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Identification of the In Vitro Translation Products of Adenovirus mRNA by Immunoprecipitation

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Adenovirus type 2 mRNA was translated in S30 extracts from Ehrlich ascites and wheat embryo cells. The in vitro products were identified by sodium dodecyl sulfate-gel electrophoresis after immunoprecipitation with specific antisera in the presence of urea. Seven virion polypeptides could be identified by immunoprecipitation. Three of these appear to be precursors to polypeptides of the virion. mRNA isolated late in adenovirus infection was separated into three size classes by zonal sedimentation. Material sedimenting at 26*S* was translated into polypeptides corresponding to the largest virion polypeptides II to IV, a 22*S* fraction corresponding to polypeptide V, and smaller polypeptides and a 15*S* fraction corresponding to polypeptide IX. A significant amount of polypeptide IX was also synthesized by the 26*S* and 22*S* RNA.

The DNA-containing adenoviruses may serve as a model for translation in eukaryotic cells since the viral mRNA, like the host-cell mRNA, is synthesized and polyadenylated in the nucleus and processed and transported to the cytoplasm where it forms polyribosomes (for a review, see 20). Several of the virion proteins have been extensively characterized (for a review, see 21), and there is evidence for regulation at the translational level of their synthesis (18). Translation in a cell-free system may unravel these control mechanisms and also reveal precursor polypeptides, since cleavage is often deficient in cell-free systems (15, 17).

Several reports have appeared suggesting in vitro synthesis of adenovirus proteins from polyribosomes from infected cells, but it has not been established unequivocally that these systems initiate translation (5, 27). Translation of mRNA in a complete heterologous cell-free system overcomes this difficulty, and no absolute restriction for translation has yet been reported (14, 25). This communication describes translation in S30 extracts from Ehrlich ascites and wheat embryo cells of adenovirus type 2 (Ad2) RNA isolated late in productive infection. Several products are identified by specific immunoprecipitation followed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis.

The translation products generated by the three size classes of late adenovirus mRNA have also been analyzed.

MATERIALS AND METHODS

RNA preparations. KB cells grown in spinner cultures at a cell density of 2×10^5 to 5×10^5 cells/ml were infected with Ad2 or mock infected as described previously (13). The cells were labeled with [5-^{3}H]uridine from 14 to 16 h postinfection. During labeling the cells were concentrated 10 times. Infected and mock-infected cells were fractionated into cytoplasm and nuclei, and in some cases the polyribosomes were isolated as previously described (13). The cytoplasm was extracted with phenol according to three different procedures (4, 9, 16). RNA dissolved in water at a concentration of 2 to 4 mg/ml was used in the translation system. Cytoplasmic RNA was, in some experiments, subjected to fractionation by affinity chromatography on oligo(dT)-cellulose essentially by the method of Aviv and Leder (3). In other experiments, poly(U)-Sepharose was used instead of oligo (dT) as described by Lindberg and Persson (12).

The poly(A)-containing RNA eluted from the oligo (dT)-cellulose columns was fractionated by centrifugation. It was dissolved in 0.1 × SSC (0.15 M NaCl plus 0.015 M sodium citrate) plus 0.5% sarcosyl to a concentration of 1 to 2 mg/ml. Nine volumes of formamide was added 150 to 200 μg of RNA. Samples were kept at 37 C in a water bath for 5 min and quickly cooled by incubation at −20 C for 1 min. Samples were then diluted to a final concentration of 0.50% formamide with 0.1 × SSC plus 0.5% sarcosyl and layered on top of a 15 to 30% (wt/vol) sucrose gradient containing 0.1 M NaCl-0.1 M EDTA (pH 7.5)-0.01 M Tris (pH 7.5)-0.5% sarcosyl-50% formamide. Centrifugation was for 48 h at 4 C in a SW 27 rotor in a LS 50 Beckman centrifuge.

Cell-free extracts. Preincubated S30 extracts from Ehrlich ascites cells were prepared as described by

Eggen and Shatkin (6). It was important to raise the magnesium ion concentration to 3.5 mM immediately after disruption of the cells to obtain an extract with high activity. Wheat-germ cell extracts were prepared according to Roberts and Paterson (22). The magnesium ion concentration was also raised to 3.5 mM in these extracts directly after grinding.

Cell-free protein synthesis. The incubation conditions for ascites and wheat embryo cell extracts were those shown in Table 1 unless stated otherwise. The standard volume was 100 μliters. Ten microliters of 0.1 M unlabeled methionine was added to duplicate samples, and the mixtures were pipetted onto 3 MM Whatman filter paper disks and placed in 10% trichloroacetic acid at 90 C for 15 min and washed in 5% trichloroacetic acid, ethanol, ethanol-ether, and ether. Radioactivity was determined in a toluene based scintillation mixture at an efficiency of 80% for ^{35}S.

Antisera. Antisera against poliovirus and against Ad2 virion proteins hexon (polypeptide II), penton (polypeptides III and IV), and major core protein (polypeptide VII) were prepared in rabbits (8); swine immunoglobulin G (IgG) directed against rabbit IgG (Dakopatts A/S, Denmark) was used in the second step in the double immunoprecipitation. The nomenclature for the adenovirus polypeptides is that of Anderson et al. (1). Antisera against Ad2 proteins corresponding to polypeptides IIIa, VI, VIII, and IX were prepared in guinea pigs (7a; unpublished data). Rabbit IgG directed against guinea pig IgG (Hoechst, Behringewerke) was then used in the second step. Two antisera against hexon (polypeptide II) were used, one prepared against native hexon (8) and one against hexon denatured with 6 M guanidine (10a). IgG was purified by passing the antisera through columns of DEAE-Sephadex A-50 in 0.01 M sodium phosphate (pH 6.5) and collecting the flow through.

TABLE 1. *Translation conditions*

Condition	Cell extract	
	Ehrlich ascites	Wheat embryo
HEPES[a] (pH 7.6)	20 mM	20 mM
KCl	120 mM	65 mM
$MgAc_2$	3.2 mM	3.2 mM
ATP (Li or K)	1 mM	1 mM
GTP (Li or K)	0.1 mM	0.2 mM
CTP (Tris)	0.06 mM	
Creatine phosphate (Tris)	2 mg/ml	4 mg/ml
Creatine kinase	100 μg/ml	40 μg/ml
[^{35}S]methionine (150 to 200 Ci/mmol)	50 to 250 μCi/ml	50 to 250 μCi/ml
S30 extract, protein concentration	5 to 10 mg/ml	5 to 10 mg/ml
RNA	20 to 60 μg/ml	20 to 60 μg/ml
Dithiothreitol	0.75 mM	0.75 mM
Amino acids	20 μM	20 μM
Incubation time	80 min	80 min
Incubation temperature	37 C	30 C

[a] HEPES, *N*-2-hydroxyethyl-piperazine-*N*'-2'-ethanesulfonic acid.

Immunoprecipitation. Cell-free translation mixtures prepared and incubated as described in Table 1 were further incubated for 30 min at 37 C with 100 μg of RNase A per ml in the presence of 0.1 M EDTA and dialyzed overnight at 4 C against 7 M urea-1 mM dithiothreitol in phosphate-buffered saline (PBS). The resulting solution was centrifuged at 16,000 × *g* for 10 min, and the supernatant was diluted with PBS to 1 M urea, adjusted to 1% with Triton-X 100, and centrifuged again at 16,000 × *g* for 10 min. At least 98% of the trichloroacetic acid-precipitable radioactivity of the original translation mixture was recovered in the supernatant before immunoprecipitation with antisera. The cell-free translation mixture was subjected to a nonspecific precipitation with anti-poliovirus rabbit IgG–swine anti-rabbit IgG, and the supernatant was used for immunoprecipitation with antibodies directed against Ad2 virion proteins. Specific and nonspecific immunoprecipitation was carried out by a double-antibody technique: 1.0 ml portions of the sample were incubated at room temperature for 1 h with 0.1 ml of antiserum (1.0 mg of protein/ml) or purified IgG (0.4 mg of protein/ml) directed against poliovirus or Ad2 virion proteins. A heterologous antiserum or IgG directed against the first IgG was added at equivalence, and the samples were kept overnight at 4 C. The immunoprecipitates were collected by centrifugation at 3,000 × *g* for 10 min, washed three times with PBS-1% Triton X-100, dissolved in 0.1 M NaOH, precipitated with trichloroacetic acid, washed twice with acetone, and dissolved for electrophoresis in gel sample buffer (11).

Electrophoresis. Portions of the translation mixtures or the marker proteins were incubated with 0.1 M NaOH for 15 min at 37 C and precipitated with equal volumes of 10% trichloroacetic acid, washed with 5% trichloroacetic acid and acetone or ethanol, and dissolved in 100 μliters of gel sample buffer (11). Slab gels contained 13% acrylamide (11) and were subject to electrophoresis at 100 V for 3 to 4 h with 10-μliter samples applied. [^{35}S]methionine-labeled Ad2 was used as marker. For autoradiography, X-ray films (Kodak X G14) were exposed to dried gels for 10 days. Immunoprecipitates were analyzed on cylindrical SDS-polyacrylamide gels (8) (13% polyacrylamide, 0.5 by 13.0 cm) with [^{3}H]methionine-labeled Ad2 as an internal marker. Electrophoresis was carried out at 100 V for 6 h. The procedures used to slice the gels and to assay radioactivity have been described (8).

Chemicals and isotopes. Fresh commercial wheat germ was supplied by a local mill (Kungsörnen, Uppsala). *N*-2-hydroxyethyl-piperazine-*N*'-2'-ethanesulfonic acid, ATP (Ba salt) and CTP (Tris salt) were from Sigma, GTP (Li salt) and creatine phosphate (Tris salt) were from Boehringer, Mannheim. ATP potassium salt was prepared by triturating the barium salt with K_2SO_4 and removing the $BaSO_4$ precipitate. [^{35}S]methionine (150 to 250 Ci/mmol) was from Amersham and ^{3}H-labeled amino acid mixture (1 mCi/ml) and [5-^{3}H]uridine (40 to 50 Ci/mmol) from New England Nuclear Corp. Oligo (dT)-cellulose was obtained from Collaborative Re-

search Inc. DEAE-Sephadex was from Pharmacia. Formamide (Merck, Darmstadt) was purified by the method of Tibbets et al. (24).

Virus. [^{35}S]methionine or [^{3}H]amino acid-labeled Ad2 was prepared as described earlier (9).

Enzymes. Creatine kinase (EC 2.7.3.2) was from Boehringer, Mannheim, and RNase A (EC 2.7.7.16) was from Sigma.

RESULTS

RNA preparation. Three different procedures (4, 9, 16) were used to extract RNA from cytoplasm of Ad2-infected KB cells at a time (16 h postinfection) when capsid proteins are synthesized at a maximal rate and the host cell protein synthesis is depressed (27). RNA from mock-infected KB cells was extracted as described by Öberg and Philipson (16). No qualitative difference in translation products could be observed by polyacrylamide electrophoresis using adenovirus RNA prepared by different methods (Fig. 1C and E). This was also the case when translation of poly(U)-Sepharose (12) and oligo(dT)-selected RNA was compared. Nor could any difference in translation be observed between RNA from cytoplasm or isolated polyribosomes. However, the relative amount of radioactivity in the different bands showed slight variation with different RNA preparations, particularly for the polypeptides of high molecular weight.

Conditions for in vitro translation. The optimal magnesium concentration for translation into trichloroacetic acid-precipitable radio-

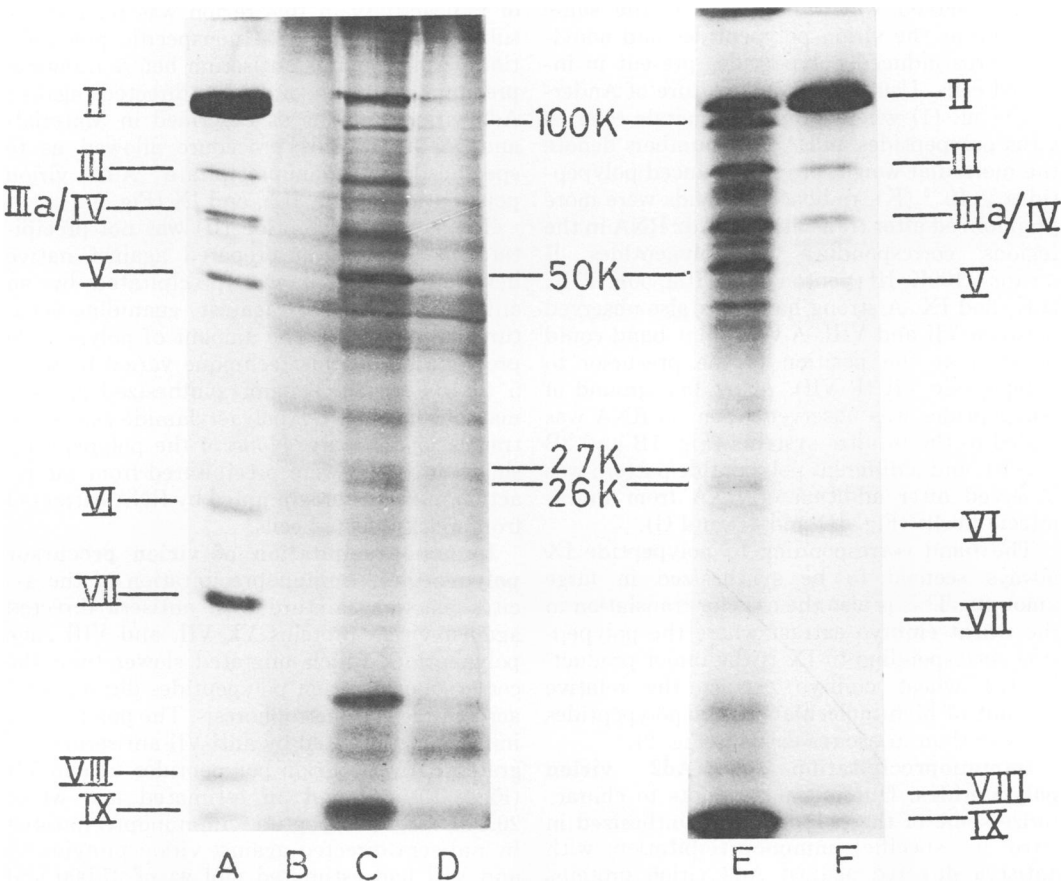

FIG. 1. *SDS-polyacrylamide gel electrophoresis of products obtained by cell-free translation of adenovirus RNA in ascites cell extracts. Preparation of samples and electrophoresis was as described in Materials and Methods. (A) Adenovirus marker; (B) no RNA added to the translation mixture; (C) translation of RNA from adenovirus-infected cells extracted by the method of Brawerman et al. (4); (D) translation of RNA from mock-infected cells; (E) translation of RNA from adenovirus-infected cells extracted by the method of Öberg and Philipson (16); (F) adenovirus marker (containing very low amounts of polypeptides V and VII).*

activity was found to be 3.2 mM at a potassium concentration of 120 mM in ascites and 65 mM in wheat embryo extracts. The optimal potassium concentration was 120 mM in ascites and 65 mM in wheat embryo extracts at 3.2 mM magnesium. Both translation systems were saturated at RNA concentrations of 100 μg per ml of incubation mixture, using oligo(dT)-selected RNA which contained around 50% of ribosomal RNA. Translation kinetics showed that the net synthesis terminated after 60 to 80 min of incubation in both systems under conditions indicated in Table 1.

Translation products. Figure 1 shows polyacrylamide gel patterns of polypeptides synthesized in an ascites cell extract with RNA from infected and mock-infected KB cells as mRNA. When RNA from adenovirus-infected cells (Fig. 1C and E) was translated, several polypeptides were observed which migrated at the same position as the virion polypeptides and nonvirion virus-induced polypeptides present in infected cells. Using the nomenclature of Anderson et al. (1) where Roman numerals refer to virus polypeptides and Arabic numbers denote the molecular weight of virus-induced polypeptides $\times 10^{-3}$ (K), radioactive bands were more pronounced after translation of Ad2 RNA in the regions corresponding to polypeptides II (hexon), 100K, III (penton base), IIIa, 50K, 27K, 26K, and IX. A strong band was also observed between VII and VIII. A very faint band could be seen at the position of the precursor to polypeptide VII (P VII). A low background of polypeptides was observed when no RNA was added to the in vitro systems (Fig. 1B and 2B and E), and a different polypeptide pattern was observed after addition of RNA from mock-infected cells (Fig. 1D and 2D and G).

The band corresponding to polypeptide IX always seemed to be synthesized in large amounts. This is also the case for translation in the wheat embryo extract where the polypeptide corresponding to IX is the major product. In the wheat embryo extract the relative amount of high-molecular-weight polypeptides is lower than in ascites extract (Fig. 2).

Immunoprecipitation of Ad2 virion polypeptides. Our initial attempts to characterize some of the polypeptides synthesized in vitro by specific immunoprecipitation with antisera directed against Ad2 virion proteins were unsuccessful. Centrifugation of the translation mixture prior to immunoprecipitation sedimented up to 50% of the acid-precipitable radioactivity, and immunoprecipitation of the supernatant directly or by a double-antibody technique showed no specificity. Conditions to solubilize the proteins in the in vitro reaction mixture and to maintain them in solution during incubation with antibody were therefore developed as described in Materials and Methods. This procedure maintained up to 98% of the acid-precipitable radioactivity in solution before immunoprecipitation. Immunoprecipitation of the solubilized in vitro ascites reaction mixture with antisera directed against Ad2 virion proteins, by either direct immunoprecipitation or by a double-antibody technique, specifically precipitated the proteins reacting with respective antisera (Fig. 3). In addition, one polypeptide migrating in polyacrylamide gels at the position of Ad2 virion polypeptide V was precipitated. This latter polypeptide was also precipitated by poliovirus antiserum (Fig. 3B) or other nonspecific antisera. The amount of radioactivity in this region was reduced by subjecting samples to a nonspecific precipitation with poliovirus antiserum before immunoprecipitation with antisera directed against Ad2 virion proteins as described in Materials and Methods. This procedure allowed us to specifically immunoprecipitate Ad2 virion polypeptides II, III, IIIa, and IX (Fig. 3).

The hexon polypeptide (II) was not precipitated by antiserum prepared against native hexon (19), but it was precipitated by an antiserum prepared against guanidine-denatured hexon (10a). The amount of polypeptide precipitated by this technique varied between 5 to 15% of the amount synthesized as estimated from the SDS-polyacrylamide gels of the translation mixture. None of the polypeptides shown in Fig. 3 were precipitated from the reaction mixtures programmed by RNA extracted from mock-infected cells.

Immunoprecipitation of virion precursor polypeptides. Immunoprecipitation of the ascites reaction mixture with antisera directed against virion proteins VI, VII, and VIII gave polypeptides which migrated slower than the corresponding virion polypeptides during polyacrylamide gel electrophoresis. The polypeptide immunoprecipitated by anti-VII antiserum migrated between virion polypeptides VI and VII (Fig. 3F) and had an estimated mol wt of 20,000. The polypeptides immunoprecipitated by antisera directed against virion proteins VI and VIII had estimated mol wt of 27,000 and 26,000, respectively (Fig. 4A). Although these two latter polypeptides migrated close to each other in polyacrylamide gels, they were distinct in size since they give sharp peaks when subjected to electrophoresis separately (Fig. 4A)

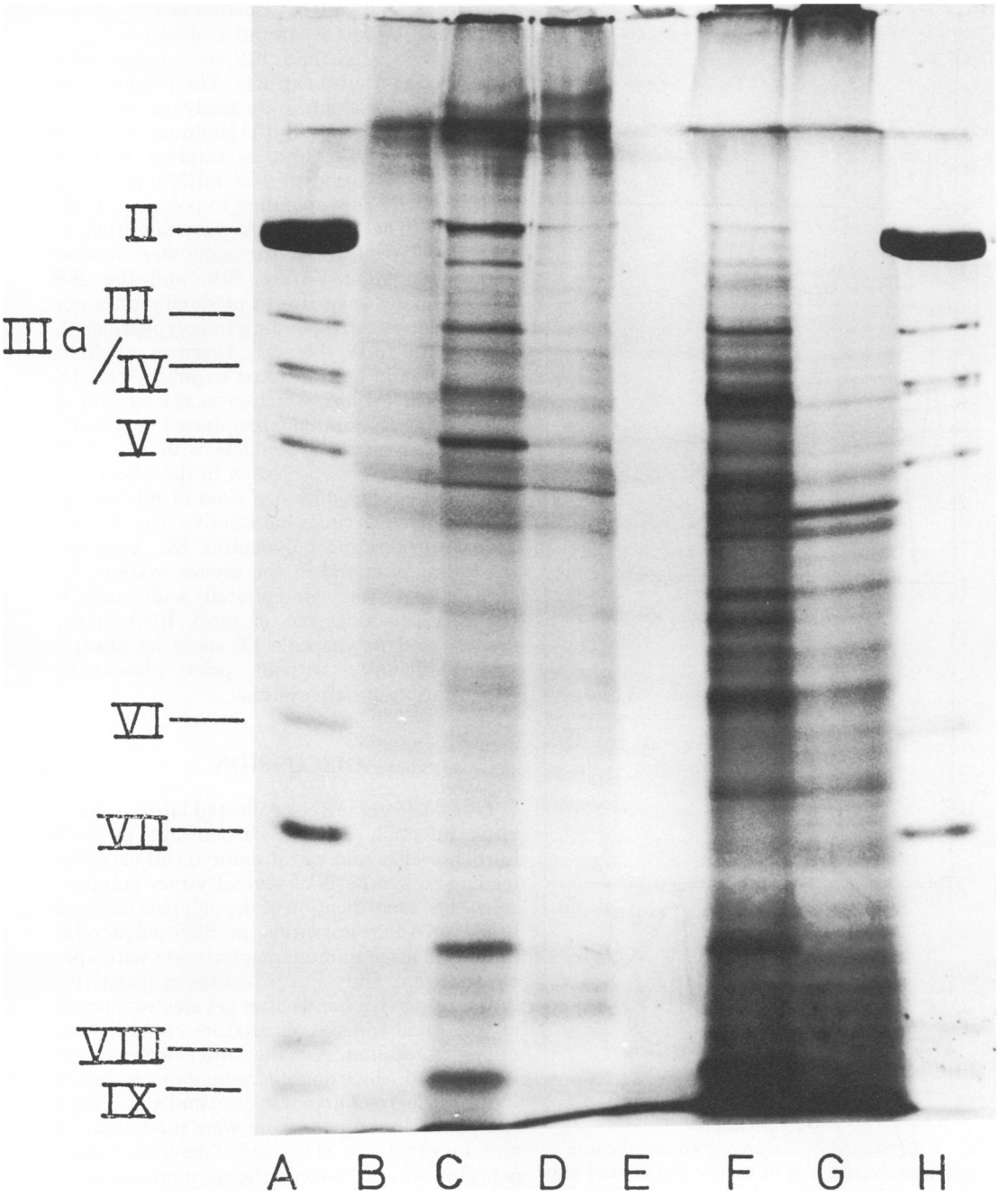

FIG. 2. *Comparison between the translation products in ascites and wheat embryo extracts by SDS-polyacrylamide gel electrophoresis. The products were analyzed as in the legend to Fig. 1, and the RNA from infected cells was extracted by the method of Brawerman et al. (4). (A) Adenovirus marker; (B) ascites cell extract, no RNA added; (C) ascites cell extract, unfractionated RNA from adenovirus-infected cells; (D) ascites cell extract, unfractionated RNA from mock-infected cells; (E) wheat embryo cell extract, no RNA added; (F) wheat embryo cell extract, unfractionated RNA from adenovirus-infected cells; (G) wheat embryo cell extract, unfractionated RNA from mock-infected cells; (H) adenovirus marker.*

but a broad band when analyzed together (Fig. 4B).

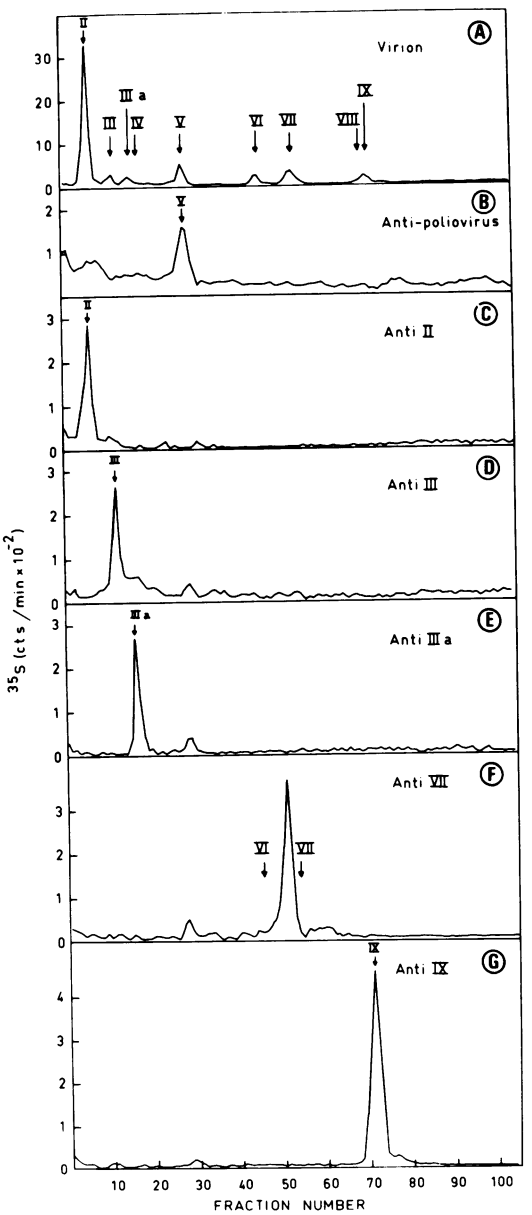

FIG. 3. *SDS-polyacrylamide gel electrophoresis of polypeptides synthesized in ascites cell extract and immunoprecipitated with antibodies directed against Ad2 virion proteins. Immunoprecipitates were prepared and processed as described in Materials and Methods and analyzed on gels. The antisera used are indicated in the figure. The precipitation with antisera against Ad2 proteins was preceded by nonspecific immunoprecipitation. [^{3}H]methionine-labeled Ad2 virion polypeptides were used as internal markers.*

Translation of different size classes of adenovirus mRNA. mRNA isolated late in adenovirus infection was separated as shown in Fig. 5, and the 26S, 22*S*, and 15*S* size classes were translated in ascites extracts. The [^{35}S]methionine-labeled products were analyzed on SDS-polyacrylamide gels with [^{3}H]amino acid-labeled adenovirus polypeptides as internal markers (Fig. 6). Translation of 15*S* mRNA gave one major product corresponding to polypeptide IX (Fig. 6A). The 22*S* mRNA was translated to several polypeptides of the same size, smaller than polypeptide V (Fig. 6B), and the 26*S* mRNA finally gave rise to predominant bands in the regions corresponding to polypeptides II, III, and IIIa to IV (Fig. 6C). However, all RNA fractions could be translated to polypeptide IX as shown in Table 2, which gives the amount of polypeptide IX immunoprecipitated by anti-IX from the translation products with 15*S*, 22*S*, and 26*S* mRNA, respectively, in the wheat embryo extract. The 15*S* size class of mRNA appeared to be five times more active than the 26*S* RNA in generating polypeptide IX. A similar result was obtained in the ascites system. No radioactivity was precipitated with anti-IX serum with endogenous or mock RNA in the system, and polypeptide IX could be precipitated efficiently without prior nonspecific precipitation in both systems.

DISCUSSION

Translation of mRNA collected late in adenovirus infection can be carried out in both Ehrlich ascites and wheat embryo cell extracts, leading to synthesis of several virion polypeptides. The identification of the polypeptides was based on their migration in SDS-polyacrylamide gels after immunoprecipitation with specific antisera. Only 5 to 15% of the radioactivity in the respective bands after gel electrophoresis of the crude translation mixture could be immunoprecipitated. A higher efficiency may not be expected, since double immunoprecipitation in the presence of urea was used and all antisera except the hexon antiserum were made against virion proteins (7a, 8) instead of denatured polypeptides. Alternatively, although more unlikely, only a portion of the radioactivity in each band corresponds to virus-induced polypeptides. The relative amounts of polypeptides formed in the two systems are different. The ascites extract can form more high-molecular-weight polypeptides, especially polypeptide II (mol wt, approximately 120,000) than the wheat embryo extract. The latter extract has, on the other

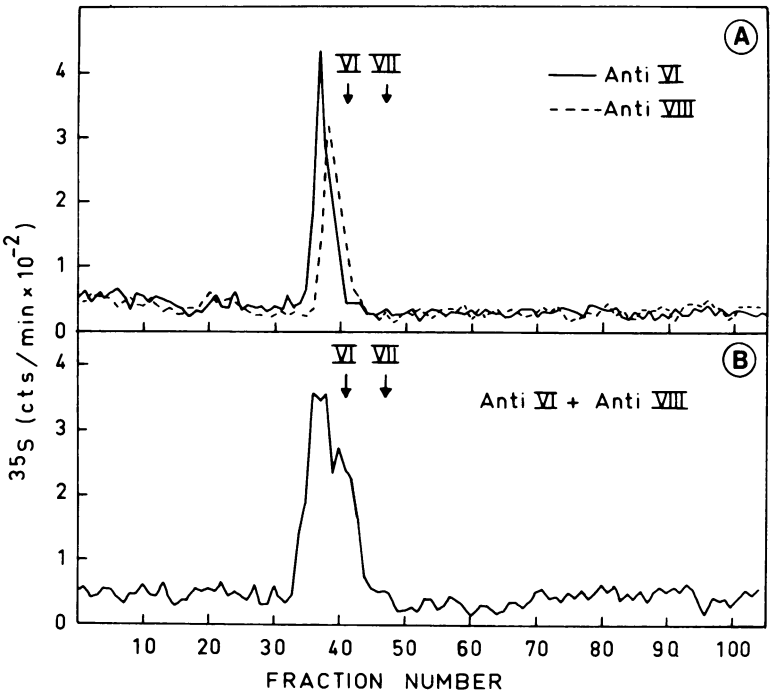

FIG. 4. *SDS-polyacrylamide gel electrophoresis of polypeptides synthesized in ascites cell extract and immunoprecipitated with antisera directed against Ad2 virion proteins VI and VIII. Samples were prepared and analyzed with internal markers as in the legend to Fig. 3. The precipitates obtained with anti-VI and anti-VIII antisera were analyzed in separate gels (A) or mixed before electrophoresis (B).*

hand, an ability to form more of the low-molecular-weight polypeptides.

Pulse-chase experiments of Ad2-infected cells have shown that during a short pulse with [^{35}S]methionine Ad2 virion polypeptides VI, VII, and VIII are not labeled after the pulse but become labeled during the chase (1, 7a, 10). Thus, polypeptides VI, VII, and VIII are probably not primary translation products, but are derived from precursor polypeptides. Anderson et al. (1) have shown that P-VII (mol wt, 20,000) has a tryptic fingerprint pattern closely related to that of Ad2 virion polypeptide VII (mol wt, 18,500). They concluded that virion polypeptide VII is derived from PVII by removal of about 15 amino acids and suggested that polypeptide 27K (mol wt, 27,000) is a precursor to polypeptide VI. A structural relationship between Ad3 polypeptide 27K (mol wt, 27,000) and Ad3 virion polypeptide VI (mol wt, 24,000) has also been established by fingerprinting of tryptic digest of these two [^{35}S]methionine-labeled polypeptides (Prage et al., unpublished data). Our results with immunoprecipitation agree with the proposed precursor-product relationship between 27K and virion polypeptide VI, PVII, and polypeptide VII, and suggest that polypeptide 26K is a precursor of virion polypeptide VIII (Fig. 3 and 4). The difference in size between precursor and product for polypeptide VIII is considerably larger (mol wt, 13,000) than for polypeptides VI (mol wt, 3,000) and VII mol wt, 1,500). Processing of precursor polypeptides involving cleavage of 10 to 20 amino acids has been implicated in the assembly process of the Ad2 virion (10) and the phage T4 (11). The processing of 26K to give polypeptide VIII might give rise to one of the small virion polypeptides (X to XII), or two polypeptides VIII may be generated from one precursor.

Separation of adenovirus mRNA in three size classes (13) and analysis of their translation products revealed that virion polypeptides II, III, and IIIa to IV were translated from the 26*S* RNA but not from the 22*S* class. The latter could possibly code for polypeptide V and several smaller polypeptides. The 15*S* RNA class is translated almost exclusively to polypeptide IX. It is not clear if any of the messengers are polycistronic, but the size of the 26*S* RNA is at least 30% larger than required to code for polypeptide II. Conclusive experiments will,

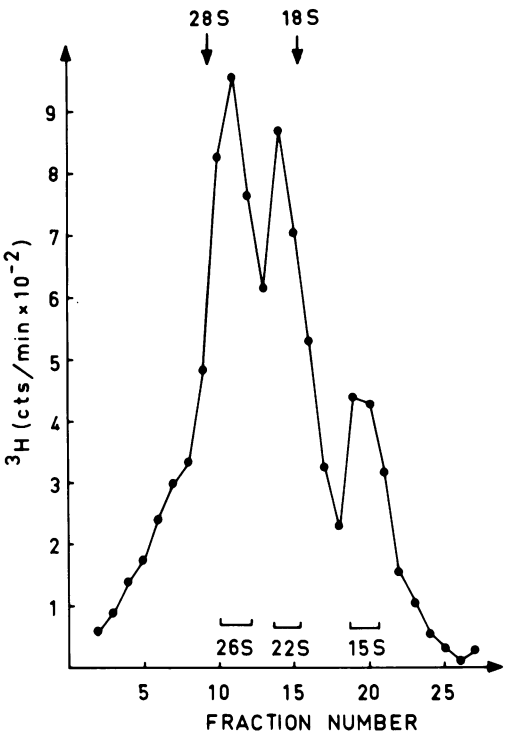

FIG. 5. *Separation of mRNA from adenovirus-infected cells. Oligo(dT)-selected mRNA extracted by the method of Holmes and Bonner (9) was separated on sucrose gradients containing formamide as described in Materials and Methods. Bars indicate the fractions pooled and precipitated with 2% potassium acetate and 2 volumes of ethanol at −2 C. The designation 26S, 22S, and 15S refers to the sedimentation rates relative to rRNA centrifuged in a parallel gradient.*

however, require isolation of distinct mRNA species.

The presence of polypeptide IX in the translation products from all size classes of RNA (Table 2) could be trivial and due to aggregation, but since the RNA was exposed to denaturing conditions before and during sucrose centrifugation, the RNA sequence coding for polypeptide IX may be part of RNA classes of different length as shown for the globin RNA sequences in heterogenous nuclear RNA (HnRNA) (23).

In conclusion, it has been shown that translation of adenovirus mRNA occurs faithfully in cell-free systems and that immunoprecipitation can be used to identify at least seven viral polypeptides, three of which appear to be precursors to the virion polypeptides. During the course of this investigation translation of adenovirus mRNA in cell-free systems was independently reported by Eron et al. (6a, 7) and Anderson et al. (2), who showed, by tryptic peptide mapping, that the in vitro translation products were similar to some virion polypeptides. Together with the present results these studies

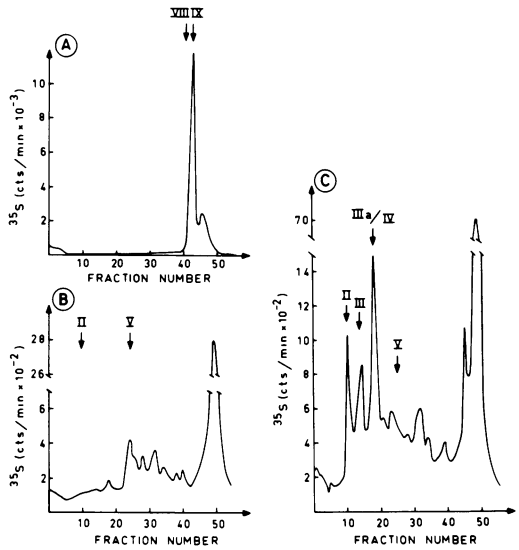

FIG. 6. *SDS-polyacrylamide disc gel electrophoresis of the translation products obtained with 15S, 22S and 26S RNA from adenovirus-infected cells. Cell-free translation mixtures were prepared and incubated as described in Table 1 using 15S, 22S, and 26S RNA in the ascites cell extract of a volume of 100 μliters and a [^{35}S]methionine concentration of 10 μCi/ml. The incubation mixtures were treated as described in Materials and Methods and dissolved in 100 μliters of sample buffer. (A) Fifty microliters of translation product from 15S RNA; (B) 20 μliters of translation product from 22S RNA; (C) 50 μliters of translation product from 26S RNA.*

TABLE 2. *Immunoprecipitation of polypeptide IX from translation products in wheat embryo extract*[a]

Translated mRNA class	Acid-precipitable translation product (counts/min × 10^{-6})	Immunoprecipitated polypeptide IX (counts/min × 10^{-4})	Immunoprecipitated polypeptide IX (% total acid-precipitable product)
26S	1.03	1.62	1.54
22S	1.32	3.01	2.28
15S	0.97	7.09	7.34
No RNA added	0.18	0.03	0.17

[a] The immunoprecipitated polypeptide was analyzed on polyacrylamide gels, and the figures in the table showing immunoprecipitates are the amount of radioactivity at the position of polypeptide IX in the gels.

suggest that most and possibly all the adenovirus polypeptides can be synthesized in vitro.

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LITERATURE CITED

1. Anderson, C. W., P. R. Baum, and R. F. Gesteland. 1973. Processing of adenovirus 2-induced proteins. J. Virol. **12**:241-252.
2. Anderson, C. W., J. B. Lewis, J. F. Atkins, and R. F. Gesteland. 1974. Cell-free synthesis of adenovirus 2 proteins programmed by fractionated messenger RNA. A comparison of polypeptide products and messenger RNA lengths. Proc. Nat. Acad. Sci. U.S.A. **71**:2756-2760.
3. Aviv, H., and P. Leder. 1972. Purification of biologically active globin messenger RNA by chromatography on oligo thymidylic acid cellulose. Proc. Nat. Acad. Sci. U.S.A. **69**:1408-1412.
4. Brawerman, G., J. Mendecki, and S. Y. Lee. 1972. A procedure for the isolation of mammalian messenger ribonucleic acid. Biochemistry **11**:637-641.
5. Caffier, H., H. J. Raskas, J. T. Parsons, and M. Green. 1971. *In vitro* and *in vivo* initiation of mammalian viral protein synthesis by methionyl-tRNA. Nature N. Biol. **229**:239-241.
6. Eggen, K. L., and A. J. Shatkin. 1972. In vitro translation of cardiovirus ribonucleic acid by mammalian cell-free extracts. J. Virol. **9**:636-645.

6a. Eron, L., R. Callahan, and H. Westphal. 1974. Cell free synthesis of adenovirus coat proteins. J. Biol. Chem. **249**:6331-6338.

7. Eron, L., H. Westphal, and R. Callahan. 1974. In vitro synthesis of adenovirus core proteins. J. Virol. **14**:375-383.

7a. Everitt, E., and L. Philipson. 1974. Structural proteins of adenoviruses. XI. Purification of three low molecular weight proteins of adenovirus type 2 and their synthesis during productive infection. Virology **62**:253-272.

8. Everitt, E., B. Sundquist, U. Pettersson, and L. Philipson. 1973. Structural proteins of adenoviruses. X. Isolation and topography of low molecular weight antigens from the virion of adenovirus type 2. Virology **52**:130-147.
9. Holmes, D. S., and J. Bonner. 1973. Preparation, molecular weight, base composition and secondary structure of giant nuclear ribonucleic acid. Biochemistry **12**:2330-2338.
10. Ishibashi, M., and J. V. Maizel, Jr. 1974. The polypeptides of adenovirus. V. Young virions, structural intermediate between top components and aged virions. Virology **57**:409-424.

10a. Jörnvall, H., U. Pettersson, and L. Philipson. 1974. Structural studies of adenovirus type 2 hexon protein. Eur. J. Biochem. **48**:179-192.

11. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophase T4. Nature (London) **227**:680-685.
12. Lindberg, U., and T. Persson. 1972. Isolation of mRNA from KB-cells by affinity chromatography on polyuridylic acid covalently linked to Sepharose. Eur. J. Biochem. **31**:246-254.
13. Lindberg, U., T. Persson, and L. Philipson. 1972. Isolation and characterization of adenovirus messenger ribonucleic acid in productive infection. J. Virol. **10**:909-919.
14. Mathews, M. B. 1973. Mammalian messenger RNA, p. 59-102. *In* P. N. Campbell and G. D. Greville (ed.), Essays in biochemistry, vol. 9. Academic Press Inc., New York.
15. Milstein, C., G. G. Brownlee, T. M. Harrison, and M. B. Mathews. 1972. A possible precursor of immunoglobulin light chains. Nature (London) **239**:117-120.
16. Öberg, B., and L. Philipson. 1969. Replication of poliovirus RNA studied by gel filtration and electrophoresis. Eur. J. Biochem. **11**:305-315.
17. Öberg, B., and A. J. Shatkin. 1972. Initiation of picornavirus protein synthesis in ascites cell extracts. Proc. Nat. Acad. Sci. U.S.A. **69**:3589-3593.
18. Perlman, S., M. Hirsch, and S. Penman. 1972. Utilization of messenger in adenovirus-2-infected cells at normal and elevated temperatures. Nature N. Biol. **238**:143-144.
19. Pettersson, U. 1971. Structural proteins of adenoviruses. VI. On the antigenic determinants of the hexon. Virology **43**:123-136.
20. Philipson L., U. Lindberg, T. Persson, and B. Vennström. 1973. Transcription and processing of adenovirus RNA in productive infection, p. 167-183. *In* G. Raspé (ed.), Advances in the biosciences, vol. 11. Pergamon Press, Oxford, England.
21. Philipson, L., and U. Pettersson. 1973. Structure and function of virion proteins of adenoviruses. Prog. Exp. Tumor Res. **18**:1-55.
22. Roberts, B. E., and B. M. Paterson. 1973. Efficient translation of tobacco mosaic virus RNA and rabbit globin 9S RNA in a cell-free system from commercial wheat germ. Proc. Nat. Acad. Sci. U.S.A. **70**:2330-2334.
23. Ruiz-Carrillo, A., M. Beato, G. Schutz, P. Fiegelson, and V. G. Allfrey. 1973. Cell-free translation of the globin message within polydisperse high-molecular-weight ribonucleic acid of avian erythrocytes. Proc. Nat. Acad. Sci. U.S.A. **70**:3641-3645.
24. Tibbetts, C., K. Johansson, and L. Philipson. 1973. Hydroxylapatite chromatography and formamide denaturation of adenovirus DNA. J. Virol. **12**:218-225.
25. Vaquero, C., L. Reibel, J. Delaunay, and G. Schapira. 1973. Translation of globin mRNA among eukaryotes. Biochem. Biophys. Res. Commun. **54**:1171-1177.
26. White, D. O., M. D. Scharff, and J. V. Maizel, Jr. 1969. The polypeptides of adenoviruses. III. Synthesis in infected cells. Virology **38**:395-406.
27. Wilhelm, J. M., and H. S. Ginsberg. 1972. Synthesis in vitro of type 5 adenovirus capsid proteins. J. Virol. **9**:973-980.