JOURNAL OF VIROLOGY, Jan. 1988, p. 33–39
0022-538X/88/010033-07$02.00/0
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Vol. 62, No. 1

Identification and Gene Mapping of a 14,700-Molecular-Weight Protein Encoded by Region E3 of Group C Adenoviruses

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Received 20 July 1987/Accepted 15 September 1987

Early region E3 of adenovirus type 5 should encode at least nine proteins as judged by the DNA sequence and the spliced structures of the known mRNAs. Only two E3 proteins have been proved to exist, a glycoprotein (gp19K) and an 11,600-molecular-weight protein (11.6K protein). Here we describe an abundant 14.7K protein coded by a gene in the extreme 3′ portion of E3. To identify this 14.7K protein, we constructed a bacterial vector which synthesized a TrpE-14.7K fusion protein, then we prepared antiserum against the fusion protein. This antiserum immunoprecipitated the 14.7K protein from cells infected with adenovirus types 5 and 2, as well as with a variety of E3 deletion mutants. Synthesis of the 14.7K protein correlated precisely with the presence or absence of the 14.7K gene and with the synthesis of the mRNA (mRNA *h*) which encodes the 14.7K protein. The 14.7K protein appeared as a triplet on immunoprecipitation gels and Western blots (immunoblots).

Functional studies on proteins derived from early region E3 of adenovirus have been limited, mainly because E3 can be deleted without apparent effect on virus growth in cultured cells (10, 21). E3 also is not required for replication of adenovirus type 5 (Ad5) in the lungs of acutely infected hamsters (25a). A glycoprotein coded by E3, called gp19K or E19, has been shown to bind strongly to class I antigens of the major histocompatibility complex and to prevent their transport to the cell surface; this suggests that gp19K protects infected cells from lysis by cytotoxic T cells (4, 5, 24, 26–28, 36). Since adenovirus transcription units tend to contain genes with similar types of functions, it is conceivable that other E3-coded proteins may also be involved in protecting adenovirus from host defense mechanisms during natural infections. If so, then these proteins are likely to be of considerable interest.

A schematic representation of region E3 is presented in Fig. 1 (the legend summarizes mRNA mapping studies). Putative E3 proteins, as well as their coding position in the E3 mRNAs, are indicated by the stippled and hatched bars (see reference 9 for a detailed discussion). Stippled bars indicate proteins that are proposed to exist, based on their conservation in the DNA sequence of Ad5 (9) and Ad2 (13, 14) and on their position in the mRNAs. Hatched bars indicate proteins that have been proved to exist. gp19K is well characterized (15, 19, 25b, 29, 30, 32, 40), and its gene has been mapped by NH_2-terminal sequence analysis (19, 30, 40), by cell-free translation of *r*-strand and E3-specific mRNA (29, 30), by analysis of E3 deletion mutants (20, 25b, 29, 34, 38, 42), by expression in expression vectors (4, 28), and by immunoprecipitation with antisera directed against synthetic peptides (1, 40) or against adenovirus-transformed cells that express E3 (17, 32, 33, 43). The E3A–11,600-molecular-weight protein (11.6K protein) gene was mapped by immunoprecipitation of the protein from virus-infected cells and from cell-free translation products of E3-specific mRNA (41). Indirect evidence suggests that the E3A-6.7K gene also exists, i.e., a 6.7K-gp19K fusion protein was immunoprecipitated by a gp19K antiserum from cells infected with a deletion mutant that fuses the 6.7K and gp19K genes (42). Since this 6.7K-gp19K fusion protein is made in this mutant, it seems likely that the 6.7K protein will be made in wild-type virus. An E14K protein was mapped to the E3 region of Ad2 by cell-free translation and by analysis of an E3 deletion mutant (31). Proteins of 13,000, 14,000, and 15,500 molecular weight have also been mapped to E3 by cell-free translation of Ad2-specific mRNA (12), but it is not known whether these proteins correspond to any of the proven E3 proteins.

In this study, we established that the E3B-14.7K gene exists. Our approach was to prepare an antiserum against a TrpE-14.7K fusion protein synthesized in *Escherichia coli*. This antiserum immunoprecipitated a triplet of 14.7K-related proteins from cells infected with viruses that retain the 14.7K gene, but not from cells infected with a mutant that lacks the gene.

MATERIALS AND METHODS

Viruses, cells, protein labeling, and immunoprecipitation. Procedures for the maintenance of KB cells, for adenovirus preparation, and for plaque assays have been described previously (11). Mutant H5/2*rec dl*708 was described by Bhat et al. (2), H5*sub*304 was described by Jones and Shenk (18), and H2*dl*801 was described by Challberg and Ketner (6). H5/2*rec dl*731 and H5/2*rec dl*748 were obtained from H. Brady, and H5*dl*1035 was obtained from G. Ketner.

Early viral proteins were labeled from 7.5 to 12 h postinfection with [^{35}S]methionine in methionine-free medium by the cycloheximide-enhanced procedure exactly as described previously (40, 41). The label was either a ^{35}S-labeled hydrolysate of *E. coli* (70% [^{35}S]methionine [1,090 Ci/mmol], 20% [^{35}S]cysteine; ICN Radiochemicals) or [^{35}S]methionine (~1,103 Ci/mmol; New England Nuclear Corp.).

Cells were rinsed twice in cold phosphate-buffered saline and lysed on ice with 10 times the pellet volume of iso-hi-pH buffer (0.14 M NaCl, 1 mM $MgCl_2$, 10 mM Tris chloride, pH 8.5) containing 0.5% Nonidet P-40 and 1 mM phenylmethylsulfonyl fluoride. Nuclei were removed, and the supernatant (5×10^6 cpm) was assayed by immunoprecipitation with 5 μl of antiserum and 50 μl of Formalin-fixed *Staphylococcus aureus* containing protein A (Pansorbin; Calbiochem-Behring, La Jolla, Calif.) or protein A-Sepharose (Sigma

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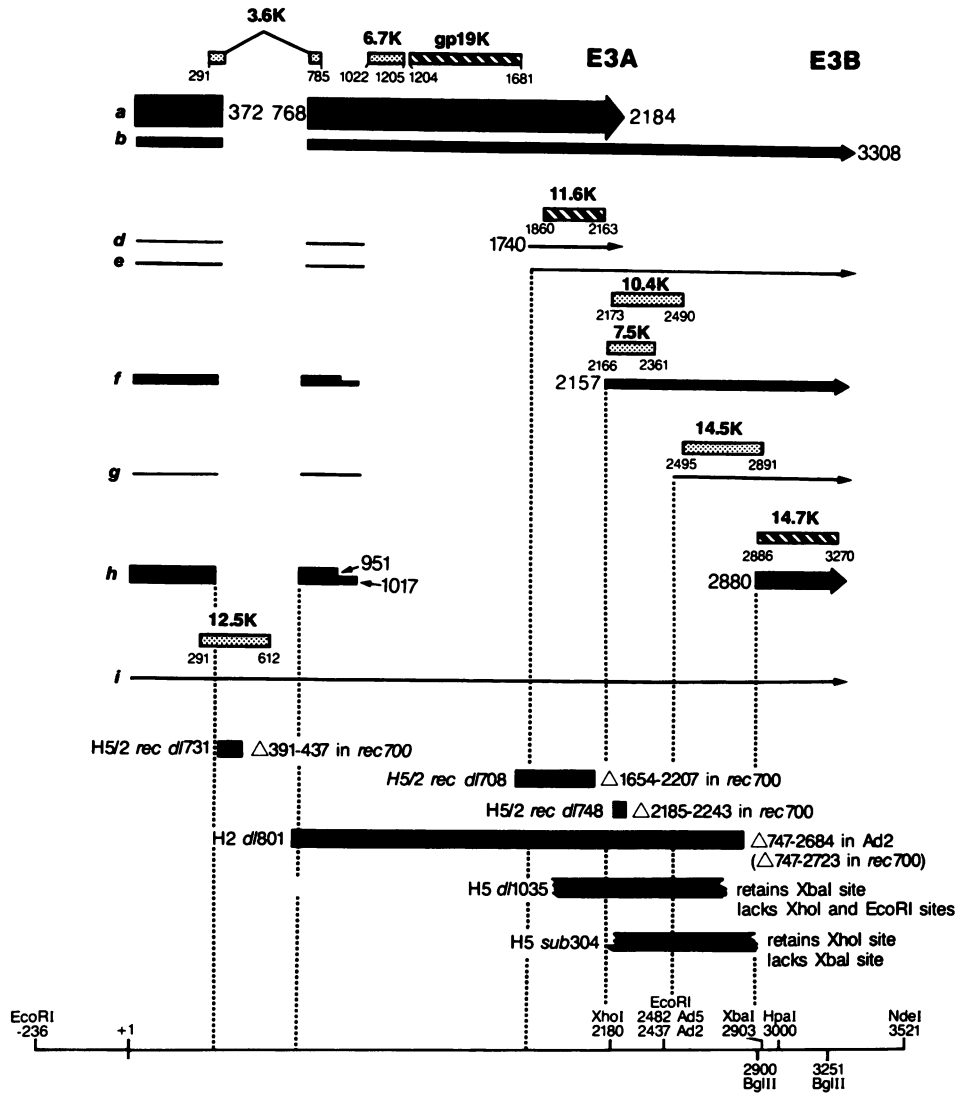

FIG. 1. Schematic representation of the E3 transcription unit. The split arrows (*a* to *i*) indicate the exon structures of the mRNAs (7, 8, 22, 30). The bars indicate the positions in the mRNAs that encode putative proteins (see reference 9 for discussion and original references). Hatched bars indicate proven proteins, and stippled bars indicate proteins that are proposed to exist. The numbers refer to nucleotide numbers in the transcription unit; nt +1 is the transcription initiation site. The schematic shown is for *rec*700, which is mainly Ad5 except that it has the Ad2 *Eco*RI D fragment (map positions 76 to 86) in place of the equivalent Ad5 EcoRI C fragment (42). In E3 of *rec*700, Ad2 numbers are used between the *Eco*RI sites at −236 to 2437, and Ad5 numbers are used downstream of the *Eco*RI site at 2482 in the Ad5 sequence (9). E3A and E3B are polyadenylation sites. Restriction sites are numbered at the 5′ base in the recognition sequence, not at the actual cleavage site. The bars at the bottom indicate the deletions in the mutants; the sequences deleted in *dl*708 (2), *dl*801 (E. Wang, M. Scott, and R. Ricciardi, personal communication), *dl*731, and *dl*748 (H. Brady and W. Wold, unpublished data) are shown. The deletions in *sub*304 and *dl*1035 were estimated from restriction enzyme cleavage patterns.

Chemical Co., St. Louis, Mo.) (3). Immunoprecipitates were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on 10 to 18% gradient gels (0.75 mm by 16 cm; acrylamide–*N*,*N*′-methylene-bisacrylamide, 29.2:0.8, wt/wt). All gels were fluorographed. ^{14}C-labeled molecular weight markers were obtained from Bethesda Research Laboratories, Inc. (Gaithersburg, Md.). The antisera used were against a TrpE-14.7K fusion protein (see below), the E1B-176R protein (37), or a purified E14K protein (31).

Western blots (immunoblots). Cells were infected as described above. At 12 h postinfection, cells were pelleted, washed with phosphate-buffered saline, and suspended in 2× Laemmli buffer (125 mM Tris chloride [pH 6.8], 20% glycerol, 4% SDS, 10% β-mercaptoethanol, 0.0025% [wt/vol] bromophenol blue). DNA was sheared by passing several times through a 20-gauge needle. The extract was boiled for 4 min, and the equivalent of 3×10^5 to 6×10^5 cells per well (1 cm) was loaded onto 10 to 18% gradient SDS-polyacrylamide gels (1.5 mm by 16 cm). After electrophoresis, proteins were transferred to nitrocellulose (39) (NC, 0.22-μm pore size; Schleicher & Schuell, Inc., Keene, N.H.) in 25 mM Tris–192 mM glycine–20% methanol overnight at 30 V and then for 1 h at 70 to 80 V. The blot was probed with the TrpE-14.7K antiserum (sixth injection) diluted 1:100 in 1% gelatin–Tris-buffered saline. The antiserum was removed, and the blot was rinsed twice with 0.05% Tween 20–Tris-buffered saline (pH 7.5). The second anti-

body was affinity-purified horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin G (heavy and light chains) (Bio-Rad Laboratories, Richmond, Calif.) diluted 1:2,000 in 1% gelatin–Tris-buffered saline. After rinsing, the 14.7K protein was visualized with the HRP Color Development Reagent (containing 4-chloro-1-naphthol) obtained from Bio-Rad.

Construction of TrpE-14.7K expression plasmid. A plasmid was constructed that synthesizes a fusion protein consisting of TrpE and Ad5 14.7K sequences. The original expression vector, pATH2, was obtained from T. J. Koerner. This vector, which is similar to the vector described by Spindler et al. (37), makes a 37,000-molecular-weight fragment of the TrpE protein under control of the *trp* promoter. A polycloning site is located at the 3′ region of the TrpE-coding sequences. A *Bgl*II-*Bgl*II fragment, nucleotides (nts) 2900 to 3251 in the E3 transcription unit of Ad5, was gel purified and cloned into the *Bam*HI site of pATH2. *E. coli* HB101 cells were transformed, and plasmids were screened for inserts in the correct orientation and containing the *Sst*I and *Hpa*I sites at nts 2985 and 3000, respectively (9). The resulting plasmid, pTrpE-14.7K, was used to transform *E. coli* RR1, which was used for expression of the fusion protein. Tryptophan (0.1 mg/ml) was included in the medium in all cloning steps to prevent loss of the plasmid.

The fusion protein synthesized by pTrpE-14.7K consists of 325 amino acids of the TrpE protein at the NH_2 terminus, followed by 119 amino acids of the 14.7K protein; the molecular weight is ~50,000. Amino acids 6 to 125 of 14,700 molecular weight are present in the fusion protein; there are 128 amino acids in the complete protein, including the initiator methionine.

Purification of TrpE-14.7K fusion protein and generation of antiserum. Methods for induction and purification of the fusion protein were obtained from T. J. Koerner and were similar to methods described by Spindler et al. (37). Overnight cultures of *E. coli* grown in M9 medium with Casamino Acids (Difco Laboratories, Detroit, Mich.) containing 20 μg of Trp per ml and 100 μg of ampicillin per ml were diluted 1:10 in the same medium lacking Trp and were grown for 90 min with great aeration. The fusion protein was induced by the addition of 3β-indoleacrylic acid (Sigma) to a final concentration of 20 μg/ml, and cultures were grown overnight. To obtain whole-cell lysates for SDS-PAGE, we suspended bacteria pellets in 2× Laemmli buffer and then boiled them for 4 min. To extract the fusion protein for purification, we lysed cells on ice for 30 min with 50 mM Tris chloride (pH 7.5)–5 mM EDTA–3 mg of lysozyme per ml. NaCl and Nonidet P-40 were added to final concentrations of 0.3 M and 0.5%, respectively, and incubation was continued for 30 min. The extract was then digested on ice for 1 h with 2 μg of DNase I (D-4763; Sigma) per ml plus 5 mM $MgCl_2$. The insoluble fraction, which consisted mostly of the fusion protein, was collected by centrifugation, suspended in 10 mM Tris chloride (pH 7.5), and stored at −70°C.

The fusion protein was solubilized in 2× Laemmli buffer and purified by electrophoresis on a preparative 10% SDS-polyacrylamide gel (3 mm by 16 cm). The band position was visualized by brief staining in 0.1% Coomassie blue (in 40% methanol–10% glacial acetic acid). The band was excised, homogenized, and mixed with an equal volume of adjuvant.

New Zealand White female rabbits were injected subcutaneously biweekly with 25 to 50 μg of protein in complete Freund adjuvant (Sigma) for the first injection and in incomplete Freund adjuvant (Sigma) for subsequent injections. Immune serum was collected 10 days postinjection.

Antisera were prepared similarly against the TrpE 37K protein synthesized from the pATH2 vector.

RESULTS

Synthesis of 14.7K protein in cells infected with wild-type and mutant adenoviruses. The TrpE-14.7K protein was purified from insoluble fractions and preparative SDS-PAGE gels as described in Materials and Methods. The 50,000-molecular-weight TrpE-14.7K band was excised from the gel and used to immunize rabbits. The resulting antiserum immunoprecipitated a strong [^{35}S]methionine-labeled protein of ~16,000 molecular weight from cells infected with Ad5 (Fig. 2A, lane e). A weaker band of 15,000 molecular weight was also immunoprecipitated. These same two bands as well as a band of 17,000-molecular weight were also obtained from *rec*700 (lane c). *rec*700 is an Ad5-Ad2-Ad5 recombinant virus which has Ad2 sequences between nts −236 and 2437 in the E3 transcription unit and has Ad5 sequences elsewhere (9, 42). The 14.7K gene is in the Ad5 portion of *rec*700. A 14.7K triplet was also obtained from Ad2 (lane g); however, all three bands had slightly greater mobility in the gel than did the Ad5 versions. This mobility difference indicates that the proteins are virus coded.

None of the 14,700-molecular-weight bands was immunoprecipitated from mock-infected extracts by immune serum (Fig. 2A, lane b). In data not shown, none of these bands was immunoprecipitated from the *rec*700 extract by preimmune serum or by antiserum against the TrpE 37K protein alone. We conclude that the 14.7K triplet is specific to virus-infected cells and to the TrpE-14.7K antiserum.

The 14.7K protein should be coded by mRNA *h* (Fig. 1),

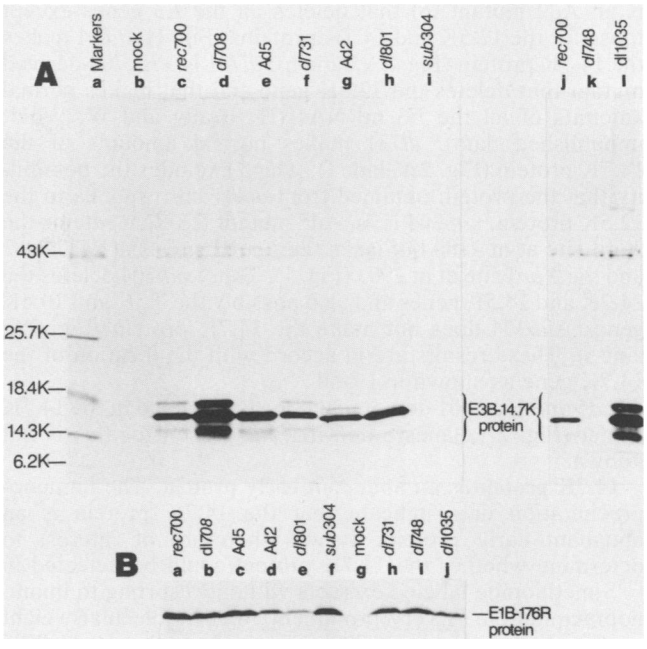

FIG. 2. Immunoprecipitation of the 14.7K protein from [^{35}S]methionine-labeled extracts of cells infected with wild-type Ad5, Ad2, and E3 deletion mutants. Proteins immunoprecipitated by antiserum against the TrpE-14.7K fusion protein are shown in panel A; panel B indicates proteins immunoprecipitated by antiserum against the TrpE-E1B-176R protein (37). An equal number of counts (5×10^6 cpm) were used for each immunoprecipitation. Eluted proteins were separated on a 10 to 18% gradient SDS-polyacrylamide gel. Marker proteins are shown to the left of panel A.

based on the DNA sequence and the structures of the known E3 mRNAs. We have constructed and characterized a variety of E3 deletion mutants derived from *rec*700 which synthesize altered levels of mRNA *h* (2). Some of these mutants were analyzed to determine whether they synthesize correspondingly altered levels of the 14.7K protein. *dl*708 made large amounts of mRNA *h*, modest amounts of mRNAs *b* and *i*, and no mRNAs *a*, *d*, *e*, and *f*. *dl*708 greatly overproduced the 14.7K triplet (Fig. 2A, lane d); this is the result expected if the 14.7K protein is coded by mRNA *h*. *dl*748 is a pre-mRNA processing mutant which synthesized mainly mRNA *f* and very little mRNA *h*; the reason for this is not clear, but it seems likely that the deletion somehow greatly increases the use of the 3′ splice site at nt 2157 for mRNA *f* such that mRNA *f* becomes virtually the only mRNA made (H. Brady and W. Wold, unpublished data). Consistent with its synthesis of very small amounts of mRNA *h*, *dl*748 made only very small amounts of the 14.7K protein (Fig. 2A, lane k) (the 14.7K protein bands are not visible in the figure, but they can be seen after longer exposures). Thus, the synthesis of mRNA *h* correlates with that of the 14.7K protein. All these mutants synthesized approximately the same levels of the E1B-176R protein encoded by early region E1B (Fig. 2B); this control rules out the possibility that the different levels of the 14.7K protein were caused by differences in the efficiency of infection or the expression of early genes. (The reduced amount of E1B-176R protein obtained from Ad2 and H2*dl*801 [Fig. 2B, lanes d and e] is presumably due to the fact that the antiserum was prepared against the Ad5 version of the protein.)

Several other mutants were analyzed whose deletions are particularly advantageous for mapping the 14.7K gene. *dl*801 is an Ad2 mutant (6) that deletes all the E3 genes except those for the 12.5K and 14.7K proteins (Fig. 1). *dl*801 makes the 14.7K protein (Fig. 2A, lane h). *dl*731 is a *rec*700-derived mutant that deletes the 12.5K gene and that makes normal amounts of all the E3 mRNAs (H. Brady and W. Wold, unpublished data). *dl*731 makes normal amounts of the 14.7K protein (Fig. 2A, lane f), which excludes the possibility that the protein obtained from *dl*801 corresponds to the 12.5K protein. *sub*304 is an Ad5 mutant (18) that retains the *Xho*I site at nt 2180 but lacks the *Eco*RI site at nt 2437/2482 and the *Xba*I site at nt 2903 (Fig. 1). Thus, *sub*304 deletes the 14.7K and 14.5K genes and also possibly the 7.5K and 10.4K genes. *sub*304 does not make the 14.7K protein (Fig. 2A, lane i). These results are in accord with the location of the 14.7K gene as shown in Fig. 1.

Ad5 and H2*dl*801 do not make the upper band in the 14.7K triplet (Fig. 2A, lanes e and h). The reason for this is not known.

14.7K protein is an abundant early protein. The immunoprecipitation data indicate that the 14.7K protein is an abundant early protein. It was therefore of interest to determine whether the 14.7K protein could be detected in [^{35}S]methionine-labeled extracts without resorting to immunoprecipitation. A very prominent 16,000-molecular-weight protein was made by *dl*708 (Fig. 3, lane f); since *dl*708 overproduces the 14.7K protein, this 16,000-molecular-weight band very likely corresponds to the 14.7K protein. Consistent with this, the 14.7K protein was present at lesser amounts in Ad5 (Fig. 3, lane b) and *rec*700 (lane c); it was absent from *sub*304 (lane e), which lacks the 14.7K gene; and it was absent from mock-infected extracts (lane a). In accord with the immunoprecipitation data, the Ad2 version of the 14.7K protein, as detected in Ad2 (Fig. 3, lane d) and *dl*801

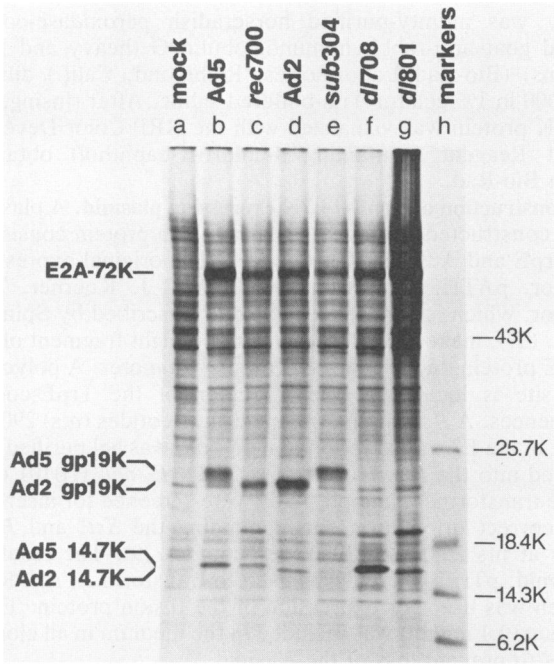

FIG. 3. SDS-PAGE of [^{35}S]methionine-labeled early proteins. [^{35}S]methionine-labeled early protein extracts were prepared from mock-infected cells or from cells infected with the indicated viruses as described in Materials and Methods. A total of 10^5 cpm were loaded per lane on a 10 to 18% gradient gel. Proteins were visualized by fluorography. Marker proteins are shown on the right.

(lane g), had slightly more mobility in the gel than did the Ad5 version seen in Ad5, *rec*700, and *dl*708.

The E3-coded gp19K is also visible on the gel in Fig. 3. The Ad2 version of this protein (lanes c and d) had greater mobility in the gel than the Ad5 version (lanes b and e). It is gratifying that the syntheses of both gp19K and 14.7K are consistent with the sequences present in the mutants and with the RNA-processing phenotypes. *sub*304, an Ad5 mutant, retains the gp19K gene but lacks the 14.7K gene; *sub*304 makes Ad5 gp19K but does not make 14.7K protein (lane e). *dl*801, an Ad2 mutant, lacks the gp19K gene but retains the 14.7K gene; *dl*801 makes the Ad2 version of 14.7K protein but does not make gp19K (lane g). *rec*700 has the Ad2 gp19K gene and the Ad5 14.7K gene; *rec*700 makes the Ad2 version of gp19K and the Ad5 version of 14.7K protein (lane c). gp19K is coded by mRNAs *a* and *b*, and 14.7K protein is coded by mRNA *h* (30). Ad5, *rec*700, and Ad2 make comparable levels of these mRNAs (2). As expected, these viruses make comparable levels of gp19K and comparable levels of 14.7K protein. *dl*708 markedly underproduces mRNAs *a* and *b* and markedly overproduces mRNA *h* (2); as expected, gp19K could not be detected in *dl*708 and 14.7K protein was overproduced (lane f).

We conclude that 14.7K protein is a fairly abundant E3-coded early protein. As a very rough estimate, 14.7K protein has about one-third the abundance of gp19K in *rec*700-infected cells. This estimate takes into account the number of methionines in these proteins (9, 13, 14) and the fact that both gp19K and the 14.7K protein resolve into multiple bands on SDS-PAGE. This estimate is roughly consistent with the relative levels of mRNAs *a* and *b* (~60% of total E3 mRNAs), which encode gp19K, and mRNA *h* (~25% of total), which encodes the 14.7K protein in *rec*700.

The three 14.7K bands all contain 14.7K protein sequences.

The three protein bands immunoprecipitated could be different versions of 14.7K protein, or they could be other proteins that were coprecipitated. To address this, we analyzed extracts from *dl*708-infected cells on a Western blot (immunoblot). All three bands are apparent (Fig. 4, lane a), indicating that they represent different versions of the 14.7K protein. None of these proteins was observed from *sub*304 (Fig. 4, lane b), consistent with the immunoprecipitation data (Fig. 2A, lane i). Western blots of the other mutants also showed the same 14.7K proteins that were detected by immunoprecipitation (data not shown).

Correspondence of 14.7K protein to E14K protein. Persson et al. (31) purified an E14K protein from Ad2-infected cells and prepared an antiserum in rabbits against the purified protein. This antiserum immunoprecipitated the same three protein bands from *dl*708-infected cells as did our antiserum against the TrpE-14.7K fusion protein (Fig. 5). Thus, the E14K protein corresponds to the 14.7K protein.

DISCUSSION

The E3 transcription unit has an open reading frame at nts 2886 to 3270 in the Ad5 and *rec*700 sequence which could encode a 14,700-molecular-weight protein (9, 14). We prepared a strong antiserum against the nearly complete Ad5 protein (amino acids 6 to 124, of a total of 128) expressed in *E. coli* as a TrpE-14.7K fusion protein. This antiserum immunoprecipitated a strong band of 16,000 molecular weight and weaker bands at 17,000 and 15,000 molecular weight from virus-infected cells. These three bands are versions of the 14.7K protein, since they were observed in Western blots probed with the fusion protein antiserum. Studies with E3 deletion mutants were entirely in accord with the location of the 14.7K gene and the abundance of its mRNA (mRNA *h*). The 14.7K protein was made by *dl*801, which has most of the E3 genes deleted but retains the 14.7K gene, and it was not made by *sub*304, which has the 14.7K gene deleted. Also, the 14.7K protein was overproduced by *dl*708, which overproduces mRNA *h*, and it was underproduced by *dl*748, which underproduces mRNA *h*. These results provide very strong evidence that the 14.7K protein is a bona fide E3 protein.

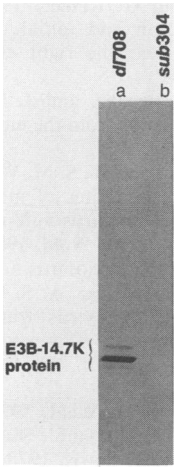

FIG. 4. Western blot of proteins extracted from cells infected with *dl*708 or *sub*304 and probed with the TrpE-14.7K antiserum. Extracts from early infection with *dl*708 or *sub*304 were separated on a 10 to 18% SDS-polyacrylamide gel, transferred to nitrocellulose, and probed with antiserum against the TrpE-14.7K fusion protein. The second antibody was affinity-purified horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin G (heavy and light chains).

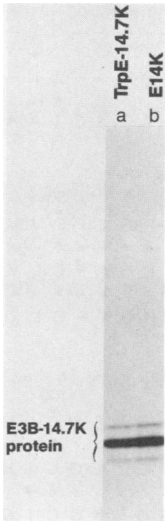

FIG. 5. Immunoprecipitation of the 14.7K protein from cells infected with *dl*708, using antiserum against the TrpE-14.7K protein and against the purified E14K protein (31). See the legend to Fig. 2 for details.

Several years ago Persson et al. (31) purified an E14K protein from Ad2-infected cells and prepared an antiserum against the purified protein. They did not detect this E14K protein in mock-infected cells or in cells infected with Ad2$^+$ ND1 (a nondefective adenovirus-simian virus 40 hybrid virus which has a large deletion in E3, including the 14.7K gene). The E14K protein was also immunoprecipitated from an Ad2-transformed rat cell line that retains region E3, but not from other cell lines that lack region E3. As shown here (Fig. 5), the antiserum against the purified E14K protein immunoprecipitated exactly the same proteins as did our antiserum against the TrpE-14.7K fusion protein. Thus, the E14K protein of Persson et al. (31) corresponds to our 14.7K protein.

Persson and colleagues (30) originally proposed that the E14K protein was coded by mRNA *h*. This proposal was based on the translation of the protein from mRNA selected by hybridization to restriction fragments that include exons in mRNA *h* and on the translation of the E14K protein from sucrose gradient fractions enriched in mRNA *h*. We showed with *dl*708 and *dl*748 that the abundance of mRNA *h* correlates with that of the 14.7K protein. Thus, the 14.7K protein is undoubtedly coded by mRNA *h*. It is of interest that the 14.7K protein apparently cannot be coded by mRNA *f*, because mRNA *f* is made in very high abundance by *dl*748, which makes only very low amounts of the 14.7K protein.

DNA sequencing studies indicate that the 14.7K gene is located in the same position in region E3 in at least two groups of adenoviruses, group C (Ad2, Ad5) (9, 14) and group B (Ad3) (35). Thus, the 14.7K gene is likely to be an important gene common to all human adenoviruses. The sequences of the 14.7K protein from Ad2, Ad5, and Ad3 are shown in Fig. 6. The sequence is highly conserved between Ad2 and Ad5, and it is also quite conserved in Ad3, especially in the C-terminal two-thirds of the protein. In many places where the amino acids are not identical, the substitutions are conservative. In contrast to five other

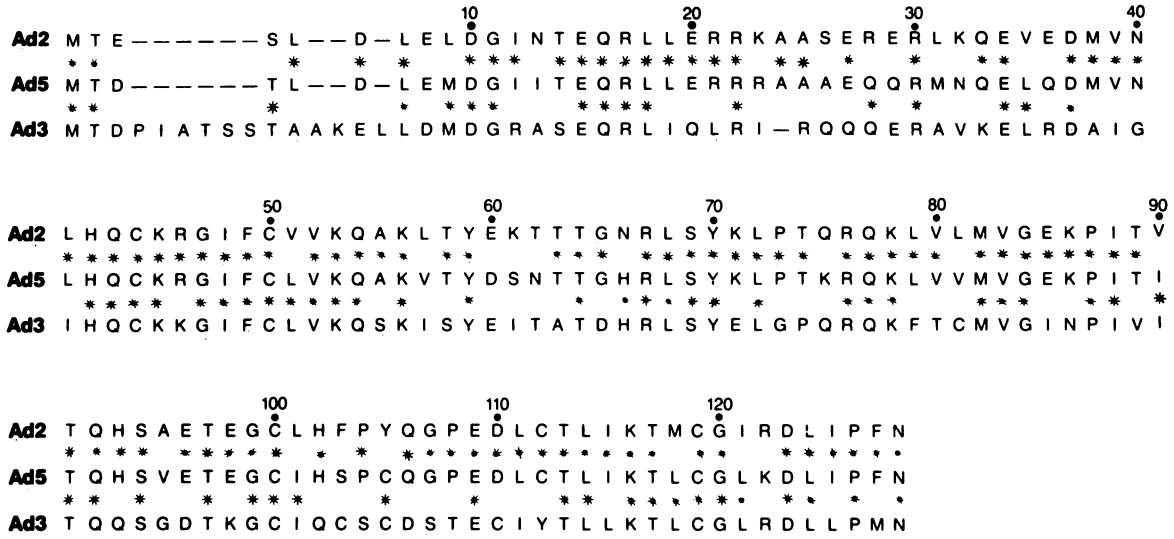

FIG. 6. Amino acid sequence of the 14.7K protein from Ad2, Ad5, and Ad3. The Ad2 sequence is from reference 14, the Ad5 sequence is from reference 9, and the Ad3 sequence is from reference 35. A computer program (25) was used to analyze the sequences.

putative E3 proteins (9), the 14.7K protein does not have any hydrophobic domains. Rather, it is quite highly charged, i.e., 26% of the amino acids in the Ad5 protein are lysine, arginine, asparagine, or glutamic acid. We showed that the Ad5 and Ad2 versions of the protein have slightly different mobilities in SDS-PAGE; this is interesting because, based on the DNA sequence, the two proteins have the same number of amino acids.

At least three bands of the 14.7K protein from *rec*700, *dl*708, *dl*731, *dl*1035, and Ad2 are visible on our gels as well as on the gels of Persson et al. (31). Curiously, only the strong middle and the weak lower band are seen in Ad5 and *dl*801. There are two explanations for these multiple bands. First, the 14.7K protein may undergo posttranslational modification. Phosphorylation or glycosylation is unlikely because the 14.7K protein does not appear to be labeled in vivo by $^{32}PO_4$, [^{3}H]glucosamine, or [^{3}H]mannose (15, 16, 23, 31, 32). Also, the 14.7K sequence does not contain the consensus sequences for N-linked glycosylation, NX(S, T). Perhaps the 14.7K protein is proteolytically cleaved, or perhaps it is acylated. Second, the weak bands of the 14.7K protein may result from low-abundance mRNAs that are spliced differently from mRNA *h*. For example, an mRNA could exist in which the protein initiates at ATG_{291} and then continues into the 14.7K sequences as a result of an appropriate in-frame splice. Ad5 and *dl*801 may not synthesize the weak upper band because they do not make this splice.

ACKNOWLEDGMENTS

We thank T. J. Koerner for the pATH2 vector and for advice, H. Brady for H5/2*rec dl*731 and H5/2*rec dl*748, M. Pursley for technical assistance, G. Ketner for H2*dl*801 and H5*dl*1035, T. Shenk for H5*sub*304, H. Persson for the E14K antiserum, A. Berk for the E1B-176R antiserum, and R. Ricciardi for communicating results before publication.

This work was supported by Public Health Service grant CA24710 from the National Institutes of Health.

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