumin), 0.05 M phosphate buffer (pH 6.5), 100 μg of sonicated denatured salmon sperm DNA per ml, and 0.1% SDS for 30 min at 42°C, hybridization was carried out with cDNA from RNA1 (23.6 kb). For this purpose RNA1 was purified by sucrose gradient centrifugation, denatured in 10 mM methyl mercury hydroxide, and randomly primed with calf thymus DNA pentamers (2 μg/μg of RNA; Pharmacia, Uppsala, Sweden). The synthesis was carried out in a 25-μl reaction volume containing 5 U of RNAsin (Amersham, Amersham, England); 0.1 mM each dGTP, dCTP, and dTTP; 50 mM Tris hydrochloride (pH 7.4); 50 mM KCl, 8 mM $MgCl_2$, 5 U of reverse transcriptase (Promega Biotec); and 2 μl of [α-^{32}P]ATP. After the incubation period the filter was washed with three changes of 2× SSC at 42°C for 30 min.

RESULTS

TGEV structural proteins. An SDS-polyacrylamide gel analysis of purified [^{35}S]methionine-labeled TGEV from a sucrose gradient peak fraction is shown in Fig. 1 (lane 1). Two closely comigrating protein species with molecular weights of 280,000 and 240,000 were observed; these were interpreted to be different forms of the peplomeric protein (E2). In addition, the nucleocapsid (N) protein (molecular weight, 48,000) and three protein species of E1 (molecular weights, 28,000, 31,500, and 33,000) were observed.

Intracellular viral proteins and the effect of tunicamycin. TGEV-infected PD5 cells were incubated with [^{35}S]methionine in the presence or absence of tunicamycin (1 μg/ml) from 8 to 9.5 h p.i. Tunicamycin, if added, was present from 4 h p.i. until the end of the labeling period. The cell lysates were analyzed after immunoprecipitation followed by SDS-polyacrylamide gel electrophoresis. Without tunicamycin the viral proteins listed above with molecular weights of

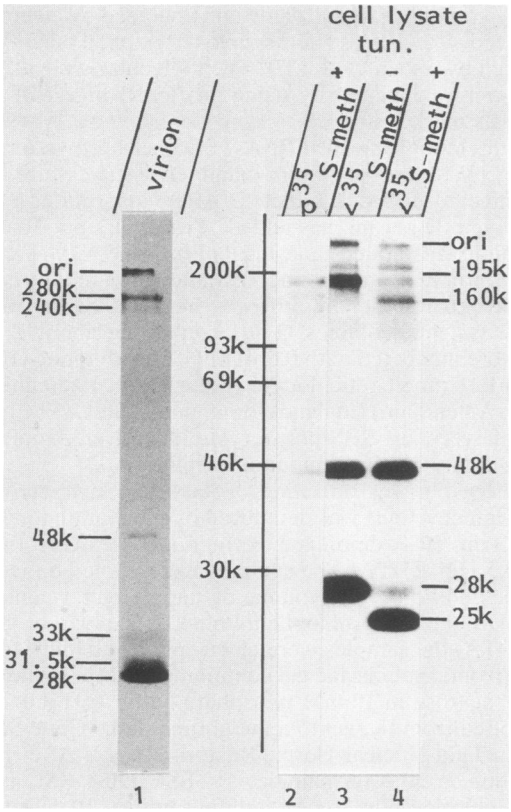

FIG. 1. Electrophoretic analysis of [^{35}S]methionine (^{35}S-meth)-labeled TGEV structural and intracellular proteins: the effect of tunicamycin. TGEV purified from culture fluids of infected PD5 cells is shown in lane 1. Intracellular proteins were obtained by immunoprecipitation of lysates of virus-infected (v) PD5 cells (p, preimmune serum). Tunicamycin (tun) was added 4 h p.i. Molecular weights (in thousands [k]) were calculated from the indicated marker proteins.

'195,000, 48,000, and 28,000 were observed (Fig. 1, lane 3). In the presence of tunicamycin the amount of the proteins with molecular weights of 195,000 and 28,000 was reduced; instead, proteins with molecular weights of 160,000 and 25,000 were detected (Fig. 1, lane 4).

Isolation of virus-specific intracellular RNAs and genome RNA. Preliminary experiments showed that the synthesis of viral RNAs had reached its maximum rate between 8 and 10 h p.i. and that there was no difference in virus production between actinomycin D-treated and untreated cells. To analyze virion RNA and intracellular RNAs, infected cells were labeled with ^{32}P from 8 to 10 h p.i. After the labeling period the virus from the culture fluid was precipitated with polyethylene glycol, the cells were lysed, and RNA was extracted with phenol. Poly(A)-containing RNAs were isolated by oligo(dT)-cellulose chromatography, denatured with glyoxal and dimethyl sulfoxide, and analyzed by urea-agarose gel electrophoresis. From the autoradiograph of the gel, the molecular weights of the RNAs were calculated by using a marker. Five major and two minor RNAs that were absent in mock-infected cells were observed with lengths of 23.6, 8.4, 3.8, 3.0, 2.6, 1.9, and 0.7 kb (Fig. 2A). To prove the virus specificity of the isolated mRNAs, a Northern blot analysis was performed. After electrophoresis of unlabeled total intracellular RNAs in a formaldehyde gel, they were transferred to a membrane filter and subsequently hybridized to a cDNA probe copied from RNA1. The same RNAs were detected as described above with the exception of the 0.7-kb RNA species (Fig. 2B). The virus-specific RNAs were designated as RNA1 and RNA3 to RNA7 (see below for an explanation of why RNA2 is lacking). RNA1 comigrated with virion RNA (Fig. 2A).

In vitro translation of mRNAs in the reticulocyte system. The intracellular RNAs from TGEV-infected cells were separated by isokinetic sucrose-gradient centrifugation. Material from each fraction was precipitated with ethanol and analyzed by urea-agarose gel electrophoresis. Fractions containing only RNA3, RNA4, and RNA7 could be obtained. The RNA6 preparation was slightly contaminated with RNA5 and RNA7 (Fig. 2C). The purified RNAs thus obtained were translated in a rabbit reticulocyte system, and the translation products were analyzed by SDS-polyacrylamide gel electrophoresis either directly or after immunoprecipitation (Fig. 3). Direct analysis of the translation lysates showed that RNA4, RNA6, and RNA7 encoded proteins with molecular weights of 24,000, 25,000, and 48,000, respectively (Fig. 3, lanes 1 through 4). After translation of RNA3 many proteins were obtained (Fig. 3, lane 11).

Identification of the translation products. The translation products of the separated TGEV mRNAs were further characterized by electrophoresis of the immunoprecipitates in SDS-polyacrylamide gels (Fig. 3, lanes 5 through 9); virus-specific proteins from cells infected with TGEV in the presence of tunicamycin were run as markers. The immunoprecipitated translation product of RNA7 comigrated with the 48,000-dalton protein present in TGEV-infected cells and with the nucleocapsid protein of purified virus. RNA6 was translated into a protein with the same electrophoretic behavior as the unglycosylated E1 protein present in tunicamycin-treated cells (molecular weight, 25,000). The translation product of RNA4, a protein with a molecular weight of 24,000, was detected neither in infected cells nor in purified virions, and it was not immunoprecipitated. By using a monoclonal antibody directed against the peplomer protein of TGEV (Fig. 3, lane 14), polypeptides with molecular weights of about 250,000 and 130,000 and smaller molecules were immunoprecipitated from the translation lysate containing RNA3 (Fig. 3, lane 10).

DISCUSSION

In TGEV (strain Purdue)-infected cells, five major poly(A)$^+$ RNAs of 23.6, 8.4, 3.8, 2.6, and 1.9 kb and two additional low-abundance RNAs of 3.0 and 0.7 kb were found. After Northern blot hybridization with a cDNA probe from RNA1, all mRNAs were detected (as expected, RNA1 and RNA3 were transferred poorly), with the exception of the RNA species of 0.7 kb. Since the other minor RNA could be detected in the blot, we assume that the 0.7-kb species is not virus specific. In Table 1 the molecular weights found by us for the TGEV RNA are compared with the values published by others (6, 7). In Table 2 the respective data for other coronaviruses such as infectious bronchitis virus (IBV) and mouse hepatitis virus (MHV) are presented. Adhering to the nomenclature used for MHV (22), the RNAs of TGEV were numbered RNA1 and RNA3 to RNA7 (Table 2). From lysates of TGEV-infected cells, three major proteins with molecular weights of 28,000 (E1), 48,000 (N), and 195,000 (E2) were immunoprecipitated. In contrast, sucrose gradient-purified virions contained two species of E2 (molecular weights, 280,000 and 240,000) and two additional E1 species (molecular weights, 31,500 and 33,000). The latter proteins

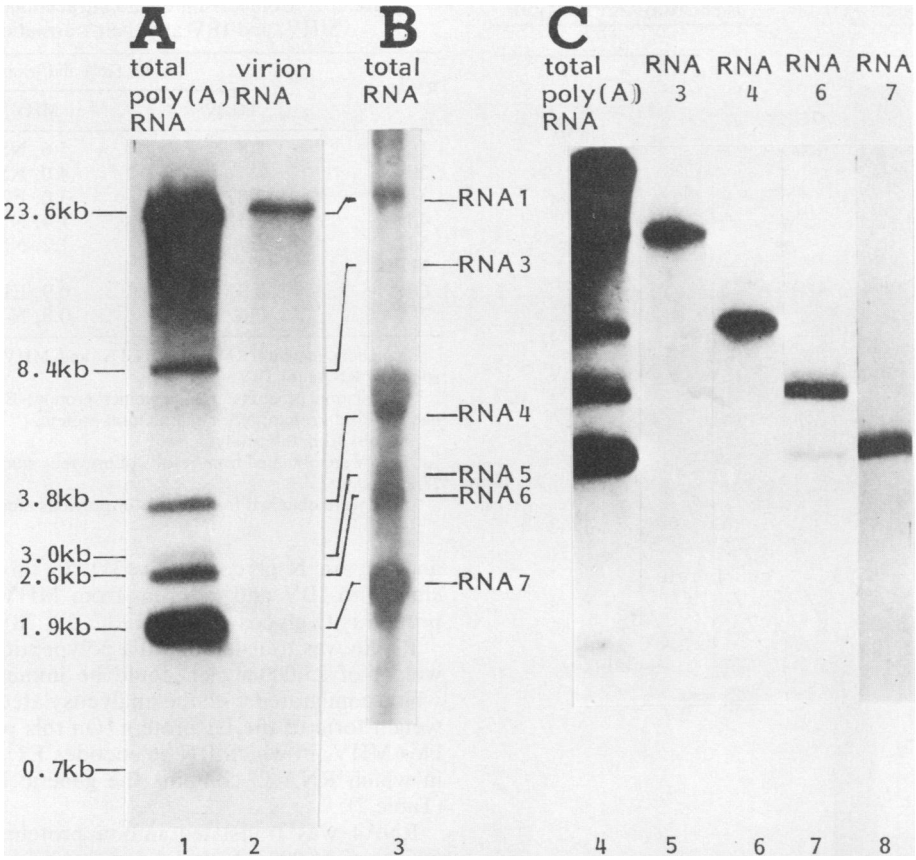

FIG. 2. (A) Electrophoresis of TGEV-specific poly(A)-containing intracellular RNAs and virion RNA. Denatured ^{32}P-labeled RNAs were analyzed in agarose-urea gels. The RNAs are indicated by numbers (Tables 1 and 2). Their lengths are also given in kilobases. Autoradiographs of the dried gels are shown. (B) Northern blot analysis of TGEV mRNAs. Unlabeled intracellular RNAs from TGEV-infected cells were separated in a formaldehyde gel, transferred to a membrane filter, and hybridized with ^{32}P-labeled cDNA to RNA1. An autoradiograph of the blot is shown. (C) Analysis of preparations of purified intracellular viral mRNAs. TGEV-infected PD5 cells were lysed at 10 h p.i., and poly(A)$^+$ RNAs were isolated and separated by isokinetic sucrose gradient centrifugation. Fractions were analyzed by 1% agarose–6 M urea gel electrophoresis. Preparations containing single RNA species are shown together with unfractionated ^{32}P-labeled poly(A)$^+$ TGEV RNA, which served as a marker.

are probably modifications of the protein with a molecular weight of 28,000. In infected cells no other E2 species besides the protein with a molecular weight of 195,000 was detected. It is possible that the protein with a molecular weight of 195,000 was modified to a protein species with a molecular weight of 280,000 or 240,000 late during virus maturation.

The presence of a nested set of RNAs with expression of only the 5′-terminal nonoverlapping region has not been formally proven for TGEV. However, we assume that TGEV is similar in this respect to IBV and MHV. Direct proof for the messenger function of intracellular RNAs came from in vitro translation experiments; identification of the products was based on their electrophoretic migration and

TABLE 1. Molecular weights of the TGEV intracellular RNAs: theoretical coding capacity and translation products

RNA	Mol wt (10^6) from:			kb[a]	Theoretical nonoverlapping coding capacity (mol wt, 10^3)[a]	Actual primary translation product (10^3)[b]
	Reference 6	Reference 7	This study			
1	6.8	6.8	7.8	23.6	500	
(2)	6.2					
3	3.15	3.15	2.8	8.4	155	250, 130
4	1.40	1.40	1.25	3.8	25	24
5	1.05		1.0	3.0	14	
6	0.94	0.94	0.86	2.6	23	25
7	0.66	0.66	0.63	1.9	63	48
8	0.39, 0.34, 0.24					

[a] The molecular weight of each nonoverlapping coding region, i.e., the difference between each RNA and the next smaller species, was divided.
[b] Obtained from the results of this study.

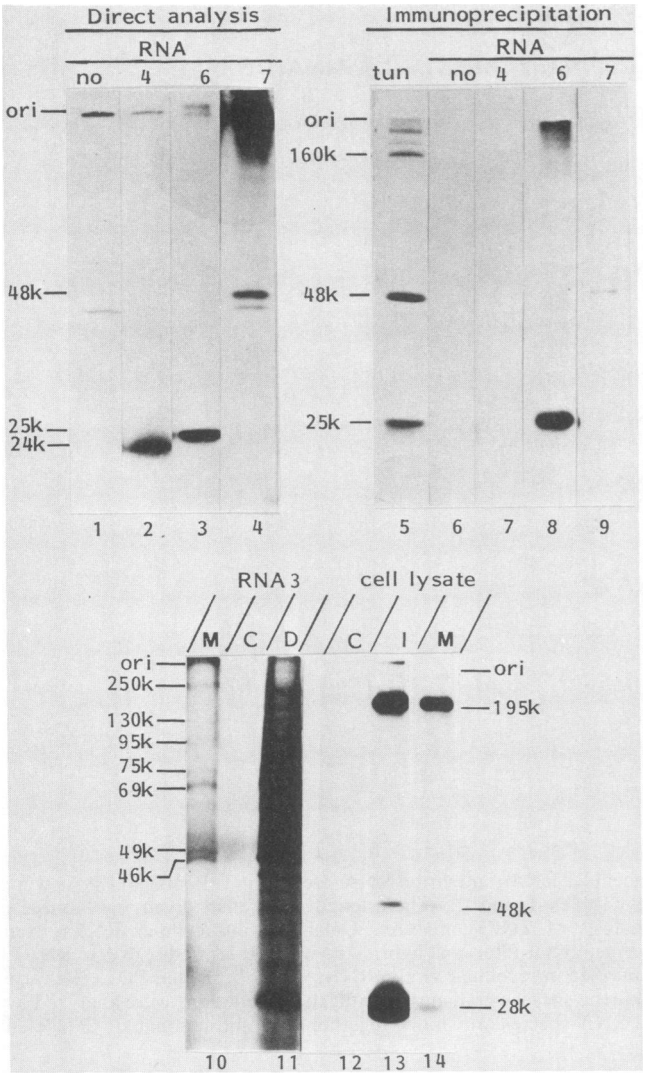

FIG. 3. Proteins translated from purified TGEV mRNAs. The products were analyzed by electrophoresis in 15% SDS-polyacrylamide gels either directly or after immunoprecipitation with a cat anti-FIPV serum. Viral structural proteins and proteins isolated from TGEV-infected cells in the presence of tunicamycin (tun) were run in parallel. Analysis of the translation products of RNA3 was performed in a 12.5% polyacrylamide gel directly (D) or after immunoprecipitation with a monoclonal antibody directed against the peplomer protein (M) or antibody-free serum (C). In lanes 12 to 14 cell lysate was immunoprecipitated with antibody-free serum (C), anti-FIPV serum (I), or with the monoclonal antibody directed against E2 (M). Molecular weights are given in thousands (k).

on immunologic recognition by an E2 protein-specific monoclonal antibody or a heterotypic polyclonal antiserum; we used an antibody preparation from a field case of feline infectious peritonitis (12, 18). Table 2 shows that RNA7 of TGEV is similar to those of IBV and MHV in that it encodes the nucleocapsid protein. To identify the other translation products some insight into the glycosylation of TGEV protein was needed. In TGEV-infected, tunicamycin-treated cells no E1 protein with a molecular weight of 28,000 and only traces of the 195,000-molecular-weight E2 protein were detected, but molecules with molecular weights of 25,000 and 160,000 accumulated instead. From the results of the experiments with tunicamycin it can be inferred that both E2 and E1 are N glycosylated (31). In this respect TGEV is similar to IBV and different from MHV, in which the E1 protein is O glycosylated (11, 17, 19, 28).

TABLE 2. Comparison of the intracellular RNAs of TGEV, MHV, and IBV and their translation products

RNA[a]	Mol wt (10^6) and translation product[b]		
	TGEV[c]	MHV[d]	IBV[e]
1/F	7.8	5.6, NS	8.1
2		4.0, NS	
3/E	2.8, E2	3.0, E2	2.6, E2
4/D	1.3, NS	1.4, NS	1.5
5/C	1.1	1.2, NS	1.3, E1
M			1.0
6/B	0.9, E1	0.9, E1	0.9, NS
7/A	0.6, N	0.8, N	0.8, N

[a] Numbers indicate RNAs for TGEV and MHV; letters indicate corresponding RNAs for IBV.
[b] Translation products: E2, peplomer protein; E1, envelope protein; N, nucleocapsid protein; NS, nonstructural protein.
[c] Results from this study.
[d] Data were obtained from results of previous studies (5, 14–16, 20, 23, 26, 27).
[e] Data were obtained from results of previous studies (1, 2, 30).

RNA6 was translated into a polypeptide with a molecular weight of 25,000 which could be immunoprecipitated and which comigrated with the unglycosylated 25,000-molecular-weight form of the E1 protein. On this point TGEV resembles MHV, in which RNA6 encodes E1, and is unlike IBV, in which RNA C contains the genetic information for E1 (Table 2).

RNA4 was translated into a protein with a molecular weight of 24,000 which did not comigrate with any intracellular viral protein nor could it be immunoprecipitated with antiserum directed against the virion proteins; it therefore is probably a nonstructural polypeptide.

Considering the biology of TGEV, the E2 protein is most interesting because antibody directed against it neutralizes the virus and protects piglets against infection (8, 10). The protein is made as a 160,000-molecular-weight unglycosylated form which can be found in tunicamycin-treated cells. In pulse-chase experiments, we found no indication for processing of the E2 (molecular weight, 195,000) protein before leaving the cell (L. Jacobs, unpublished data). This is at variance with the situations in IBV and MHV, in which precursor polypeptides are processed into smaller subunits (20, 24, 29, 33). No molecule with a molecular weight of 160,000 was recognized in RNA3-primed cell-free lysates, but products with molecular weights of 250,000 and 130,000 and of still smaller sizes were immunoprecipitated with a virus-neutralizing monoclonal antibody directed against E2. The largest species found in the immune precipitates is probably an aggregate, the products of species with molecular weights of 130,000 and smaller could be incomplete translation products. For the moment, however, the fact that they were recognized by a monoclonal antibody directed against E2 should suffice as evidence that RNA3 encodes E2.

No virus-specific translation product was identified for RNA5.

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