JOURNAL OF VIROLOGY, Apr. 1979, p. 297-310
0022-538X/79/04-0297/14$02.00/0

Vol. 30, No. 1

Adenovirus Type 2 Early Polypeptides Immunoprecipitated by Antisera to Five Lines of Adenovirus-Transformed Rat Cells

WILLIAM S. M. WOLD* AND MAURICE GREEN

Institute for Molecular Virology, Saint Louis University School of Medicine, St. Louis, Missouri 63110

Received for publication 27 October 1978

We have identified adenovirus type 2 (Ad2)-induced early polypeptides (EPs) and have attempted to determine which EPs are coded by each of the four early gene blocks. [^{35}S]methionine-labeled EPs were resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Cycloheximide pretreatment followed by labeling in hypertonic medium (210 to 250 mM NaCl) facilitated the detection of EPs. Seven *major* (reproducible bands in autoradiograms) EPs were detected with molecular weights of 74,000 (74K), 21K, 19K, 15K, 13.5K, 11.5K, and 11K. *Minor* (weaker bands) EPs of 55K, 52K, 42K, 18K, 12K, 8.8K, and 8.3K were also often seen. To identify and map the genes for virus-coded EPs, we prepared antisera against five lines of adenovirus-transformed cells that retain different fractions of the viral genome. The lines were F17, 8617, F4, and T2C4 transformed by Ad2 virions and 5RK (clone I) transformed by transfection with the Ad5 *Hsu*I-G fragment (map position 0 to 8). The early gene blocks retained and expressed (in part) as RNA in these cells were as follows: 5RK(I), block 1 (70% of left 8% of genome); F17, block 1; 8617, blocks 1 and 4; F4, blocks 1, 2, and 4; T2C4, blocks 1, 2, 3, and 4. The following *major* EPs were immunoprecipitated: 15K by all antisera; 53K and 14.5K by F17, T2C4, 8617, and F4 antisera; 11.5K by T2C4, 8617, and F4 antisera; 44K, 42K, 19K, and 13.5K by T2C4 antisera; 11K by 8617 antisera. *Minor* EPs of 28K, 18K, and 12K were precipitated by all antisera except 5RK(I). The 53K and 15K EPs were precipitated also from Ad2 early infected monkey cells by the F17 antiserum and by sera from hamsters bearing tumors induced by Ad1-simian virus 40. The relationships between some of the immunoprecipitated EPs were investigated by the partial proteolysis procedure. All 53K EPs are the "same" (i.e., highly related), all 15K EPs are the "same," and all 11.5K EPs are the "same." The 15K EP is highly related to the 14.5 K EP. Although less certain, all 28K EPs appeared related, as did all 18K EPs. The T2C4-specific 44K EP is probably a dimer of the 21K glycopolypeptide. The T2C4-specific 13.5K EP and the 8617-specific 11K EP appear unrelated to any other polypeptides. These immunoprecipitation data provide evidence that early gene block I (map position 1 to 11) may encode *major* 53K, 15K, and 14.5K polypeptides, and *minor* 28K, 18K, and 12K polypeptides, and that all or some of the gene for 15K and 14.5K lies within map position 1 to 8. The surprisingly complex pattern of polypeptides coded by early gene block I raises the possibility that some polypeptides may be coded by overlapping "spliced" mRNA's. The possible block locations of the genes for the 21K, 13.5K, and 11.5K polypeptides are discussed.

The early genes of adenovirus type 2 (Ad2) and the closely related adenovirus type 5 (Ad5) are transcribed before initiation of viral DNA synthesis at 6 to 7 h postinfection (reviewed in 17, 51, 72). The early genes are distributed in four noncontiguous gene blocks (4, 6, 12, 14, 16, 18-20, 22, 50). Blocks 1 and 3 are located within approximate map positions 1 to 11 and 76 to 86, respectively, and are transcribed in the rightward direction. Blocks 2 and 4 are located within approximate map positions 62 to 68 and 92 to 98, respectively, and are transcribed in the leftward direction. The left portion of block 1 contains transforming genetic information, because cells can be transformed by restriction endonuclease fragments such as *Hsu*I-G (map position 0 to 8) (63), and because all virus-transformed cells retain at a minimum these sequences as DNA (21, 24, 63) and express them as RNA (10, 19, 20, 22). Recent studies indicate that all four

early gene blocks encode a variety of overlapping "spliced" presumptive mRNA's (4, 38; L. Chow, T. Broker, and J. Lewis, personal communication).

The early polypeptides (EPs) induced during early stages of infection are of interest because they function in viral DNA replication, probably cell transformation, and possibly regulation of viral gene expression and inhibition of host DNA synthesis. EPs may be virus coded or cell coded and virus induced. Studies with temperature-sensitive and host-range mutants (i.e., mutants H5*ts*125, H5*ts*36, and H5*hr* group I) have provided evidence that at least three virus-coded polypeptides function in viral DNA replication (28, 40, 70) and that at least three virus-coded polypeptides (H5*ts*36, H5*hr* group I, and H5*hr* group II) may play a role in cell transformation (29, 31). Virus-coded EPs are of further interest because they serve as models of how polypeptides are synthesized from spliced mRNA's in eucaryotic cells.

The identification and analysis of EPs are difficult because many host-cell polypeptides are synthesized. Several laboratories have analyzed the polypeptides labeled in vivo with [^{35}S]methionine in infected and mock-infected cells, using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). These studies have identified EPs with molecular weights of 70,000 to 80,000 (70K–80K), 42K, 35K, and 26.5K, and as many as seven polypeptides ranging in apparent molecular weight from 11K to 21K (3, 8, 9, 32–34, 36, 47, 53, 56, 57, 69). The 70K–80K polypeptide is the only EP that has been extensively studied. It is a single-stranded DNA-binding protein (DBP) (59, 65) that is coded by early gene block 2 (30, 44, 66). DBP is a phosphoprotein (2, 37, 43, 45, 55) that has a variable apparent molecular weight by SDS-PAGE (37). It functions in viral DNA replication, as indicated by genetic studies (67, 68), cytological studies (62), and studies with in vitro complexes that synthesize viral DNA (1, 53). DBP has been purified to homogeneity (45, 61) and shown to be a nonglobular protein with a native molecular weight of 73K (61). There is evidence that the synthesis of DBP is regulated during the course of infection (7, 22, 27, 62). Several single-stranded DNA-binding polypeptides of 40K–50K have also been identified that have been shown by peptide mapping to be related to the 73K DBP (45, 54, M. Green et al., Cold Spring Harbor Symp. Quant. Biol., in press); whether these have biological functions is not known.

The 21K polypeptide detected by SDS-PAGE is a glycopolypeptide that probably is virus coded (35, 36) and that has been localized to the plasma membrane of infected cells (8). The 11K protein is localized mainly in the cell nucleus (9, 32, 53) and is a major component of an in vitro complex that synthesizes Ad2 DNA (53).

Ad2 and Ad5 EPs have also been studied by cell-free translation. Saborio and Öberg (57) identified 14 polypeptides by cell-free translation of early mRNA followed by immunoprecipitation with a rabbit antiserum prepared against Ad2 early infected KB cells. Lewis and colleagues (32, 44) translated early mRNA purified by hybridization to Ad2 restriction endonuclease fragments; this method establishes that EPs are virus coded and indicates the map positions of their genes. The most interesting EPs are those coded by early gene block 1, because these are candidate "transformation proteins." Lewis et al. (32, 34) concluded that block 1 codes several related polypeptides of 40K–50K plus a 15K polypeptide, block 2 codes DBP, block 3 codes 13K, 14K, and 15.5K polypeptides, and block 4 codes 11K, 17K, 19K, and 21K polypeptides. Lupker and colleagues (46) identified polypeptides of 14K, 38K, 42K, 48K, and 51K coded by gene block 1. Pettersson and Mathews (49) mapped a 12K polypeptide gene at approximate position 10 in gene block 1.

Radioimmunoprecipitation studies have also been done to identify transformation proteins and other EPs by use of sera from hamsters bearing tumors induced by adenovirus virions or adenovirus-transformed cells, antisera against extracts of adenovirus-transformed cells, or antisera against extracts of adenovirus early infected cells. Gilead and colleagues (26) precipated 53K and 15K polypeptides from Ad2 early infected KB cells, using both antisera against the Ad2-transformed cell line F17 and sera from hamsters bearing tumors induced by Ad1-simian virus 40 (SV40). Levinson and Levine (41, 42) precipitated a 58K polypeptide from Ad5-infected and -transformed cells, using the Ad1-SV40 sera, as well as sera from hamsters bearing tumors induced by 14b cells, a line of Ad5-transformed hamster cells. The F17 and 14b cells are interesting because they express only gene block 1 as RNA; thus, the 58K, 53K, and 15K polypeptides are candiate transformation polypeptides coded by early gene block 1.

In this report we present further attempts to identify EPs. In some experiments we enhanced the synthesis of EPs by treating cells with cycloheximide prior to labeling (33) and by labeling in hypertonic medium (11, 15, 48, 58). We have done radioimmunoprecipitation studies using antisera prepared against four lines of Ad2 virion-transformed rat cells (F17, F4, T2C4, and

8617) (23; A. Freeman, personal communication) and against one line (5RK, clone 1) transformed by transfection with Ad5 *Hsu*I-G fragment (map position 0 to 8) (64). All lines have been shown to synthesize RNA from block 1 (10, 20, 22), and thus these antisera are good reagents for identifying transformation and other polypeptides coded by this important gene block. Some lines also synthesize RNA from block 2 (T2C4 and F4), block 3 (T2C4), and block 4 (F4, T2C4 and 8617) (20, 22), and therefore the immunoprecipitation data provide evidence on where the other polypeptides might be coded. We also present our initial studies on the chemical relationships between the immunoprecipitated polypeptides, in which we used the partial proteolysis peptide mapping procedure (13).

MATERIALS AND METHODS

Preparation of Ad2-infected and mock-infected [^{35}S]methionine-labeled protein extracts, with and without cycloheximide pretreatment, labeled in isotonic and hypertonic media. KB cells were grown at 37°C in suspension culture in Eagle minimal essential medium (MEM) containing 5% horse serum. Cells were infected at 6×10^6 cells/ml with Ad2 (strain 38-2) at 100 PFU/cell in MEM without horse serum. After 1 h of adsorption, cells were suspended at 3.5×10^5 cells/ml in methionine-free MEM with 5% horse serum without cycloheximide or containing 25 μg of cycloheximide per ml. At 4 h postinfection, arabinosyl cytosine (ara-C) was added (20 μg/ml) to all cultures. About 0.5 h prior to labeling, cells were washed thrice (using centrifugation) in warm methionine-free MEM with 5% horse serum and 20 μg of ara-C per ml. For labeling under isotonic conditions, cells were incubated at 6.5×10^5 cells/ml in the above medium containing 25 μCi of L-[^{35}S]methionine (100 to 200 Ci/mmol) per ml and 10 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid, pH 7.2, for various periods and at different times postinfection, as indicated in the text. The presence of ara-C in the medium starting at 4 postinfection, and during the washing and labeling of cells, ensured that no late viral proteins were synthesized. For labeling under hypertonic conditions, cells were suspended as described above in MEM containing different final concentrations of NaCl (210 to 270 mM). After 15 min of incubation, proteins were labeled with [^{35}S]methionine. After labeling, cells were washed twice with cold phosphate-buffered saline. Cells were suspended in cold 20% trichloroacetic acid for 15 min at 4°C, washed once each in cold 10% and 1% trichloroacetic acid, washed twice in cold 0.05% trichloroacetic acid, and suspended in "SDS-PAGE buffer" (50 mM Tris-hydrochloride [pH 6.8], 10% glycerol, 1% SDS, 1% β-mercaptoethanol, and 0.005% bromophenol blue). Viscous extracts that were difficult to dissolve were adjusted to 0.05 to 0.1 N NaOH, and on occasion were also sonically treated. In most experiments, 1 to 2 mM phenylmethylsulfonyl fluoride was included in the phosphate-buffered saline, the SDS-PAGE buffer, and the immunoprecipitation buffer (see below). The same procedures were used for infected and mock-infected cells.

CV-1 (monkey) cell monolayers (usually in 75-cm^2 plastic flasks) were maintained in MEM with 5% calf serum in an atmosphere of 5% CO_2. Either confluent or subconfluent monolayers were infected with no apparent difference in the synthesis of Ad2-induced EPs. Cells were infected with Ad2 in minimal volumes of MEM with no calf serum. After 2 h of adsorption, MEM with 5% calf serum, with or without 25 μg of cycloheximide per ml, was added to a final volume of 25 ml and incubation was continued. Cells were labeled with [^{35}S]methionine, and proteins were extracted as described above for KB cells.

SDS-PAGE. Basically, the procedures of Studier (60) and Laemmli (39) were used. Gradient polyacrylamide gels with 0.1% SDS were formed as slabs between glass plates; slabs were 0.75 mm thick and 100 or 270 mm long. The gradient concentrations are presented in the figure legends. A stacking gel of 5% polyacrylamide and 1.13% bisacrylamide containing either 10 or 20 sample wells was cast above the separating gel. Solubilized proteins in SDS-PAGE buffer were heated at 100°C for 2 min. Each well was loaded with solubilized proteins, and electrophoresis was performed with a constant current of 20 mA. After electrophoresis, gels were dried under vacuum and autoradiographed using X-ray film.

Radioimmune precipitates (see below) dissolved in SDS-PAGE buffer were heated at 100°C for 4 min before electrophoresis. After electrophoresis, gels were dehydrated in dimethyl sulfoxide, soaked in a solution of 2,5-diphenyloxazole in dimethyl sulfoxide (5), dried, and exposed to X-ray film at −70°C; fluorograms were then developed.

Preparation of antisera. Antisera against extracts of F17, F4, T2C4, 8617, and 5RK (clone 1) cells were prepared in rats as described previously (26). The viral DNA and RNA sequences present in each of these cell lines are given in Table 1. The hamster antiserum against extracts of Ad1-SV40-induced hamster tumor cells (25) was a gift of R. V. Gilden. Preimmune sera were taken from the rats or hamsters used to prepare antisera. All antisera were fractionated into immunoglobulin G fractions. Some batches of antisera were absorbed for 18 h at 4°C with one-half volume of sonically treated KB cells prior to fractionation. Absorption did not affect the immunoprecipitation results.

Radioimmune precipitation. [^{35}S]methionine-labeled infected and mock-infected cells were washed thrice in cold phosphate-buffered saline. For total protein extracts, cells were suspended at about 5×10^6 cells/ml in immunoprecipitation buffer (10 mM sodium phosphate, pH 7.2, 150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, and 0.1% SDS), treated at full power in a Raytheon DF-100 sonic oscillator for three 100-s intervals at 0 to 4°C, incubated for 15 min at 20°C, and clarified by centrifugation at $30,000 \times g$ for 20 min. The preparation of nuclear and cytoplasmic extracts for radioimmunoprecipitation, and the double-antibody immunoprecipitation protocol, have been described (26). Precipitates were dissolved in 20 μl of

TABLE 1. *Summary of polypeptides immunoprecipitated from Ad2 early infected KB cells by antisera to Ad2- or Ad5-transformed rat cells*

Transformed cell antiserum[a]	Polypeptides immunoprecipitated[b]		Viral DNA and RNA in transformed cells[c]
	Major	Minor	
5RK(I)	15K		70% of left 8%
F17	53K 15K 14.5K	28K 18K 12K	Block 1
8617	53K 15K 14.5K 11.5K 11K	28K 18K 12K	Blocks 1 and 4
T2C4	53K 44K 42K 21K 15K 14.5K 13.5K 11.5K	28K 19K 18K 12K	Blocks 1, 2, 3, and 4
F4	53K 15K 14.5K 11.5K	28K 18K 12K	Blocks 1, 2, and 4

[a] F17, T2C4, and F4 cell lines were isolated by Gallimore (23). The 8617 cell line was isolated by A. Freeman (personal communication). The 5RK(I) cell line was isolated by van der Eb et al. (64). Antisera were prepared in rats against extracts of the transformed cell lines.

[b] Apparent molecular weights were estimated by SDS-PAGE, with polypeptides of known molecular weights used as standards (33). "Major" polypeptides were reproducibly observed in autoradiograms. "Minor" polypeptides were relatively weak bands observed in many but not all autoradiograms.

[c] The approximate locations of the four early gene blocks are as follows: block 1, map position 1 to 11; block 2, map position 62 to 68; block 3, map position 76 to 86; and block 4, map position 92 to 98 (4, 6, 12, 16, 19, 20, 22; L. Chow, T. Broker, and J. Lewis, personal communication). The viral DNA and RNA sequences present in F17, 8617, T2C4, and F4 cells were estimated by Gallimore et al. (24) and Flint et al. (20), respectively. 5RK(I) cells retain 70% of Ad5 *Hsu*I-G fragment (map position 0 to 8) as DNA and express 50% of G-fragment as RNA (10). The viral DNA sequences are integrated into cellular DNA with all cell lines, as indicated by Southern "blotting" analysis (unpublished data).

SDS-PAGE buffer, and equal volumes of each precipitate were analyzed by SDS-PAGE as described above.

Partial proteolysis. The relationships between the immunoprecipitated polypeptides were investigated by use of the partial proteolysis procedure (13). The polypeptides were resolved by SDS-PAGE, the gels were dried, and autoradiograms were developed. Polypeptide bands were cut from the gel, and the gel slices were inserted into wells (4 mm wide and 0.75 mm thick, containing soaking buffer which consisted of 0.125 M Tris-hydrochloride, pH 6.8, 0.1% SDS, and 1 mM EDTA) of a second slab gel and hydrated by soaking for 30 min. The proteolysis gel consisted of a 4-cm stacking gel of 9% acrylamide (pH 6.8) and a 5-cm running gel of 17% acrylamide (pH 8.8). *Staphylococcus aureus* V8 protease was diluted in soaking buffer containing 10% glycerol and 0.025% bromophenol blue, and 10 μl was added to the wells containing the gel slices. The amounts of protease used are given in the figure legends. Electrophoresis was carried out with the gel apparatus connected to a circulating water bath at 20°C. The sample was subjected to electrophoresis (25 mA per gel) until the bromophenol blue band had almost reached the bottom of the stacking gel. The power was shut off for 30 min to allow the protease to digest the [^{35}S]methionine-labeled polypeptides that had migrated from the gel slices. Electrophoresis was then continued until the dye reached the bottom of the running gel, and the gels were dried and fluorographed.

RESULTS

Preferential synthesis of Ad2-induced EPs in hypertonic medium, with and without cycloheximide pretreatment. As noted above, the detection of EPs by SDS-PAGE is difficult within the background of host-cell polypeptides. We previously described the use of cycloheximide pretreatment to facilitate the detection of EPs (33). We have now labeled cells in hypertonic medium, a procedure that enhances the synthesis of viral polypeptides relative to host polypeptides (11, 15, 48, 58). Figure 1 shows an SDS-PAGE electropherogram of polypeptides from infected and mock-infected KB cells, with and without cycloheximide pretreatment, labeled in normal medium (isotonic) and in medium at 250 mM NaCl (hypertonic). EPs are designated according to apparent molecular weight and are identified on the basis of a polypeptide band being present in lanes representing infected cell extracts but absent from lanes representing comparable mock-infected cell extracts. EPs marked with solid lines (DBP, 21K, 19K, 15K, 13.5K, 11.5K, and 11K) are "major"; i.e., they were generally prominent and were reproducibly observed. EPs marked with dashed lines are "minor"; i.e., they were seen in many but not all experiments.

As many as eight EP bands, which varied considerably in intensity, were visible in extracts of infected cells labeled in isotonic medium and not pretreated with cycloheximide (Fig. 1, lanes A and B). Two bands, DBP and 21K, were very

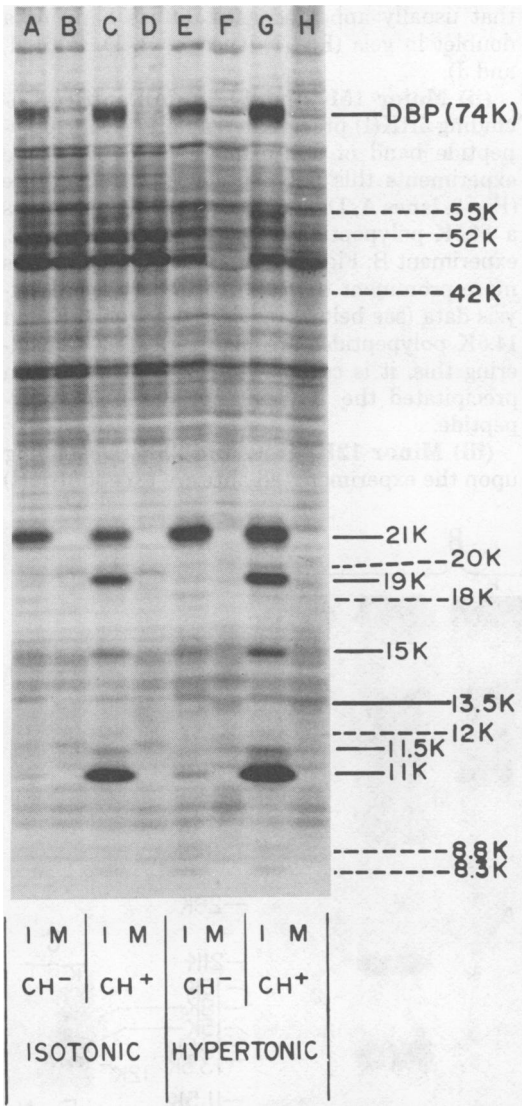

FIG. 1. *Effect of hypertonic labeling and cycloheximide pretreatment on the relative synthesis of adenovirus type 2 early polypeptides (EPs) and host polypeptides. Standard protocols were used to label cells with 25 μCi of [^{35}S]methionine/ml from 7.5 to 8.5 h postinfection in normal medium (isotonic) and in medium with 250 mM NaCl (hypertonic). Total cell proteins were resolved by SDS-PAGE (27-cm gel), and the dried gel was autoradiographed. Cells labeled in isotonic medium incorporated an average of 1.9 cpm/cell; infected and mock-infected cells labeled in 250 mM NaCl incorporated an average of 1.6 and 0.3 cpm/cell, respectively. EPs marked with a solid line are "major" EPs, and EPs marked with dashed lines are "minor" EPs (see text). (I) Infected, (M) mock infected, (CH⁻) no cycloheximide pretreatment, (CH⁺) with cycloheximide pretreatment.*

prominent. Bands of medium intensity included 11K, 13.5K, 15K, 18K, and 19K. Faint bands were 11.5K and 8.3K; the latter band was not always detectable. Cycloheximide pretreatment increased the density of all EP bands (in most experiments 21K was also increased) (lanes C and D). The same EPs were present and were more prominent in extracts of cells labeled in hypertonic medium. Cycloheximide pretreatment plus hypertonic labeling (lanes G and H) yielded the most pronounced major and minor EP bands, and revealed additional EPs of 55K and 52K. A 42K band could also be seen occasionally. These 55K, 52K, and 42K EPs are of particular interest because they may be polypeptides coded by gene block 1 (see Discussion). Hypertonic labeling seemed to reduce the synthesis of most host polypeptides and produced roughly the same increase in the relative density of bands of all major EPs and most minor EPs. Cycloheximide pretreatment increased the relative density of all EPs, but had a much more pronounced effect on 11K and 19K.

Radioimmunoprecipitation of EPs from Ad2 early infected human cells. Immunoprecipitation studies were done to further identify EPs. Antisera were prepared against extracts of four lines of Ad2-transformed rat cells (F17, F4, F25, and 8617) (23) and one line (5RK, clone 1) of rat cells transformed by Ad5 *Hsu*I-G fragment (64). We expected these antisera to contain antibodies against virus-coded EPs synthesized in the transformed rat cells, and we expected these antibodies to precipitate [^{35}S]methionine-labeled EPs from Ad2-infected KB cells. The viral DNA and RNA sequences present in these transformed rat cells are known (see Table 1), and therefore we were able to obtain evidence on which early gene block might encode the precipitated EPs.

The five rat antisera precipitated several polypeptides ranging in apparent molecular weight from 53K to 11K. Many immunoprecipitation experiments have been done, but exactly the same "minor" polypeptides were not always observed, despite the use of the same preparations of antisera. However, any given protein extract always yielded the same array of polypeptides. This suggests that the variability in the "minor" polypeptides may be due to subtle biological differences in the protein extracts, and not to our immunoprecipitation proedure nor to differences in antisera. We present here the results obtained in four independent experiments, performed with four different extracts of [^{35}S]methionine-labeled infected cells; these results illustrate most of the polypeptides that we have

observed. Figure 2 shows three experiments in which immunoprecipitates were subjected to electrophoresis on 5 to 21% linear gradient acrylamide gels. These gels show the 53K as well as most of the smaller polypeptides. Figure 3 shows an experiment in which immunoprecipitates were run on a 10 to 18% acrylamide gel; this gel gives good resolution of the smaller polypeptides. Below we summarize (also see Table 1) the "major" and "minor" polypeptides identified in Fig. 2 and 3. None of the immunoprecipitated polypeptides was precipitated from infected cells by preimmune rat serum (Fig. 2, lanes F, G, and L; Fig. 3, lane E) or from mock-infected cells by any of the immune or preimmune sera (data not shown; see 26).

(i) Major 53K. All antisera except 5RK(I) reproducibly precipitated a 53K polypeptide that usually appeared as a diffuse band or a doublet in gels (Fig. 2, lanes A, B, D, E, H, I, and J).

(ii) Major 15K and 14.5K. All antisera including 5RK(I) precipitated a fairly strong polypeptide band of approximately 15K. In some experiments this polypeptide appeared diffuse (Fig. 2, lanes A, D, and K). In other experiments a 14.5K polypeptide was also observed (Fig. 2, experiment B; Fig. 3). Sometimes the 14.5K was more prominent than the 15K. Partial proteolysis data (see below) indicate that the 15K and 14.5K polypeptides are highly related. Considering this, it is curious that 5RK(I) antiserum precipitated the 15K but not the 14.5K polypeptide.

(iii) Minor 12K, 18K, and 28K. Depending upon the experiment, all antisera except 5RK(I)

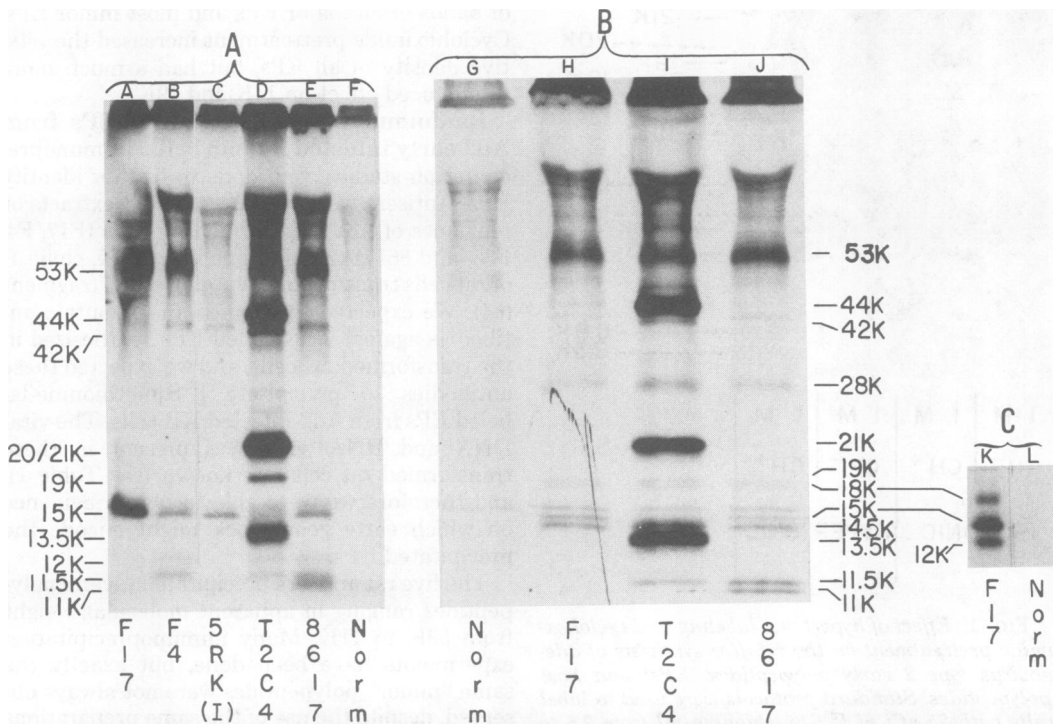

FIG. 2. *Radioimmunoprecipitation of early polypeptides (EPs) from three separate extracts of adenovirus type 2 early infected KB cells by antisera against five lines of adenovirus-transformed rat cells. Procedures are described in Materials and Methods. Adenovirus type 2 early infected KB cells were labeled from 6 to 9 h postinfection with [^{35}S]methionine. Arabinosyl cytosine (20 μg/ml) was added at 4 h postinfection, and cells were labeled in the presence of 20 μg of arabinosyl cytosine/ml. Cells were not pretreated with cycloheximide. Cytoplasmic protein extracts were prepared and precipitated with immunoglobulin G prepared from the five transformed cell antisera and from preimmune (Norm) rat sera. Each immunoglobulin G was incubated with equal volumes of extract, and equal volumes of each precipitate were subjected to electrophoresis in the 10-cm 5 to 21% linear gradient slab gel. Gels were dried and fluorographed (5). Table 1 lists the polypeptides precipitated by each antiserum and gives viral DNA and RNA sequences contained in each of the transformed cell lines. None of the EPs was precipitated by these antisera from mock-infected KB cells (data not shown). Three separate experiments with three separate protein extracts are indicated under the braces A, B, and C. F17 antiserum, lanes A, H, and K; F4 antiserum, lane B; 5RK(I) antiserum, lane C; T2C4 antiserum, lanes D and I; 8617 antiserum, lanes E and J; preimmune (normal) rat serum, lanes F, G, and L.*

precipitated fairly weak polypeptide bands of approximately 12K, 18K, and 28K. The 18K and 12K polypeptides precipitated by the F17 antiserum are clearly shown in Fig. 2, experiment C, lane K. The 12K polypeptide is visible in Fig. 2, experiment A (the F17-specific 12K cannot be seen), but not in Fig. 2, experiment B, or in Fig. 3. The 18K polypeptide cannot be seen in Fig. 2, experiment A, but is visible in experiments B and C of Fig. 2 and in Fig. 3. The 28K polypeptide, which usually appeared as a weak diffuse band, is shown in Fig. 2, experiment B. The 28K polypeptide was usually more apparent when immunoprecipitation was done by the "*Staphylococcus* A protein procedure."

(iv) Major 44K, 42K, and 21K and minor 19K polypeptides. Very strong 44K and 21K polypeptide bands were precipitated by the T2C4 antiserum (Fig. 2, lanes D and I). The 44K is probably a dimer of the 21K (see below), which is a glycopolypeptide (36). Polypeptides of 42K and 19K were also reproducibly precipitated by the T2C4 antiserum. Several weak, diffuse bands ranging from 21K to 40K were also often precipitated by the T2C4 antiserum (Fig. 2). Although T2C4 cells express a portion of early gene block 2 as RNA (20), the T2C4 antiserum did not precipitate DBP, which is coded by block 2. Consistent with this, we were unable to detect DBP in T2C4 cells by immunofluorescence procedures using an antiserum to DBP (62).

(v) Major 13.5K polypeptide. The T2C4 antiserum reproducibly precipitated a strong 13.5K polypeptide (Fig. 2, lanes D and I; Fig. 3, lane B), which usually appeared in gels as a doublet.

(vi) Major 11.5K polypeptide. The F4, T2C4, and 8617 antisera reproducibly precipitated a relatively weak band of 11.5K (Fig. 2 and 3).

(vii) Major 11K polypeptide. An 11K polypeptide was reproducibly precipitated by the 8617 antiserum (Fig. 2, lanes E and J; Fig. 3, lane C), but not by any of the other antisera.

Partial proteolysis analysis of the immunoprecipitated polypeptides. The chemical relationships between the EPs precipitated by the different antisera were investigated by use of the partial proteolysis procedure (13). This procedure is quite good for establishing that highly related polypeptides are, in fact, highly related. This is because all or most of the partial proteolysis bands co-migrate. In contrast, the procedure provides only suggestive evidence on whether polypeptides are partially related, or even completely unrelated, especially when the polypeptides differ considerably in apparent size.

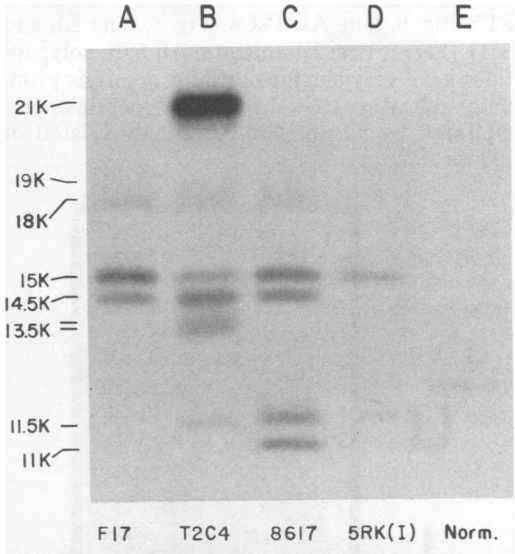

FIG. 3. *Low-molecular-weight early polypeptides immunoprecipitated from adenovirus type 2 early infected KB cells by antisera to four lines of adenovirus-transformed cells. Adenovirus type 2-[^{35}S]methionine-labeled cytoplasmic extracts were prepared and immunoprecipitated as described in the legend to Fig. 2. Cells were not pretreated with cycloheximide. The precipitated polypeptides were resolved by electrophoresis through a 27-cm 10 to 18% linear gradient slab gel. The gel was dried and fluorographed. (A) F17 antiserum, (B) T2C4 antiserum, (C) 8617 antiserum, (D) 5RK(I) antiserum, (E) normal (preimmune) rat serum.*

This is because partially related polypeptides may have only one or a few proteolysis bands that co-migrate, and it is difficult to be sure that the co-migration of these bands is not coincidental. Therefore, the major point addressed by the followng experiments is whether all polypeptides with the same mobility (e.g., all 53K polypeptides), precipitated by all antisera, are actually the same polypeptide (or a highly related form).

(i) 53K polypeptide. The 53K polypeptide precipitated by all antisera usually appeared as a diffuse band in gel electropherograms and, as shown in Fig. 4, sometimes as a doublet. The appearance of the doublet and the diffuse nature of the band raised the possibility that there may be two unrelated polypeptides of approximately 53K. To investigate this we analyzed the upper and lower 53K bands (precipitated by the F17 antiserum) by partial proteolysis. As shown in Fig. 5, the upper and lower 53K bands gave very similar partial proteolysis products at the three different levels of protease used. Therefore, there is no evidence for two unrelated 53K polypeptides. Figures 6 and 7 show partial proteolysis of the 53K polypeptides precipitated by the

F17 (Fig. 6, lane A), T2C4 (Fig. 6, lane E), and 8617 (Fig. 7, lane B) antisera. All 53K polypeptides gave very similar partial proteolysis products, indicating that the 53K polypeptides precipitated by all antisera are highly related or identical.

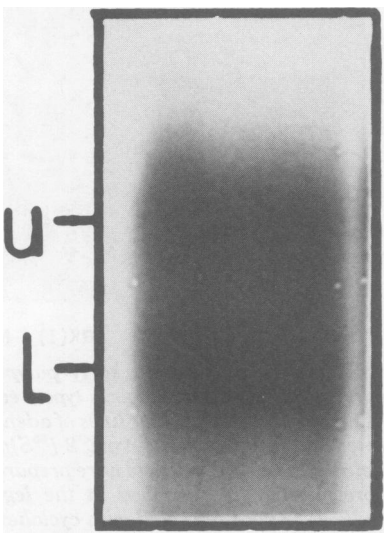

FIG. 4. *Close-up illustration of a 53K "doublet" immunoprecipitated by F17 immunoglobulin G. The gel was a 10-cm 10% slab gel. The photograph shows the needle hole in the X-ray films used to mark the location of the upper (U) and lower (L) bands of the doublet in the dried gel. The upper band (within the top four needle holes) and the lower band (within the lower four needle holes) were cut from the gel, and the polypeptides were analyzed by the partial proteolysis procedure. The results are shown in Fig. 5.*

(ii) 28K polypeptide. Similar peptide patterns were produced by partial proteolysis of the 28K polypeptide precipitated by the F17 (Fig. 6, lane B), T2C4 (Fig. 6, lane G), and 8617 (Fig. 7, lane C) antisera. Thus, the 28K polypeptides precipitated by the different antisera appear to be highly related.

(iii) 18K polypeptide. Only small amounts of the 18K polypeptide were immunoprecipitated, so it was difficult to detect and photograph all the proteolysis products. Partial proteolysis of all 18K polypeptides yielded one prominent band with the same gel mobility (Fig. 6, lanes C and J; Fig. 7, lane D). Thus, all 18K polypeptides *probably* are related.

(iv) 15K polypeptide. Very similar partial proteolysis products were generated from the 15K polypeptides precipitated by the F17 (Fig. 6, lane D), 5RK(I) (Fig. 7, lane A), and 8617 (Fig. 7, lane E) antisera. In other experiments, the 15K polypeptide precipitated by the T2C4 antiserum also gave a similar proteolysis pattern. Therefore, the prominent 15K polypeptides precipitated by all antisera appear to be highly related.

(v) Relationship between 53K, 28K, 18K, 15K, and 14.5K polypeptides. The 15K and 14.5K polypeptides precipitated by the F17 antiserum gave very similar proteolysis products, strongly suggesting that these polypeptides are highly related. (Fig. 8). It was difficult to draw clear conclusions about the other polypeptides. The major proteolysis band of the 15K polypeptide co-migrated with the major proteolysis band of the 18K polypeptide and with one (second from bottom) proteolysis band of the 53K

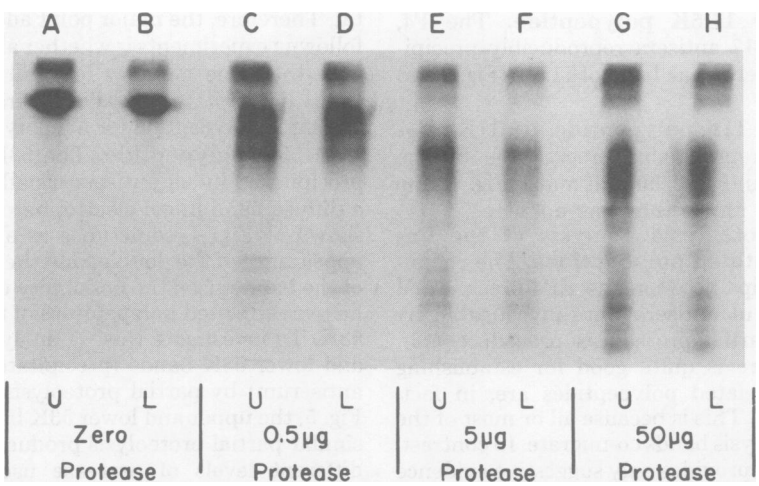

FIG. 5. *Partial proteolysis of the upper (U) and lower (L) bands of the 53K "doublet" illustrated in Fig. 4. The partial proteolysis procedure is described in Materials and Methods. (A, C, E, and G) Upper band, (B, D, F, and H) lower band, (A and B) no protease, (C and D) 0.5 μg of protease per lane, (E and F) 5 μg of protease per lane, (G and H) 50 μg of protease per lane.*

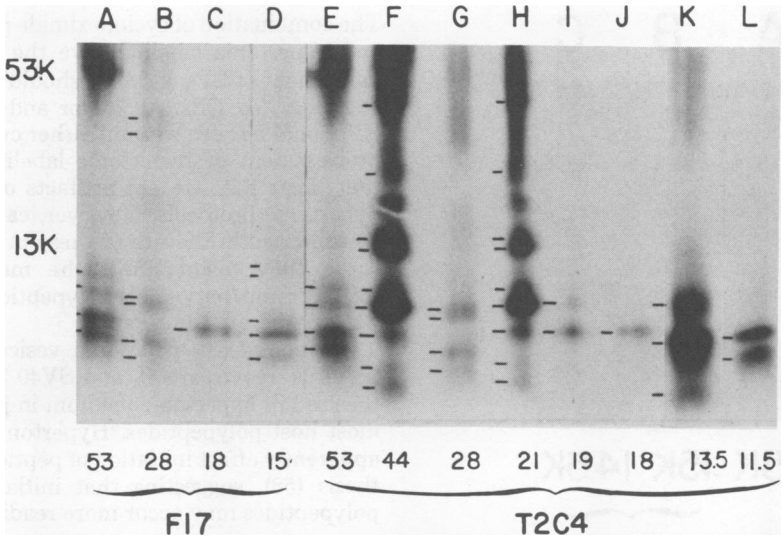

FIG. 6. *Partial proteolysis of early polypeptides immunoprecipitated by F17 and T2C4 immunoglobulin G. Protease was used at 20 μg per lane. The partial proteolysis polypeptide bands reproducibly observed are marked in the photograph. The numbers at the bottom of the figure indicate the polypeptide represented in each lane, i.e., lane A, 53K; lane B, 28K; lane C, 18K, etc. The numbers 53K and 13K at the left side of the figure indicate where 53K and 13K polypeptides migrate without treatment with protease.*

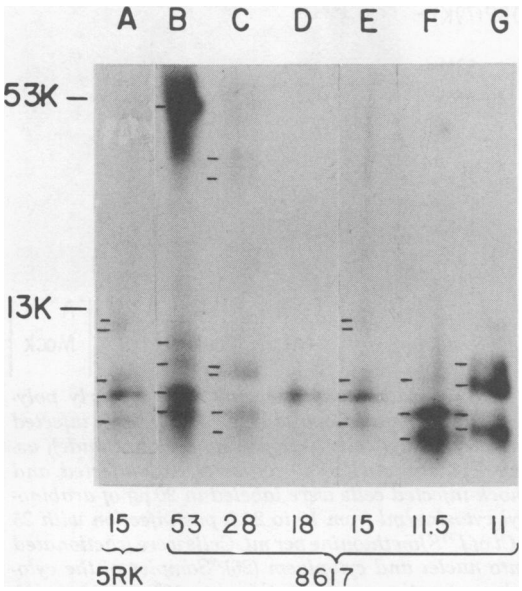

FIG. 7. *Partial proteolysis of early polypeptides immunoprecipitated by 5RK(I) and 8617 immunoglobulin G. Protease was used at 20 μg per lane. See the legend to Fig. 6 for details.*

polypeptide. Therefore, it is *possible*, although far from certain, that the 15K, 18K, and 53K polypeptides are related. The partial proteolysis pattern of the 28K polypeptide appeared to differ from those of the other polypeptides, which tends to suggest that the 28K polypeptide may be unrelated, or at least not highly related, to the other polypeptides.

(vi) 44K and 21K polypeptides. The 44K and 21K polypeptides precipitated by the T2C4 antiserum gave very similar partial proteolysis patterns (Fig. 6, lanes F and H), indicating that these polypeptides are highly related. The 21K (and 44K) polypeptide appears to be unrelated to any of the other polypeptides, except possibly the 19K.

(vii) 13.5 polypeptide. Partial proteolysis of the T2C4-specific 13.5K polypeptide reproducibly yielded three very diffuse bands (Fig. 6, lane K) that were not seen with the other polypeptides. This result suggests that the 13.5K polypeptide is unrelated to any of the other polypeptides.

(viii) 11.5K polypeptide. The 11.5K polypeptides precipitated by the T2C4 (Fig. 6, lane L) and 8617 (Fig. 7, lane F) antisera both gave two strong characteristic partial proteolysis bands. Thus, the two 11.5K polypeptides are highly related. The 11.5K polypeptide probably is not highly related to any of the other polypeptides, including the 53K, 28K, 18K, 15K, and 14.5K.

(ix) 11K polypeptide. The 11K polypeptide precipitated by the 8617 antiserum gave a distinct proteolysis pattern (Fig. 7, lane G) and therefore probably is unrelated (not highly related) to any of the other polypeptides.

Although not shown here, partial proteolysis of the 53K, 15K, and 11.5K polypeptides precip-

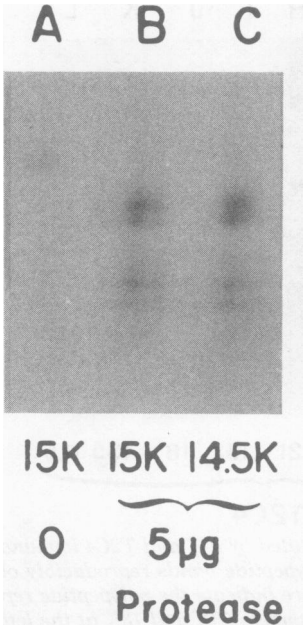

FIG. 8. *Partial proteolysis of 15K and 14.5K polypeptides immunoprecipitated by F17 immunoglobulin G. (A) 15K, no protease, (B) 15K, 5 μg of protease, (C) 14.5K, 5 μg of protease.*

itated by the F4 antiserum indicated that these polypeptides correspond to the same polypeptides precipitated by the other antisera.

Immunoprecipitation of 53K and 15K polypeptides from Ad2-infected monkey cells. As shown in lane C of Fig. 9, the 53K and 15K polypeptides were precipitated by the F17 antiserum from Ad2 early infected CV-1 (monkey) cells. The 53K and 15K polypeptides were also precipitated from CV-1 cells by a serum from hamsters bearing tumors induced by Ad1-SV40 hybrid virus (lane G). The Ad1-SV40 antiserum also precipitated polypeptides of 79K and 40K–46K, which probably represent the Ad2-coded DBP and its known 40K–50K subspecies. We have reported previously (26; Green et al., in press) that the same-sized polypeptides were precipitated from Ad2 early infected KB cells by the Ad1-SV40 antiserum. The precipitation of the 53K and 15K polypeptides from both human and monkey cells supports the notion that these polypeptides are virus coded.

DISCUSSION

We have identified Ad2-induced EPs by use of cycloheximide pretreatment and hypertonic labeling procedures that enhance the synthesis of EPs relative to host polypeptides, and by radioimmunoprecipitation with antisera against EPs in adenovirus-transformed and tumor cells. The combination of cycloheximide pretreatment and hypertonic labeling gave the greatest enhancement of EPs and thus should be useful for studying Ad2 EPs. All major and some minor EPs could be seen without either cycloheximide pretreatment or hypertonic labeling, implying that these EPs are not artifacts of the CH or hypertonic protocols. However, caution should be exercised in the use of these procedures because they might affect the metabolism of mRNA's and/or possible polypeptide precursors which generate the EPs.

Polypeptides of poliovirus, vesicular stomatitis virus, reovirus (48), and SV40 (15) are synthesized in hypertonic medium in preference to most host polypeptides. Hypertonic conditions apparently affect initiation of peptide chain synthesis (58), suggesting that initiation of viral polypeptides may occur more readily than initi-

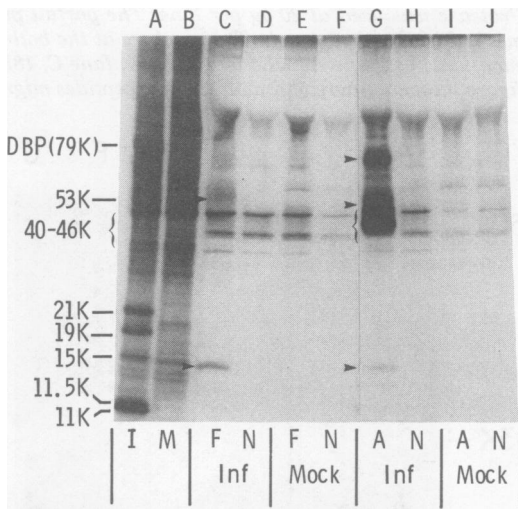

FIG. 9. *Radioimmunoprecipitation of early polypeptides from adenovirus type 2 (Ad2) early infected monkey (CV-1) cells (isotonic, no cycloheximide), using F17 and Ad1-SV40 antisera. Ad2-infected and mock-infected cells were labeled in 20 μg of arabinosyl cytosine/ml from 10 to 24 h postinfection with 25 μCi of [^{35}S]methionine per ml. Cells were fractionated into nuclei and cytoplasm (26). Samples of the cytoplasm fractions representing 4 × 10^6 cpm were immunoprecipitated using the rat F17 (F) and nonimmune (N) immunoglobulin G and the hamster Ad1-SV40 (A) and nonimmune (N) immunoglobulin G. The precipitates were analyzed by SDS-PAGE on 10-cm 5 to 21% linear gradient slabs. (A and B) Marker Ad2-infected and mock-infected (cycloheximide-treated) KB cell extracts. (C) Infected against F17. (D) Infected against nonimmune rat. (E) Mock against F17. (F) Mock against nonimmune rat. (G) Infected against Ad1-SV40. (H) Infected against nonimmune hamster. (I) Mock against Ad1-SV40. (J) Mock against nonimmune hamster.*

ation of cell polypeptides. The enhanced synthesis of Ad2 and Ad12 (11) EPs in hypertonic medium implies that initiation of adenovirus EPs differs from initiation of most host polypeptides; this is of interest because adenovirus EPs are thought to be synthesized entirely by host-cell mechanisms. Possibly, adenovirus early mRNA's have different structures than most cell mRNA's with respect to initiation of translation. Also, possibly one EP could modify the cell translation machinery so that adenovirus early mRNA's are translated more efficiently.

The increase by cycloheximide of Ad2 EP and mRNA (71) synthesis relative to host mRNA and polypeptide synthesis also implies that adenovirus early gene products are metabolized differently from most host-cell gene products. The EP and mRNA enhancement may be a general effect of inhibition of protein synthesis, because pretreatment of infected cells with either cycloheximide or pactamycin increased early protein synthesis in cell-free translation of early RNA (57). In our experiments, cycloheximide increased the labeling of 11K and 19K EPs much more than the other EPs (33). The large enhancement of 11K EP by cycloheximide pretreatment occurred in both infected human and infected monkey cells. Both these polypeptides are probably coded by early gene block 4 (see below), which suggests that cycloheximide may have a particularly strong effect on the metabolism of the products of this gene block.

Table 1 lists the polypeptides immunoprecipitated by the five transformed cell-specific antisera. In general, polypeptides of about the same gel mobility were detected by SDS-PAGE (see Fig. 1) and by immunoprecipitation. The data from the partial proteolysis analysis of the immunoprecipitated polypeptides permit the following firm conclusions. (i) The "same" (i.e., highly related) 53K polypeptide is precipitated by the F17, 8617, T2C4, and F4 antisera, but not by 5RK(I); there is no evidence for more than one 53K polypeptide. (ii) The "same" 15K polypeptide is precipitated by all five antisera, and the 15K polypeptide precipitated by the F17, 8617, T2C4, and F4 antisera is highly related to the 14.5K polypeptide precipitated by these antisera. (iii) The "same" 11.5K is precipitated by the 8617, T2C4, and F4 antisera. (iv) The 44K is highly related to the 21K glycopolypeptide, and is probably a dimer. The partial proteolysis data also allow some tentative conclusions. (i) It is probable that the 53K, 21K, 13.5K, 11.5K, and 11K major EPs are "unrelated" (i.e., not highly related) to one another or to any other EPs (including DBP); the 15K major polypeptide is "unrelated" to the 21K, 13.5K, 11.5K, and 11K polypeptides. (ii) It is also probable that the "same" 28K and 18K polypeptides are precipitated by F17, 8617, T2C4, and F4 antisera. It is difficult to draw conclusions about the relationships of the 53K, 28K, 18K, and 15K polypeptides, although the data tend to suggest that the 53K, 18K, and 15K polypeptides may be related and the 28K polypeptide may be unrelated to the others.

An indication of the gene locations for some of these polypeptides can be obtained by correlating the polypeptides precipitated by each antiserum with the gene blocks that are represented in each cell line. We emphasize that this approach strongly suggests, but does not prove, that immunoprecipitated EPs are virus coded, because we cannot exclude the possibility that the antisera might contain antibodies against virus-induced cell-coded polypeptides. However, we point out that synthesis of these EPs was enhanced by cycloheximide pretreatment and hypertonic labeling, which is further evidence that the EPs are virus coded. The strongest conclusions are possible about block 1, because all lines carry this block, and because F17 and 5RK(I) cells carry *only* this block. All antisera except 5RK(I) precipitated major polypeptides of 53K, 15K, and 14.5K, and minor polypeptides of 28K, 18K, and 12K. Thus, these polypeptides probably are coded within block 1. The 5RK(I) antiserum precipitated only the 15K polypeptide, which is highly related to the 15K and 14.5K polypeptides precipitated by the other antisera. 5RK(I) cells contain 70% of Ad5 *Hsu*I-G fragment and express 50% of G-fragment as RNA (10). Therefore, the 15K and 14.5K polypeptides may be coded, at least in part, within map position 0 to 8. Lewis et al. (44) mapped a 44K-50K polypeptide within map position 0 to 4.5 and a 15K within map position 4.5 to 11. These authors also observed 44K-50K and 15K polypeptides after translation of Ad2-specific RNA from F17 and 8617 cells (44). Lupker et al. (46) mapped a series of polypeptides (51K, 48K, 42K, 38K) and a 14K polypeptide within map position 0 to 4.5. Our 15K/14.5K, or one or more of the weak 15K-20K or 10K-15K polypeptides precipitated by the F17 and other antisera, could correspond to the 15K polypeptide of Lewis et al. (44) or the 14K polypeptide of Lupker et al. (46).

It is not known whether the 53K polypeptide corresponds to the 38K-51K polypeptide gene that maps at position 0 to 4.5 (32, 44, 46). The 5RK(I) antiserum did not precipitate the 53K polypeptide, which may suggest that the 53K polypeptide is not synthesized in 5RK(I) cells, which in turn may suggest that some or all of

the 53K gene lies to the right of map position 8. On the other hand, 5RK(I) cells may produce 53K polypeptide but in insufficient quantities to raise antibodies in rats. The 53K polypeptide could correspond to a 55K polypeptide linked to the 5′ termini of adenovirus DNA (52). There is strong evidence that this 55K polypeptide is synthesized during early stages of infection and thus is coded by a viral early gene or by a cell gene (Green et al., in press; T. Yamashita, M. Arens, and M. Green, J. Virol., in press). Peptide mapping experiments are in progress investigating the relationship of the 53K polypeptide to the 55K and to the 38K–51K polypeptides.

The T2C4, 8617, and F4 antisera precipitated an 11.5K polypeptide that appears to be unrelated to any of the other polypeptides. This polypeptide should be coded by either gene block 1 or 4, because 8617 cells do not carry blocks 2 or 3.

The 11K polypeptide was precipitated only by the 8617 antiserum, suggesting that it maps in gene block 1 or 4. Lewis et al. (32, 44) observed an 11K polypeptide after translation of *Eco*RI-C-specific Ad2 early mRNA. Possibly, their 11K and our 11K correspond, which would map our 11K in gene block 4. Our 11K is probably the 11K nucleoprotein described by several authors (9, 32, 53, 57), because it was precipitated much more strongly from the nucleoplasm than from the cytoplasm (data not shown).

It is difficult to assign tentative locations to the genes for the 21K glycopolypeptide and the 13.5K polypeptide precipitated by the T2C4 antiserum. Probably these are coded by gene block 3 or 4, although block 1 cannot be excluded. Lewis and colleagues (32, 44) mapped polypeptides of 13K, 14K, and 15.5K in block 3, and polypeptides of 11K, 21K, 19K, and 17K in block 4.

ACKNOWLEDGMENTS

We are grateful to P. Gallimore and A. van der Eb for providing us with the Ad2- and Ad5-transformed rat cell lines. We thank C. Devine for excellent technical assistance. We also thank H. Thornton for cell culture assistance and for the preparation of antisera against transformed cell extracts, R. V. Gilden for the Ad1-SV40 antiserum, and K. Brackmann, Y. Jeng, and Z. Gilead for participation in some of the experiments.

This work was supported by grants RO1-AI-01725, RO1-CA-21824, and partially by R23-CA-24710 from the National Institutes of Health. M.G. is a recipient of Research Career Award 5K5-AI-4739 from the National Institutes of Health.

ADDENDUM IN PROOF

We have recently established by two-dimensional chymotryptic or tryptic peptide mapping of polypeptides immunoprecipitated by F17 antiserum that (i) 15K is highly related to 53K, (ii) 53K is unrelated to the 38K–51K polypeptides coded by map positions 0 to 4.5, (iii) 18K and 14.5K are related to 15K, (iv) 28K and 12K are unrelated to each other or to any other F17-specific polypeptides, and (v) the two mobility forms of 53K are identical (M. Green, W. Wold, K. Brackmann, and M. Cartas, submitted for publication). We have also established that the 53K polypeptide linked to the termini of Ad2 DNA is unrelated to the Ad2-coded 53K, 38K–51K, or DBP (or 40K–50K subspecies of DBP) polypeptides (M. Green, K. Brackmann, W. Wold, and M. Cartas, submitted for publication).

LITERATURE CITED

1. **Arens, M., T. Yamashita, R. Padmanabhan, T. Tsuruo, and M. Green.** 1977. Adenovirus deoxyribonucleic acid replication. Characterization of the enzyme activities of a soluble replication complex. J. Biol. Chem. **252**:7947–7954.
2. **Axelrod, N.** 1978. Phosphoproteins of adenovirus 2. Virology **87**:366–383.
3. **Bablanian, R., and W. C. Russell.** 1974. Adenovirus polypeptide synthesis in the presence of non-replicating poliovirus. J. Gen. Virol. **24**:261–279.
4. **Berk, A. J., and P. A. Sharp.** 1978. Structure of the adenovirus 2 early mRNAs. Cell **14**:695–711.
5. **Bonner, W. M., and R. A. Laskey.** 1974. A film detection method for tritium-labeled proteins and nucleic acids in polyacrylamide gels. Eur. J. Biochem. **46**:83–88.
6. **Büttner, W., Z. Veres-Molnár, and M. Green.** 1976. Preparative isolation and mapping of adenovirus-2 early messenger RNA species. J. Mol. Biol. **107**:93–114.
7. **Carter, T. H., and R. A. Blanton.** 1978. Possible role of the 72,000-dalton DNA-binding protein in regulation of adenovirus type 5 early gene expression. J. Virol. **25**: 664–674.
8. **Chin, W. W., and J. V. Maizel, Jr.** 1976. The polypeptides of adenovirus. VII. Further studies of early polypeptides *in vivo* and localization of E2 and E2A to the cell plasma membrane. Virology **71**:518–530.
9. **Chin, W. W., and J. V. Maizel, Jr.** 1977. The polypeptides of adenovirus. VIII. The enrichment of E3 (11,000) in the nuclear matrix fraction. Virology **76**:79–89.
10. **Chinnadurai, G., K. Fujinaga, M. H. Rho, A. J. van der Eb, and M. Green.** 1978. Transcription of the transforming region of adenovirus type 5 in a rat cell line transformed by a small restriction fragment. J. Virol. **28**:1011–1014.
11. **Chinnadurai, G., Y.-H. Jeng, Z. Gilead, and M. Green.** 1977. Identification of early proteins induced by highly oncogenic human adenovirus 12 during lytic infection and in hamster tumors. Biochem. Biophys. Res. Commun. **74**:1199–1205.
12. **Chow, L. T., J. M., Roberts, J. B. Lewis, and T. R. Broker.** 1977. A map of cytoplasmic RNA transcripts from lytic adenovirus type 2, determined by electron microscopy of RNA:DNA hybrids. Cell **11**:819–836.
13. **Cleveland, D. W., S. G., Fischer, M. W. Kirschner, and U. K. Laemmli.** 1977. Peptide mapping by limited proteolysis in sodium dodecyl sulfate and analysis by gel electrophoresis. J. Biol. Chem. **252**:1102–1106.
14. **Craig, E. A., and H. J. Raskas.** 1976. Nuclear transcripts larger than the cytoplasmic mRNAs are specified by segments of the adenovirus genome coding for early functions. Cell **8**:205–213.
15. **Englund, J. M., M. K. Howett, and K. B. Tan.** 1975. Effect of hypertonic conditions on protein synthesis in cells productively infected with simian virus 40. J. Virol. **16**:1101–1107.
16. **Evans, R. M., N. Frazer, E. Ziff, J. Weber, M. Wilson, and J. E. Darnell.** 1977. The initiation sites for RNA transcription in Ad2 DNA. Cell **12**:733–739.
17. **Flint, S. J.** 1977. The topography and transcription of the adenovirus genome. Cell **10**:153–166.
18. **Flint, S. J.** 1977. Two "early" mRNA species in adenovi-

rus type 2-transformed rat cells. J. Virol. **23**:44–52.
19. **Flint, S. J., S. M. Berget, and P. A. Sharp.** 1976. Adenovirus transcription. III. Mapping of viral RNA sequences in cells productively infected by adenovirus type 5. Virology **72**:443–455.
20. **Flint, S. J., P. H. Gallimore, and P. A. Sharp.** 1975. Comparison of viral RNA sequences in adenovirus 2-transformed and lytically infected cells. J. Mol. Biol. **96**:47–68.
21. **Flint, S. J., J. Sambrook, J. F. Williams, and P. A. Sharp.** 1976. Viral nucleic acid sequences in transformed cells. IV. A study of the sequences of adenovirus 5 DNA and RNA in four lines of adenovirus 5-transformed rodent cells using specific fragments of the viral genome. Virology, **72**:456–470.
22. **Flint, S. J., and P. A. Sharp.** 1976. Adenovirus transcription. V. Quantitation of viral RNA sequences in adenovirus 2-infected and transformed cells. J. Mol. Biol. **106**:749–771.
23. **Gallimore, P. H.** 1974. Interactions of adenovirus type 2 with rat embryo cells. Permissiveness, transformation and *in vitro* characteristics of adenovirus transformed rat cells. J. Gen. Virol. **25**:263–273.
24. **Gallimore, P. H., P. A. Sharp, and J. Sambrook.** 1974. Viral DNA in transformed cells. II. A study of the sequences of adenovirus 2 DNA in nine lines of transformed rat cells using specific fragments of the viral genome. J. Mol. Biol. **89**:49–72.
25. **Gilden, R. V., J. Kern, A. E. Freeman, C. E. Martin, R. C. McAllister, H. C. Turner, and R. J. Huebner.** 1968. T and tumour antigens of adenovirus group C-infected and transformed cells. Nature (London) **219**: 517–518.
26. **Gilead, Z., Y.-H. Jeng, W. S. M. Wold, K. Sugawara, H. M. Rho, M. L. Harter, and M. Green.** 1976. Immunological identification of two adenovirus 2-induced early proteins possibly involved in cell transformation. Nature (London) **264**:263–266.
27. **Gilead Z., K. Sugawara, G. Shanmugam, and M. Green.** 1976. Synthesis of the adenovirus-coded DNA binding protein in infected cells. J. Virol. **18**:454–460.
28. **Ginsberg, H. S., M. J. Ensinger, R. S. Kauffman, A. J. Mayer, and U. Lundholm.** 1974. Cell transformation: a study of the regulation with types 5 and 12 adenovirus temperature-sensitive mutants. Cold Spring Harbor Symp. Quant. Biol. **39**:419–426.
29. **Graham, F. L., T. Harrison, and J. Williams.** 1978. Defective transforming capacity of adenovirus type 5 host-range mutants. Virology **86**:10–21.
30. **Grodzicker, T., C. Anderson, J. Sambrook, and M. B. Mathews.** 1977. The physical locations of structural genes in adenovirus DNA. Virology **80**:111–126.
31. **Harrison, T., F. Graham, and J. Williams.** 1977. Host-range mutants of adenovirus type 5 defective for growth in HeLa cells. Virology **77**:319–329.
32. **Harter, M. L., and J. B. Lewis.** 1978. Adenovirus type 2 early proteins synthesized in vitro and in vivo: identification in infected cells of the 38,000- to 50,000-molecular-weight protein encoded by the left end of the adenovirus type 2 genome. J. Virol. **26**:736–749.
33. **Harter, M. L., G. Shanmugam, W. S. M. Wold, and M. Green.** 1976. Detection of adenovirus type 2-induced early polypeptides using cycloheximide pretreatment to enhance viral protein syntheses. J. Virol. **19**: 232–242.
34. **Ishibashi, M., W. W. Chin, and J. V. Maizel, Jr.** 1977. The polypeptides of adenovirus. IX. Partial purification of early proteins and observations on late proteins of type 5 adenovirus. Virology **83**:1–15.
35. **Ishibashi, M., and J. V. Maizel, Jr.** 1974. The polypeptides of adenovirus. VI. Early and late glycopolypeptides. Virology **58**:345–361.
36. **Jeng, Y.-H., W. S. M. Wold, K. Sugawara, and M. Green.** 1978. Evidence for an adenovirus type 2-coded early glycoprotein. J. Virol. **28**:314–323.
37. **Jeng, Y.-H., W. S. M. Wold, K. Sugawara, Z. Gilead, and M. Green.** 1977. Adenovirus type 2 coded single-stranded DNA binding protein: in vivo phosphorylation and modification. J. Virol. **22**:402–411.
38. **Kitchingman, G. R., S.-P. Lai, and H. Westphal.** 1977. Loop structures in hybrids of early RNA and the separated strands of adenovirus DNA. Proc. Natl. Acad. Sci. U.S.A. **74**:4392–4395.
39. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
40. **Lassam, N. J., S. T. Bayley, and F. L. Graham.** 1978. Synthesis of DNA, late polypeptides, and infectious virus by host-range mutants of adenovirus 5 in nonpermissive cells. Virology **87**:463–467.
41. **Levinson, A. D., and A. J. Levine.** 1977. The isolation and identification of the adenovirus group C tumor antigens. Virology **76**:1–11.
42. **Levinson, A. D., and A. J. Levine.** 1977. The group C adenovirus tumor antigens: identification in infected and transformed cells and a peptide map analysis. Cell **11**:871–879.
43. **Levinson, A. D., E. H. Postel, and A. J. Levine.** 1977. *In vivo* and *in vitro* phosphorylation of the adenovirus type 5 single-strand-specific DNA-binding protein. Virology **79**:144–159.
44. **Lewis, J. B., J. F. Atkins, P. R. Baum, R. Solem, R. F. Gesteland, and C. W. Anderson.** 1976. Location and identification of the genes for adenovirus type 2 early polypeptides. Cell **7**:141–151.
45. **Linné, T., H. Jörnvall, and L. Philipson.** 1977. Purification and characterization of the phosphorylated DNA-binding protein from adenovirus-type-2-infected cells. Eur. J. Biochem. **76**:481–490.
46. **Lupker, J. H., A. Davis, H. Jochemsen, and A. J. van der Eb.** 1977. Identification of adenovirus type 5 specific transforming proteins. INSERM **69**:223–232.
47. **Neuwald, P. D., J. Meyer, J. V. Maizel, Jr., and H. Westphal.** 1977. Early gene expression of adenovirus 2: R-loop mapping of mRNA and time course of viral DNA, mRNA, and protein synthesis. J. Virol. **21**:1019–1030.
48. **Nuss, D. L., H. Oppermann, and G. Koch.** 1975. Selective blockage of initiation of host protein synthesis in RNA-virus-infected cells. Proc. Natl. Acad. Sci. U.S.A. **72**:1258–1262.
49. **Pettersson, U., and M. B. Mathews.** 1977. The gene and messenger RNA for adenovirus polypeptide IX. Cell **12**:741–750.
50. **Pettersson, U., C. Tibbetts, and L. Philpson.** 1976. Hybridization maps of early and late messenger RNA sequences on the adenovirus type 2 genome. J. Mol. Biol. **101**:479–501.
51. **Philipson, L., U. Petterson, and U. Linberg.** 1975. Molecular biology of adenoviruses. Virol. Monogr. **14**: 1–115.
52. **Rekosh, D. M. K., W. C. Russell, A. J. D. Bellet, and A. J. Robinson.** 1977. Identification of a protein linked to the ends of adenovirus DNA. Cell **11**:283–295.
53. **Rho, H. M., J.-H. Yeng, W. S. M. Wold, and M. Green.** 1977. Association of adenovirus type 2 early proteins with a soluble complex that synthesizes adenovirus DNA *in vitro*. Biochem. Biophys. Res. Commun. **79**: 422–428.
54. **Rosenwirth, B., C. Anderson, and A. J. Levine.** 1976. Tryptic fingerprint analysis of adenovirus types 2, 5, and 12 DNA-binding proteins. Virology **69**:617–625.
55. **Russell, W. C., and G. E. Blair.** 1977. Polypeptide phosphorylation in adenovirus-infected cells. J. Gen. Virol. **34**:19–35.
56. **Russell, W. C., and J. J. Skehel.** 1972. The polypeptides

of adenovirus-infected cells. J. Gen. Virol. **15**:45–57.

57. **Saborio, J. L., and B. Öberg.** 1976. In vivo and in vitro synthesis of adenovirus type 2 early proteins. J. Virol. **17**:865–875.
58. **Saborio, J. L., S.-S. Pong, and G. Koch.** 1974. Selective and reversible inhibition of initiation of protein synthesis in mammalian cells. J. Mol. Biol. **85**:195–211.
59. **Shanmugam, G., S. Bhaduri, M. Arens, and M. Green.** 1975. DNA binding proteins in the cytoplasm and in a nuclear membrane complex isolated from uninfected and adenovirus 2 infected cells. Biochemistry **14**:332–337.
60. **Studier, F. W.** 1973. Analysis of bacteriophage T7 early RNAs and proteins on slab gels. J. Mol. Biol. **79**:237–248.
61. **Sugawara, K., Z. Gilead, and M. Green.** 1977. Purification and molecular characterization of adenovirus type 2 DNA-binding protein. J. Virol. **21**:338–346.
62. **Sugawara, K., Z. Gilead, W. S. M. Wold, and M. Green.** 1977. Immunofluorescence study of the adenovirus type 2 single-stranded DNA binding protein in infected and transformed cells. J. Virol. **22**:527–539.
63. **van der Eb, A. J., and A. Houweling.** 1977. Transformation with specific fragments of adenovirus DNAs. II. Analysis of the viral sequences present in cells transformed with a 7% fragment of adenovirus 5 DNA. Gene **2**:133–146.
64. **van der Eb, A. J., C. Mulder, F. L. Graham, and A. Houweling.** 1977. Transformation with specific fragments of adenovirus DNA. I. Isolation of specific fragments with transforming activity of adenovirus 2 and 5 DNA. Gene **2**:115–132.
65. **van der Vliet, P. C., and A. J. Levine.** 1973. DNA-binding proteins specific for cells infected by adenoviruses. Nature (London) New Biol. **246**:170–174.
66. **van der Vliet, P. C., A. J. Levine, M. J. Ensinger, and H. S. Ginsberg.** 1975. Thermolabile DNA binding proteins from cells infected with a temperature-sensitive mutant of adenovirus defective in viral DNA synthesis. J. Virol. **15**:348–354.
67. **van der Vliet, P. C., and J. S. Sussenbach.** 1976. An adenovirus 5 gene function required for initiation of viral DNA replication. Virology **80**:98–110.
68. **van der Vliet, P. C., J. Zandberg, and H. S. Jansz.** 1977. Evidence for a function of the adenovirus DNA-binding protein in initiation of DNA synthesis as well as the elongation of nascent DNA chains. Virology **80**: 98–110.
69. **Walter, G., and J. V. Maizel, Jr.** 1974. The polypeptides of adenovirus. IV. Detection of early and late virus-induced polypeptides and their distribution in subcellular fractions. Virology **57**:402–408.
70. **Williams, J. F., C. S. H. Young, and P. E. Austin.** 1974. Genetic analysis of human adenovirus type 5 in permissive and nonpermissive cells. Cold Spring Harbor Symp. Quant. Biol. **39**:427–437.
71. **Wold, W. S. M., M. Green, K. H. Brackmann, C. Devine, and M. A. Cartas.** 1977. Adenovirus 2 early nuclear and mRNA: kinetic estimation of *l* and *r* DNA strand fractions complementary to different abundance classes of viral RNA. J. Virol. **23**:616–625.
72. **Wold, W. S. M., M. Green, and W. Büttner.** 1978. Adenoviruses, p. 673–768. *In* D. P. Nayak (ed.), The molecular biology of animal viruses. Marcel Dekker, Inc., New York.