JOURNAL OF VIROLOGY, Apr. 1982, p. 30–41
0022-538X/82/040030-12$02.00/0

Vol. 42, No. 1

Identification and Purification of a Protein Encoded by the Human Adenovirus Type 2 Transforming Region

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Received 15 September 1981/Accepted 18 November 1981

The human adenovirus type 2 (Ad2) transforming genes are located in early regions E1a (map position 1.3 to 4.5) and E1b (map position 4.6 to 11.2). We have identified and purified to near homogeneity a major 20,000-molecular-weight (20K) protein and have shown that it is coded by E1b. Using an Ad2-transformed cell antiserum which contained antibody to E1b-coded proteins, we immunoprecipitated 53K and 19K proteins from the nucleoplasm and 53K, 19K, and 20K proteins from the cytoplasmic S-100 fraction of Ad2 productively infected and Ad2-transformed cells. The 19K protein was present in both the nucleoplasm and the cytoplasm, whereas the 20K protein was found only in the cytoplasm. The 53K and 19K proteins are known Ad2 E1b-coded proteins. The 20K protein was purified to near homogeneity in 20 to 50% yields by sequential DEAE-Sephacel chromatography and reverse-phase high-performance liquid chromatography. Purified 20K protein shares most of its methionine-labeled tryptic peptides with E1b-53K, as shown by reverse-phase high-performance liquid chromatography, and therefore is closely related to the 53K protein. The 19K protein does not appear to share tryptic peptides with either 20K or 53K protein. To provide more direct evidence that 20K protein is virus-coded, we translated E1b-specific mRNA in vitro. Both immunoprecipitation analysis and high-performance liquid chromatography purification of the translated product identified a 20K protein that has the same tryptic peptides as the 20K protein isolated from infected and from transformed cells. These findings suggest that the Ad2 20K protein is a primary translation product of an Ad2 E1b mRNA.

The transforming region of human adenovirus type 2 (Ad2) is located at the left end of the viral genome in early region 1 (E1) at map position 1.3 to 11.2 (the linear duplex Ad2 genome of M_r 23 $\times$ 10^6 [13] is divided into 100 map units). E1 comprises two families of overlapping mRNAs and proteins, encoded in E1a (map position 1.3 to 4.5) and E1b (map position 4.6 to 11.2) (4, 7–9, 16, 23, 38). As shown by cell-free translation, E1a of Ad2 (and closely related Ad5) encodes a family of four closely related proteins of molecular weights 35,000 (35K) to 58K and a 28K protein; E1b encodes proteins of 53K (referred to also as 52K to 58K [65K for Ad5] by different laboratories) and 19K (6, 27; T. Matsuo, W. Wold, S. Hashimoto, A. Rankin, J. Symington and M. Green, Virology, in press) (referred to also as 15K [9, 17, 18, 26, 37]). Very recently, small amounts of an 18K protein were detected in the translation products of E1b (9). Virion protein IX is coded within map position 9.5 to 11.2 (31).

We have shown that antisera to Ad2-transformed rat cells immunoprecipitate major 53K (6, 12, 16, 39) and 19K (6) proteins from Ad2-infected cell extracts, as well as several 17K to 23K polypeptides (6, 12, 16, 39) which varied in amount depending upon the particular antiserum. In earlier publications, the molecular weights of these polypeptides were estimated with Ad2 virion proteins used as standards, as has been done by most laboratories working on Ad2 early proteins. It has recently become apparent that the accepted molecular weights of the small virion proteins are larger than originally published by another laboratory; for example, protein IX, originally estimated as 12K, is now assigned a molecular weight of 15K (32). We have therefore obtained new estimates of the molecular weights of the Ad2 early proteins, using a variety of commercially available molecular weight standards. We estimate that the previously described (12, 16, 39) major E1b-15K is about 20K. The other "reproducibly immunoprecipitated" members of the 17K to 23K family that are related to E1b-53K are 21K, 22K, and 23K (see Fig. 1). The E1b-19K is different from our initially described E1b-15K (12, 16, 39) and is unrelated to 53K (6). Our E1b-19K is probably the same as the protein described as 19K by one laboratory (27), but described as 15K by two other laboratories (9, 17) on the basis of old

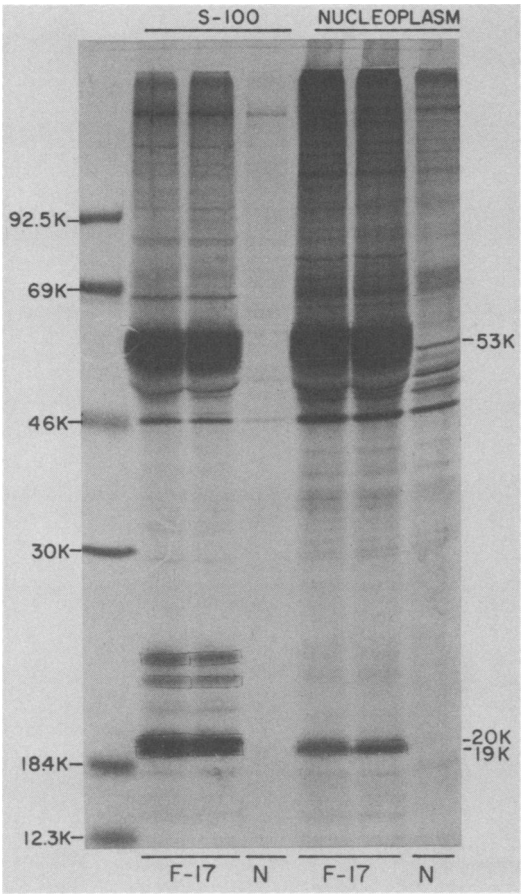


FIG. 1. Polyacrylamide gel electrophoresis of [^{35}S]methionine-labeled proteins immunoprecipitated from Ad2 early infected cells by antiserum to F17 cells (an Ad2-transformed cell line). M_r markers are [^{14}C]methyl-labeled phosphorylase b (92.5K), bovine serum albumin (69K), ovalbumin (46K), carbonic anhydrase (30K), lactoglobulin A (18.4K), and cytochrome *c* (12.3K). F17, Rat antiserum to F17 cells; N, normal rat serum.

values for Ad2 virion standards. The 53K and 19K proteins do not share methionine-labeled tryptic peptides (6; Matsuo et al., in press). The 53K and 19K polypeptides most likely are coded by the E1b 22S and 13S mRNA, respectively (9, 17). Other laboratories have reported proteins of similar sizes in immunoprecipitates of Ad2- or Ad5-infected or -transformed cells (21, 25, 33, 35).

In this study, using antisera to the Ad2-transformed cell line F17, we identified a major protein of apparent molecular weight 20K that is present exclusively in the cytoplasm of both Ad2-infected cells and transformed cells; this protein is probably a member of the 17K to 23K family. We have purified this 20K protein to near homogeneity by high-performance liquid chromatography (HPLC) and have shown that it shares most of its tryptic peptides with E1b-53K, but not with E1b-19K. By translation in vitro, we obtained evidence that the 20K protein is probably a primary translation product of an E1b-coded mRNA.

MATERIALS AND METHODS

Preparation of [^{35}S]methionine-labeled cytoplasmic S-100 and nucleoplasmic fractions. Ad2 (strain 38-2) was grown in suspension cultures of KB cells in Eagle minimal essential medium (MEM) containing 5% horse serum (15). For the preparation of Ad2 early S-100 fraction and nucleoplasm, 15 to 20 liters of KB cells (4 × 10^5 cells per ml) were centrifuged and infected with 500 PFU per cell of Ad2 in 0.05 the initial volume of MEM. After 1 h at 37°C, the cells were diluted to the initial volume with MEM plus 5% horse serum and 25 μg of cycloheximide per ml. At 5 h postinfection, 1-β-D-arabinofuranosylcytosine (araC) was added to 20 μg/ml and the cells were centrifuged and washed twice with warm methionine-free MEM containing 5% horse serum and 20 μg of araC per ml. The cells were resuspended at 10^6 cells per ml in methionine-free MEM containing 5% horse serum and 20 μg of araC per ml, and were labeled with [^{35}S]methionine (5 mCi, 1,044 Ci/mmol) from 6 to 18 h postinfection. The following steps were performed at 0 to 4°C. Cells were centrifuged and washed twice with phosphate-buffered saline containing 1 mM phenylmethylsulfonyl fluoride. The cell pellet was incubated for 20 min in 160 ml of buffer A (10 mM Tris-hydrochloride [pH 8.5]–250 mM sucrose–3.7 mM $CaCl_2$–12 mM $MgCl_2$–1 mM phenylmethylsulfonyl fluoride–1% Nonidet P-40). Nuclei were centrifuged at 800 × *g* for 10 min at 4°C and washed with 80 ml of buffer A. The cytoplasmic fraction (combined supernatants) was centrifuged at 100,000 × *g* in a Beckman Ti50.2 rotor after the addition of KCl to 0.4 M and sodium deoxycholate to 1%. The supernatant is the S-100 fraction. Nuclei were lysed in 50 ml of 10 mM Tris-hydrochloride (pH 8.5)–5 mM $MgCl_2$–0.5 mM dithiothreitol–0.1 mM EDTA–10% glycerol by addition of 4 M $(NH_4)_2SO_4$ to 0.3 M rapidly with vigorous stirring. The viscous mixture was sonicated for 2 min, and Nonidet P-40 and sodium deoxycholate were added to 0.5% and 1.0%, respectively. The nuclear lysate was centrifuged at 100,000 × *g* in the Beckmann Ti50.2 rotor. The supernatant is the nucleoplasm.

The Ad2-transformed rat cell line 8617 (11) was grown in suspension in MEM containing 2.5 to 5% dialyzed horse or calf serum and 0.5 to 2% fetal calf serum. One liter of cells (10^6 cells per ml) was labeled with [^{35}S]methionine (5 mCi, 1,044 Ci/mmol) for 13 h. Cells were harvested, and the S-100 fraction and nucleoplasm were prepared as described above.

Immunoprecipitation analysis. [^{35}S]Methionine-labeled S-100 fraction and nucleoplasm were immunoprecipitated with antiserum to F17 cells, an Ad2-transformed cell line that contains only E1 genetic information. The immunoprecipitate was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and autoradiography (14).

Two-dimensional peptide maps. Excised bands of [^{35}S]methionine-labeled polypeptides, identified on

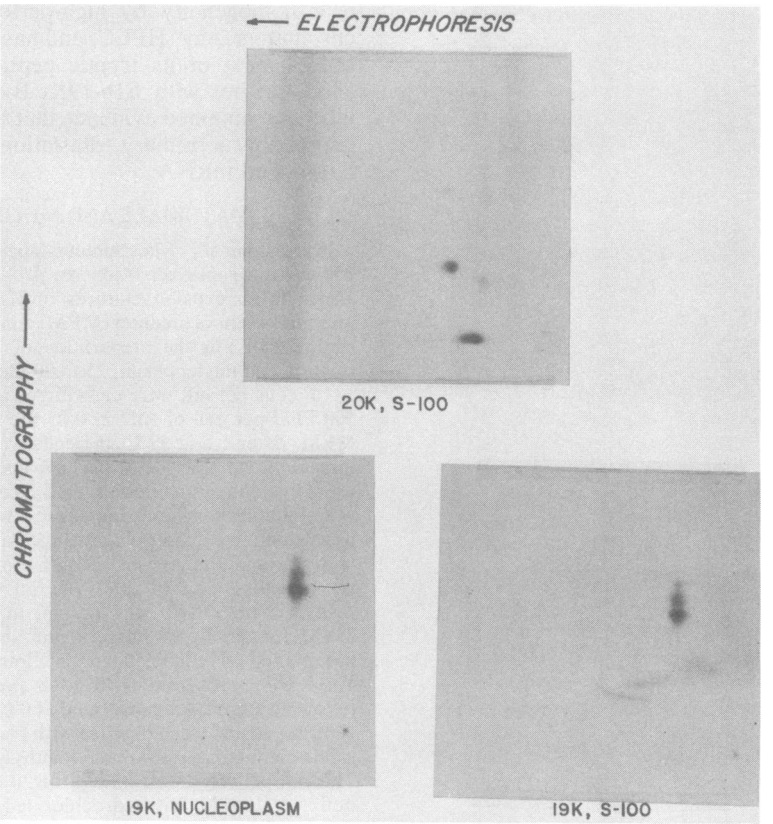


FIG. 2. Tryptic peptide maps of [^{35}S]methionine-labeled 19K and 20K proteins immunoprecipitated from the Ad2-infected cell S-100 fraction and nucleoplasm shown in Fig. 1.

dried gels by autoradiography, were digested with trypsin and analyzed by two-dimensional electrophoresis (pH 1.7)–thin-layer chromatography (16).

HPLC of tryptic fragments of [^{35}S]methionine-labeled polypeptides. Tryptic peptides were analyzed by reverse-phase HPLC on a C_{18} column (Altex, ultrasphere-ODS, 4.6 by 250 mm), essentially as described by Rubinstein et al. (34), but with a 0 to 40% instead of a 0 to 20% linear 1-propanol gradient.

Purification of Ad2 20K protein by DEAE-Sephacel chromatography and reverse-phase HPLC. All steps were performed at 0 to 4°C. We describe below a typical purification. The S-100 fraction prepared as described above from 7×10^9 [^{35}S]methionine-labeled Ad2 early infected cells was loaded on a 2.5 by 22 cm DEAE-Sephacel column equilibrated with buffer B (10 mM Tris [pH 8.5]–15 mM NaCl–1 mM dithiothreitol–1 mM phenylmethylsulfonyl fluoride–0.5% Nonidet P-40–10% glycerol). After being washed with 200 ml of buffer B, the column was eluted with 600 ml of a 15 to 500 mM NaCl gradient in buffer B. Fractions (6 ml) were collected, and 25-μl and 500-μl portions were used to measure radioactivity and for immunoprecipitation with F17 antiserum, respectively. Fractions containing 20K protein were pooled and concentrated by using a membrane filter (YM5, Amicon Corp). One-fourth of the concentrate was loaded on a C_8 column (Altex, ultrasphere-octyl 4.6 by 250 mm) and eluted with a linear 120-min gradient of 0 to 60% 1-propanol in 0.5 M pyridine formate, pH 4.1, at a flow rate of 0.33 ml/min, essentially as described by Kimura et al. (22). Fractions (1.0 ml) were collected, and portions were analyzed for radioactivity and for immunoprecipitation with F17 antiserum.

In vitro translation of Ad2 E1b-specific mRNA. Polyadenylate-terminated polysomal RNA from Ad2-infected cells cultured in the presence of cycloheximide was prepared as described (20). E1b mRNA was selected by hybridization to cloned Ad2 *Hin*dIII-C (map position 8.0 to 17.0). The mRNA was translated in vitro by use of a micrococcal nuclease-treated reticulocyte lysate (30) as described elsewhere (Matsuo et al., in press).

RESULTS

Immunoprecipitation of Ad2 E1b proteins from the cytoplasmic S-100 fraction and from the nucleoplasm of Ad2 early infected cells. Ad2 early infected cells were treated with cycloheximide from 1 to 5 h postinfection (29) to enhance the synthesis of early proteins (19) and were labeled

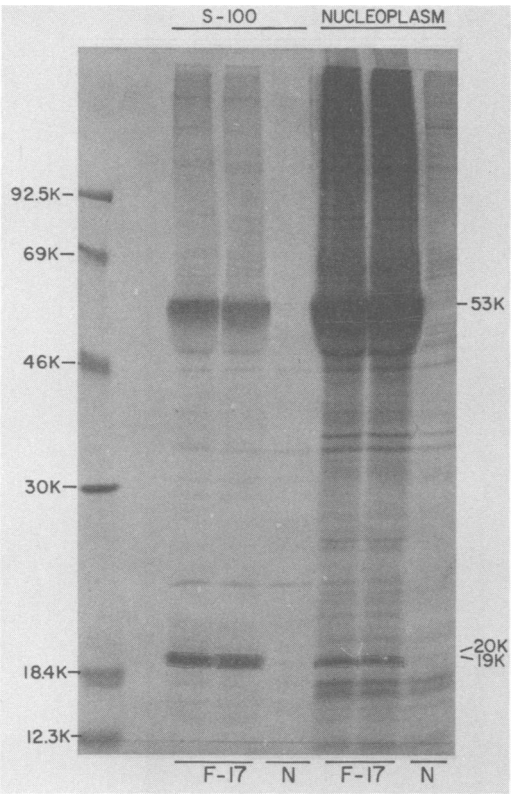


FIG. 3. Polyacrylamide gel electrophoresis of [^{35}S]methionine-labeled proteins from the Ad2-transformed cell line 8617 immunoprecipitated by antiserum to F17 cells. M_r markers are given in the legend to Fig. 1.

with [^{35}S]methionine from 6 to 18 h postinfection in the presence of araC (to block the late stages of infection). The S-100 fraction and nucleoplasm were isolated and immunoprecipitated with a rat antiserum to the Ad2-transformed rat cell line F17 (the antiserum contained high levels of antibody to E1b proteins). The immunoprecipitate was analyzed by SDS-PAGE (Fig. 1). Major 53K and 19K proteins were immunoprecipitated from the nucleoplasm, and major 53K and 19K to 20K doublet (and less pronounced 22K and 23K) proteins were immunoprecipitated from the S-100 fraction; the 22K and 23K polypeptides shared peptides with the 20K protein and could represent post-translation products of 20K protein. None of these proteins was immunoprecipitated from infected cells by normal rat serum (Fig. 1) or from uninfected KB cells by F17 antiserum (not shown).

The 19K protein in the nucleoplasm and the upper and lower portions of the 19K to 20K protein in the cytoplasm were peptide mapped to establish their identity. As shown in Fig. 2, the 19K protein in the nucleoplasm and the lower portion of the 19K to 20K protein in the S-100 are the same species of polypeptide. The 20K upper band is a different protein. About one-half of the 19K protein was found in the nucleoplasm, whereas virtually all of the 20K protein was present in the S-100 fraction. The ratio of total methionine-labeled 53K to 19K to 20K is 4:2:1, as estimated from the amount of radioactive tryptic peptides eluted from the gel band. The 19K protein, which possesses two characteristic methionine-containing tryptic peptide spots, has been translated in vitro with E1b-specific mRNA as template (Matsuo et al., in press) and is probably coded by the E1b 13S mRNA (9, 17; Matsuo et al., in press). The 53K protein, which we have described in detail previously (12, 16, 39), is coded by the E1b 22S mRNA (9, 17). As we show below, the major 20K protein is a new virus-coded protein that is closely related to 53K protein.

Immunoprecipitation of E1b proteins from 8617 cells. To determine whether the cytoplasmic 20K protein that is synthesized early during productive infection is synthesized also by a stable line of Ad2-transformed cells, we analyzed [^{35}S]methionine-labeled 8617 cells by immunoprecipitation with F17 antiserum (Fig. 3). A similar distribution of 53K, 19K, and 20K proteins was found in 8617 cells (Fig. 3) as was found in Ad2 early infected cells (Fig. 1). The 19K protein was present mainly in the nucleoplasm and the 20K protein was found exclusively in the cytoplasm, as documented by peptide map analysis (Fig. 4). Since cytoplasmic 20K protein excised from a gel is often contaminated by 19K protein (see Fig. 4), the peptide map of 20K protein purified by HPLC is shown as a reference in Fig. 4.

Purification of the 20K protein. The 20K protein was purified to near homogeneity, starting with the S-100 fraction of early infected cells, by use of two steps, DEAE-Sephacel chromatography and reverse-phase HPLC. The [^{35}S]methionine-labeled S-100 fraction from 7×10^9 cells was loaded on a DEAE-Sephacel column, washed with 15 mM NaCl, and eluted with a linear 15 to 500 mM NaCl gradient. The distribution of radioactivity and of F17-immunoprecipitable counts per minute is shown in Fig. 5. There are two broad peaks of labeled proteins, one at the beginning of the flow-through (fractions 10 to 40) and the other in the middle of the gradient (fractions 140 to 180). Each immunoprecipitate was analyzed by SDS-PAGE (Fig. 5). The 20K protein (identified by peptide map) eluted between the two major radioactive peaks. Fractions 92 to 135, which contained most of the 20K protein, were pooled and concentrated, and

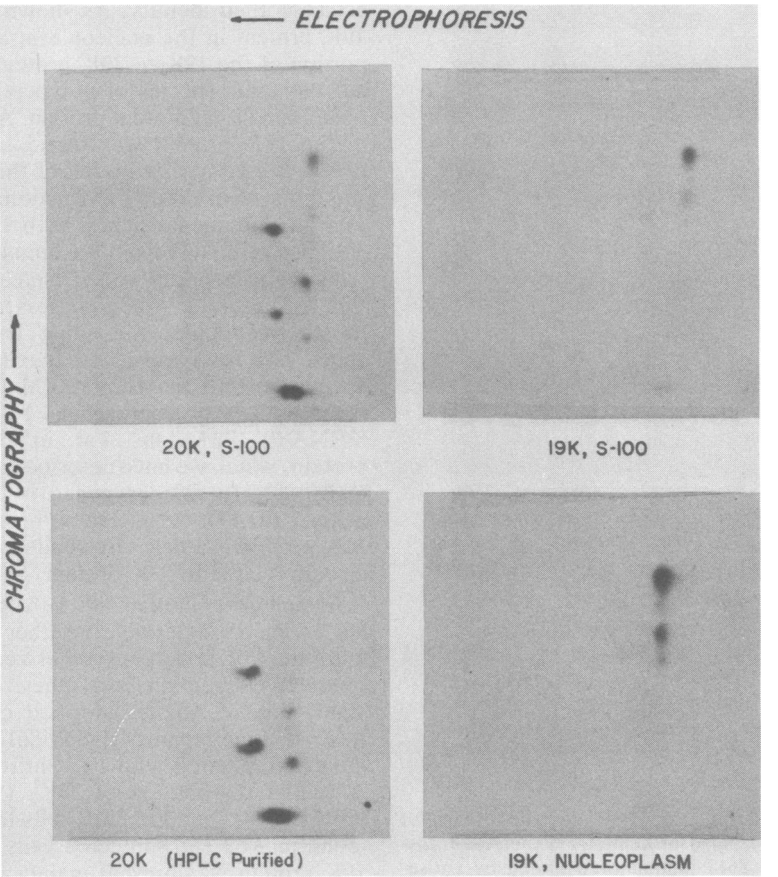


FIG. 4. Tryptic peptide maps of [^{35}S]methionine-labeled 19K and 20K proteins immunoprecipitated from the S-100 fraction and nucleoplasm of the 8617 cells shown in Fig. 3. The map of the 20K protein purified by HPLC from an Ad2-infected cell S-100 fraction is shown as reference.

one-fourth of the material was purified by reverse-phase HPLC (22). A peak of protein (determined by an automated fluorescence detection system [5] and co-eluting radioactivity) was found in HPLC fractions 27 to 31 (Fig. 6). About 95% of the radioactive protein in these fractions had an apparent molecular weight of about 20K by SDS-PAGE (Fig. 7A). The 20K protein was immunoprecipitated from the same HPLC fractions by F17 antiserum (Fig. 7B). The peptide maps of the upper (Fig. 7C) and lower (Fig. 7D) portions of the protein band in fraction 28 from Fig. 7A revealed the presence of only the 20K protein. The fractions containing 20K protein were pooled, concentrated, subjected to preparative SDS-PAGE, and stained with Coomassie blue. Each of the four lanes contained a single band of ~4 μg of 20K protein (Fig. 8). The 20K band is somewhat smeared as a result of the age of the methionine-labeled preparation (about 5 months).

Close relation of 20K and E1b-53K proteins as shown by HPLC peptide map analysis. A peptide map of [^{35}S]methionine-labeled 53K protein, eluted from a gel band such as that shown in Fig. 1, was established by reverse-phase HPLC (34) and compared with that of HPLC-purified 20K protein (Fig. 9). The 53K profile reveals about 11 [^{35}S]methionine-labeled tryptic peptides. The tryptic peptides of the 20K protein appear to correspond to peptides of 53K protein (Fig. 9), suggesting that 20K protein is closely related to E1b-53K.

An Ad2 E1b mRNA encodes 20K protein. To learn whether 20K protein is coded by an Ad2 E1b mRNA or is a post-translational product of 53K protein, we selected E1b-specific mRNA by hybridization to the cloned Ad2 DNA *Hin*dIII-C (map position 8.0 to 17.0) fragment and translated it with a reticulocyte extract. The [^{35}S]methionine-labeled translation product was analyzed both by immunoprecipitation with F17 antise-

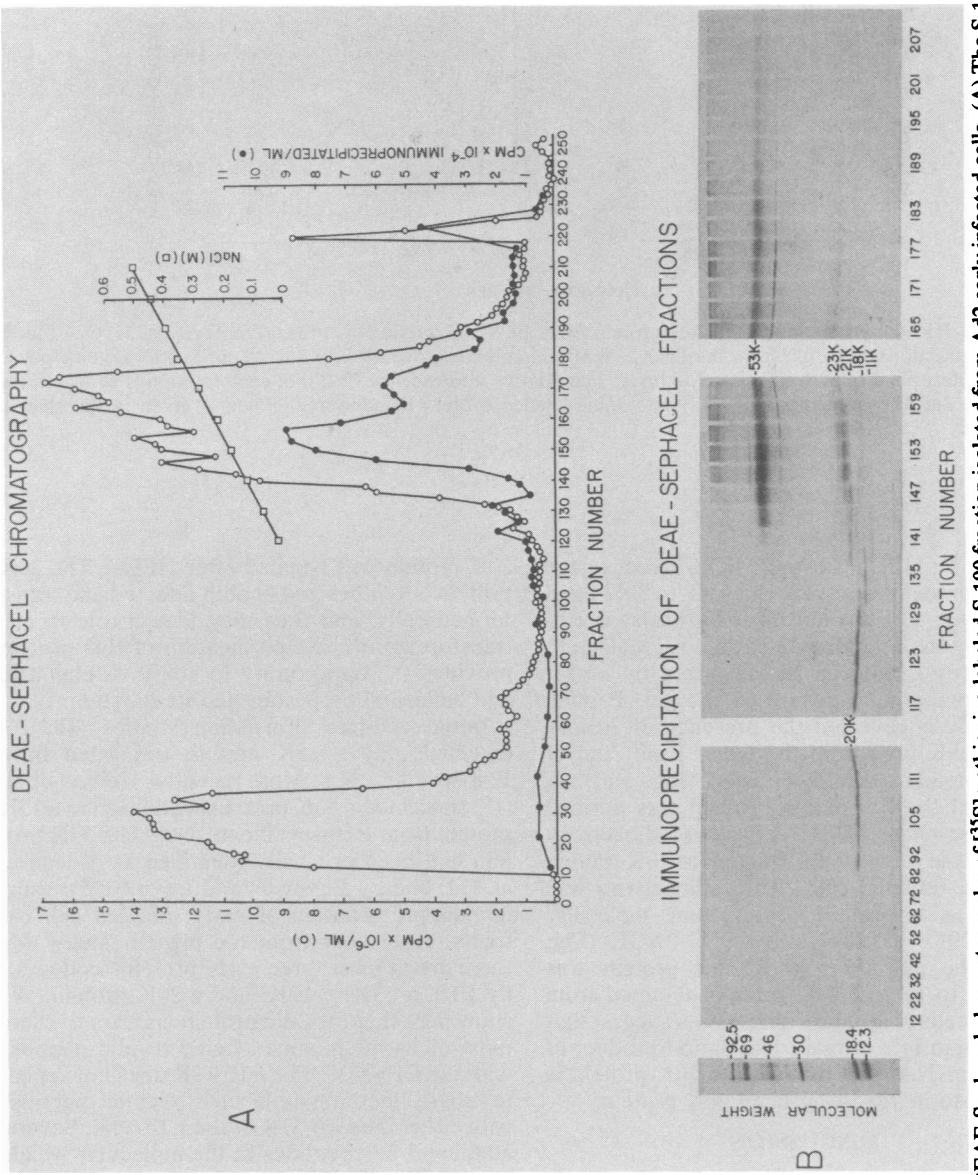


FIG. 5. DEAE-Sephacel chromatography of [^{35}S]methionine-labeled S-100 fraction isolated from Ad2 early infected cells. (A) The S-100 fraction from 7×10^9 [^{35}S]methionine-labeled Ad2 early infected cells was chromatographed on a DEAE-Sephacel column as described in Materials and Methods. Aliquots of every other fraction were analyzed for total radioactivity and for F17 antiserum-immunoprecipitable radioactivity. (B) The immunoprecipitates from A were analyzed by SDS-PAGE and autoradiographed. M_r markers are given in the legend to Fig. 1.

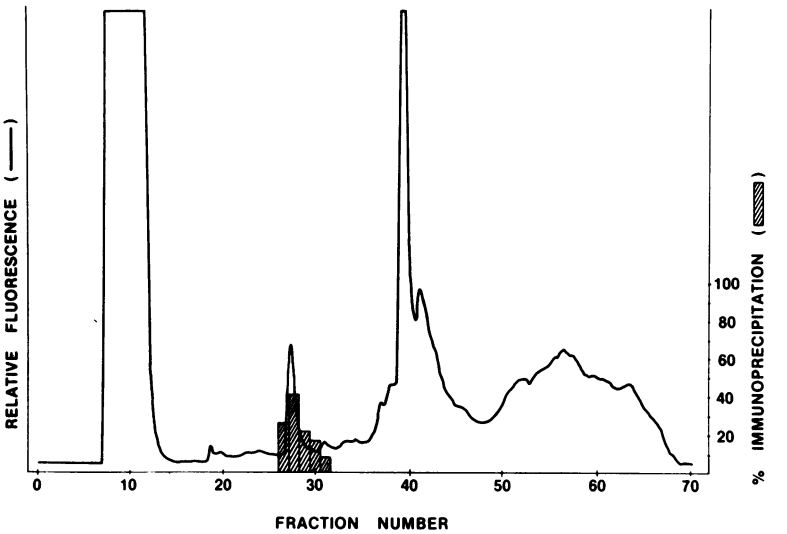


FIG. 6. HPLC purification of the 20K protein from the S-100 fraction of Ad2 early infected cells. One-fourth of pooled fractions 92 to 135 from the DEAE-Sephacel column shown in Fig. 5 were purified by reverse-phase HPLC as described in Materials and Methods. The relative fluorescence (5, 22) of each fraction is shown in the solid line, and the percentage of [^{35}S]methionine-labeled protein immunoprecipitated is given in the shaded rectangles.

rum and by HPLC analysis. SDS-PAGE of the immunoprecipitate revealed a 19K to 20K band (Fig. 10A); 53K protein is not translated efficiently by the reticulocyte extract (9; Matsuo et al., in press), but can be visualized by longer autoradiographic exposure of the gel. Peptide map analysis revealed the presence of 20K as well as 19K protein in the upper band, and of 19K protein in the lower band (Fig. 10B). A portion of the translation product was purified by reverse-phase HPLC. A peak of radioactivity eluted in the same HPLC fraction as 20K protein from Ad2-infected cells. The radioactivity was present in a protein of apparent molecular weight 20K, as shown by SDS-PAGE (Fig. 11A). The peptide map of this protein was identical to that of 20K protein obtained from infected cells (Fig. 11B). These data suggest that 20K protein is a primary translation product of an E1b mRNA, and that in vivo 20K protein is identical to in vitro-translated 20K protein.

DISCUSSION

We have shown that a 20K protein is coded by the E1b region of Ad2, and we have purified this protein to near homogeneity by using DEAE-Sephacel chromatography and HPLC. The yields of 20K protein are about 20 to 50%; e.g., starting with 7×10^9 early infected cells (1.0 to 1.5 g of protein) containing approximately 100 to 150 μg of the 20K protein, 30 to 50 μg of purified 20K protein was isolated after HPLC. The 20K protein is synthesized in both infected and transformed cells, and thus may play a role in cell transformation. The purification of this protein provides the opportunity to study its chemical and biological properties and its function.

Initial cell-free translation studies (18, 26) detected only a 15K protein translated from E1b-specific RNA. More recently, Halbert et al. (17) translated a 53K protein in addition to a 15K protein from E1b-specific mRNA. The 53K protein had been originally identified by Gilead et al. (12) and by Levinson and Levine (25), using immunoprecipitation of early infected cell extracts. It is clear from the present study that there are at least three early proteins coded for by E1b, a 53K, a 19K, and a 20K protein. We show here that the 20K protein appears to share most of its methionine-labeled tryptic peptides with the E1b-53K. The E1b-19K does not appear to share methionine-labeled tryptic peptides with either the E1b-53K or the E1b-20K. Several additional polypeptides in the molecular weight range 17K to 23K that share tryptic methionine peptides with 20K and 53K proteins have also been immunoprecipitated from infected cell extracts (6); it is not known whether these polypeptides are also independent translation products or whether they represent post-translational processing products of 20K or 53K protein. Recently, Esche et al. (9) have translated 15K

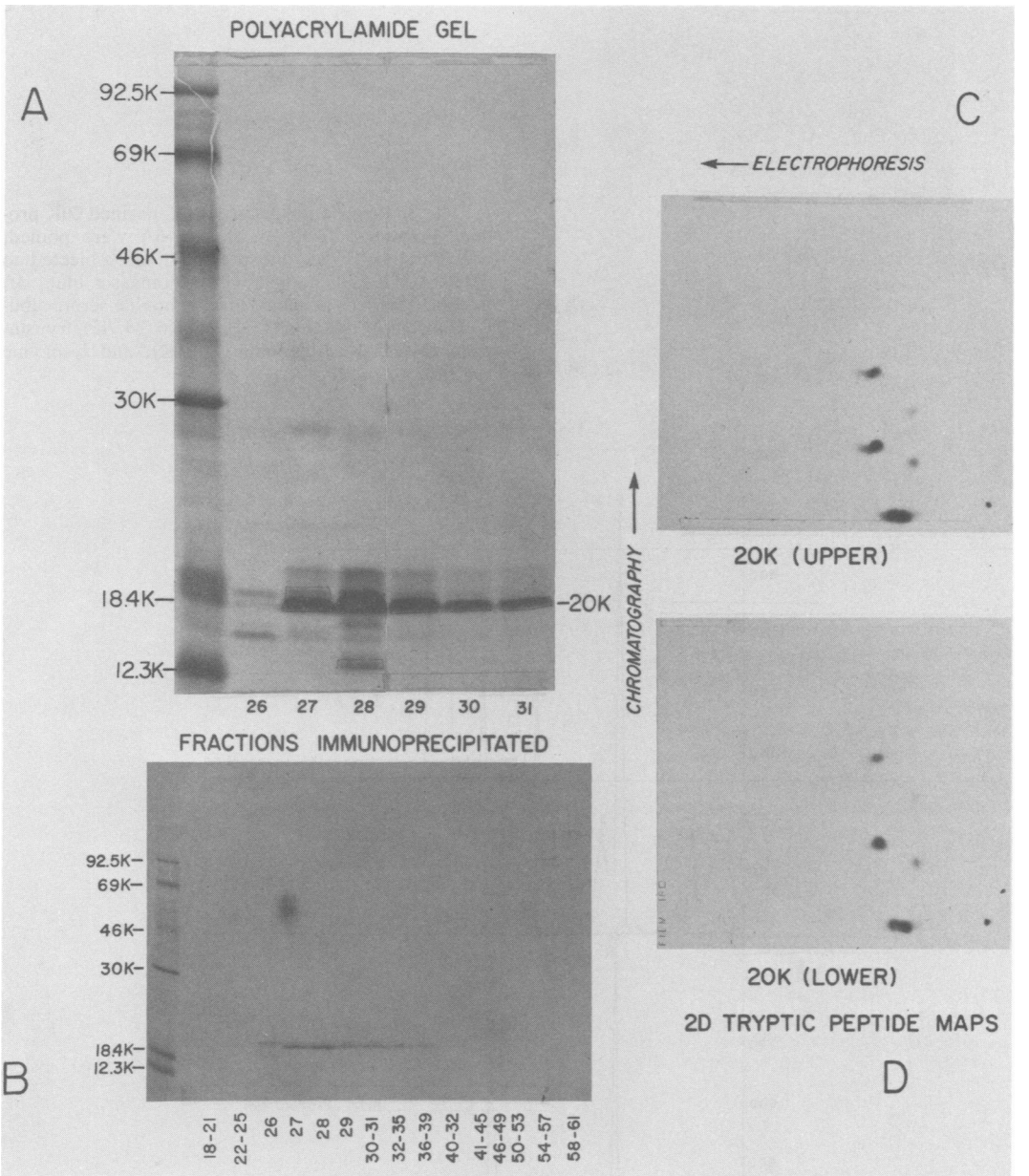


FIG. 7. Analysis of HPLC fractions by polyacrylamide gel electrophoresis, immunoprecipitation, and tryptic map determination. (A) Polyacrylamide gel electrophoresis of HPLC fractions containing 20K protein. An aliquot of each fraction in A was analyzed by SDS-PAGE. Fractions 26 to 31 contained a 20K protein. M_r markers are given in the legend to Fig. 1. (B) HPLC fractions indicated in the abscissa were immunoprecipitated by F17 antiserum and analyzed by SDS-PAGE (the dark area between 46K and 69K is a film artifact). (C and D) Tryptic peptide maps of upper and lower bands, respectively, of fraction 28.

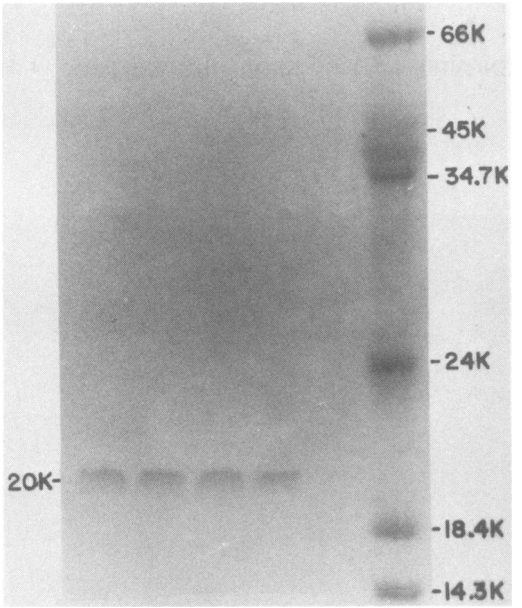
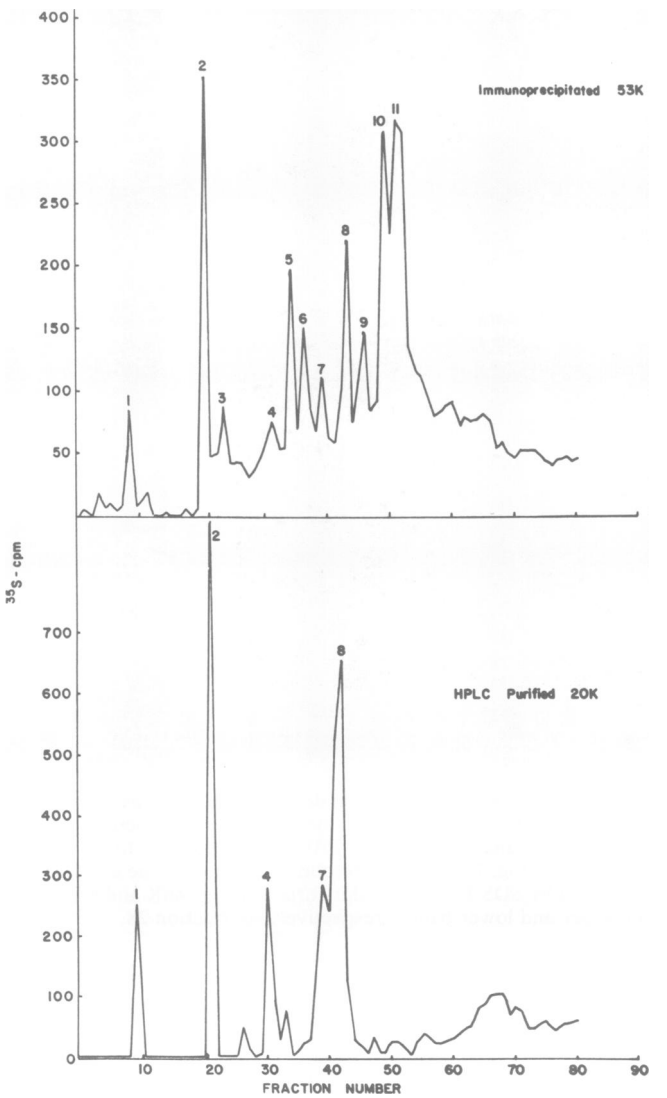


FIG. 8. Preparative gel of HPLC-purified 20K protein. Fractions 27 to 31 of Fig. 6A were pooled, concentrated by acetone precipitation, subjected to SDS-PAGE, and stained with Coomassie blue. M_r markers (Sigma Chemical Co.) are bovine serum albumin (66K), egg albumin (45K), pepsin (34.7K), trypsinogen (24K), β-lactoglobulin (18.4K), and lysozyme (14.3K).



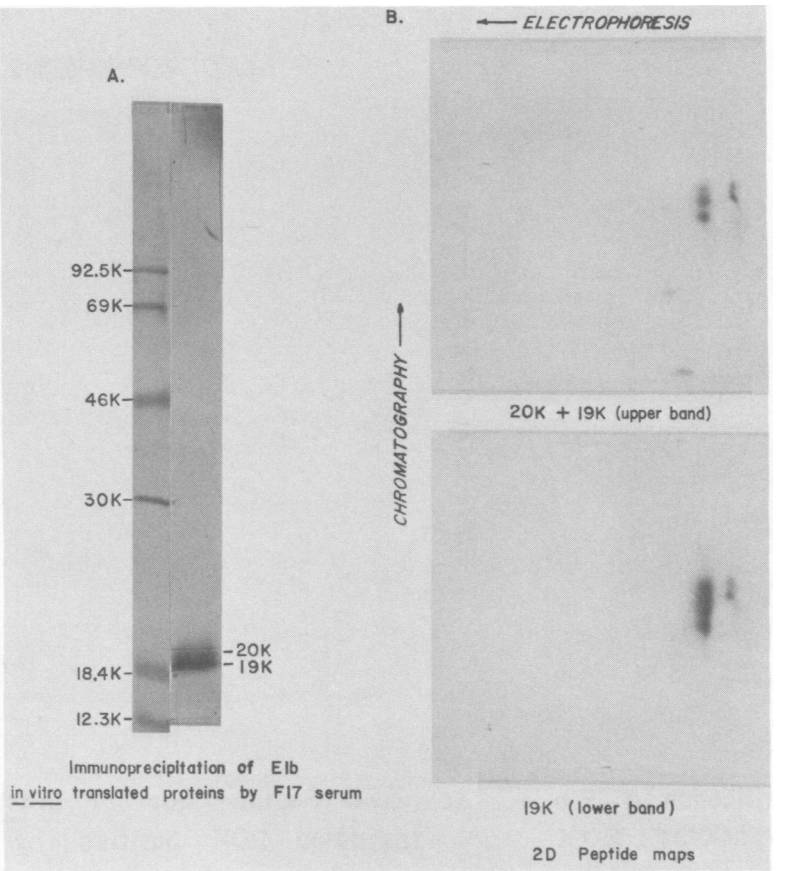


FIG. 10. Translation in vitro of Ad2 E1b mRNA. (A) Ad2 E1b-selected mRNA was translated with a reticulocyte extract by using [^{35}S]methionine as labeled amino acid and was analyzed by SDS-PAGE. (B) The upper and lower portions of the 19K–20K band in A were digested with trypsin and peptide mapped.

and 18K polypeptides from E1b-specific mRNA; these polypeptides were not peptide mapped, and it is not known whether the 15K and 18K polypeptides correspond to our 19K and 20K polypeptides.

The genome locations of the coding sequences for E1b-20K and E1b-19K proteins are not known, and it is difficult to reconcile the peptide maps of these proteins with the structures of the known E1b mRNAs (4, 8, 23) and with the fact that translation of eucaryotic mRNAs usually initiates at the 5′ proximal AUG (24). E1b is believed to contain only two promoters (10, 36), one at map position 4.6, which initiates two mRNAs of 22S and 13S, and the other at map position 10.2, which initiates an mRNA of 9S (9, 38). The 22S mRNA encodes a 53K protein, the 13S encodes a 15K protein, and the 9S mRNA encodes virion protein IX (1, 9, 17). The E1b 22S and E1b 13S mRNAs are thought to initiate at nucleotide 1,699 on the Ad2 DNA sequence (3, 20a). The first translation-initiation codon (ATG) is present 13 nucleotides downstream at nucleotide 1,711, and this should be the initiation codon for the 53K and 19K or 20K polypeptides. This has been shown to be the case with the E1b-15K protein (2), which presumably corresponds to our 19K polypeptide. If 53K, 20K, and 19K proteins all initiate translation at nucleotide 1,711, they should share two large methio-

FIG. 9. Reverse-phase HPLC of tryptic fragments from [^{35}S]methionine-labeled Ad2 E1b-53K and HPLC-purified 20K protein. E1b-53K was obtained by immunoprecipitation of an Ad2 early S-100 fraction and SDS-PAGE. The band of 53K, revealed by autoradiography, was trypsinized directly and analyzed by HPLC, as described in Materials and Methods. The 20K protein was purified from an Ad2 early S-100 fraction by reverse-phase HPLC, trypsinized, and analyzed as described above.

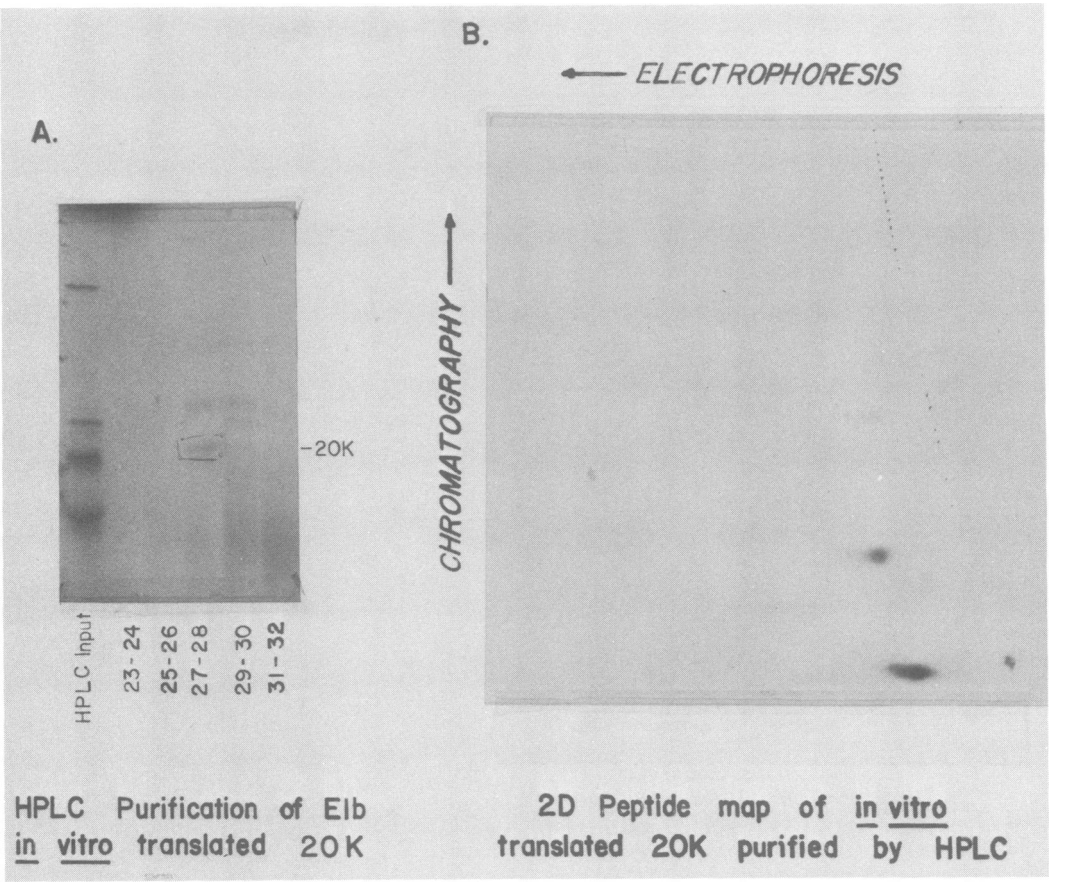


FIG. 11. Isolation of the 20K protein from the in vitro translation product of Ad2 E1b-specific mRNA. (A) A portion of the [^{35}S]methionine-labeled translation product analyzed in Fig. 9 was subjected to reverse-phase HPLC, and each fraction was analyzed by SDS-PAGE. (B) The band of 20K in fractions 27–28 was trypsinized and peptide mapped.

nine-containing tryptic peptides, based on the DNA sequence of Ad5 (28) and the almost identical DNA sequence of Ad2 (T. Gingeras and R. Robert, personal communication), and based on the report that the 5′-terminal exons of the 22S and 13S mRNAs are colinear for about 550 nucleotides (8). Since 53K and 20K proteins do not share methionine-containing tryptic peptides with 19K proteins, all three proteins probably do not initiate at nucleotide 1,711. It seems likely that the E1b-53K is coded by a second open reading frame between an ATG at nucleotide 2,016 and a TGA at nucleotide 3,501, which is predicted from the DNA sequence of the E1b region of Ad2 (T. Gingeras and R. Roberts, personal communication). The 19K and 53K should not share sequences because they are translated in different reading frames; in accord with this prediction, our peptide maps of 19K and 53K indicate that these polypeptides do not share sequences. The 20K polypeptide which shares sequences with the 53K must be translated in the same reading frame as 53K, but its mRNA may have a spliced structure that allows it to initiate or terminate differently from 53K; this may be the twice-spliced mRNA detected as a minor species by L. Chow, T. Broker, and colleagues (personal communication). Alternatively, it is possible that the 20K initiates at the same ATG as 53K (2,016), but on the 13S mRNA rather than on the 22S mRNA; i.e., the 13S could be translated to either the 19K or the 20K protein.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grants 9 RO1 CA29561 and 2 RO1 CA21824 from the National Cancer Institute. M.G. holds Research Career Award 5 KO6 AI04739.

We thank S. Udenfriend for suggesting the general strategy for purification of 20K, B. Jones for instruction in HPLC technology, G. Chinnadurai, J. Symington, and W. S. M. Wold for reviewing the manuscript, and C. E. Mulhall for editorial assistance.

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