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Simian Virus 40-Specific Polypeptides in Ad2+ND1- and Ad2+ND4-Infected Cells

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A comparison of the proteins synthesized in human cells at late times after infection with adenovirus (Ad2) and with the adeno-simian virus 40 (SV40) hybrid viruses revealed polypeptides of 30,000 and 92,000 molecular weight specific for the hybrid viruses Ad2+ND1 and Ad2+ND4, respectively. Cell-free translation of SV40-specific mRNA, prepared from these cells by hybridization of total cytoplasmic RNA to SV40 DNA, showed that the mRNA's specifying these two polypeptides were at least partially encoded by the SV40 portion of the hybrid viruses. Cell-free translation of SV40-specific mRNA prepared from monkey cells infected with SV40 produced polypeptides of 40,000, 43,000, 48,500, and 92,000 molecular weight. The SV40 and Ad2+ND4 92,000-molecular-weight polypeptides made in vitro were very similar in electrophoretic mobility in sodium dodecyl sulfate-polyacrylamide gels to the polypeptide precipitated by Tegtmeyer (1974) with SV40 anti-T serum.

The efficient growth of adenovirus on monkey cells requires a simian virus 40 (SV40) early gene product (24). Analysis of human adenovirus serotypes adapted to growth on monkey cells revealed the existence of various adeno-SV40 hybrid viruses, among them the five non-defective viruses described by Lewis et al. (12, 13). Each of these five viruses grows normally, without helper adenovirus 2 (Ad2), on human cells, and three of them (Ad2+ND1, Ad2+ND2, and Ad2+ND4) also grow to high titers on monkey cells. These three hybrids each have a small section of the Ad2 genome replaced by a segment of the early region of the SV40 genome (8, 11, 19), and each expresses one or several SV40 early antigens (13, 14). Ad2+ND1 contains the region 0.11 to 0.28 fractional units on the conventional SV40 map and expresses only SV40 U antigen. Ad2+ND4 contains the region 0.11 to 0.59, practically the entire SV40 early region, and expresses SV40 U, TSTA, and T antigens.

The SV40 T antigen has now been purified manyfold (16, 20), and recently Tegtmeyer (27) has reported the selective precipitation of a 100,000-molecular-weight (100K) polypeptide from SV40-infected cells by SV40 anti-T sera.

It has become possible to identify virus-coded polypeptides by cell-free protein synthesis programed by mRNA selected by hybridization to viral DNA (5, 15, 23). Purified SV40 early mRNA has recently been shown to program the synthesis in vitro of a polypeptide of 85 to 90K mol (22, 26), which can be specifically precipitated with SV40 anti-T serum.

We have used the translation of hybridization-selected mRNA to identify the polypeptide products of SV40-specific mRNA from SV40-infected monkey cells and from Ad2+ND1- and Ad2+ND4-infected human cells. The results described below are consistent with the hypothesis that the early region of SV40 programs the synthesis of a 90 to 100K polypeptide corresponding, at least in part, to the SV40 T antigen. A polypeptide of similar apparent size is expressed by Ad2+ND4 in vivo, and its mRNA hybridizes to SV40 DNA. A polypeptide of 30K (7, 17), which may be a fragment of the larger Ad2+ND4 polypeptide, is expressed in Ad2+-ND1-infected cells, and its mRNA also hybridizes to SV40 DNA.

MATERIALS AND METHODS

Cells and viruses. KB cells (originally derived from a human carcinoma) were grown in suspension, using Joklik modified Eagle medium (Grand Island Biological Co., catalogue no. F-12) supplemented with 5% horse serum. HeLa cells (also derived from a human carcinoma) and CV-1 cells (a continuous line of African green monkey kidney cells) were propagated as monolayers in Dulbecco modified Eagle medium (GIBCO, catalogue no. C-21) supplemented with penicillin (100 U/ml), streptomycin (100 μg/ml), and 10% calf serum (HeLa) or 10% fetal calf serum (CV-1). SV40 strain 777, obtained from J. Sambrook, was propagated at low multiplicity (less than 1 PFU/cell) on CV-1 cells.

Ad2+ND1, originally obtained from A. M. Lewis, has been propagated in KB cells at moderate multiplicities (1 to 50 PFU/cell), as has the Ad2 (originally obtained from U. Pettersson). Ad2+ND4 (pool B377) was used in the laboratory of A. M. Lewis (National Institutes of Health) to infect KB cells for the preparation of Ad2+ND4 RNA and for labeling with [^{35}S]methionine to observe virus-induced protein synthesis in vivo. For comparison with Ad2+ND4 protein synthesis, other cultures of KB cells were infected with the Ad2 stock of A. M. Lewis (pool B378). Virus titers were determined by plaque assay on CV-1 cells (SV40), HeLa cells (Ad2+ND1), or human embryonic kidney cells (Ad2 and Ad2+ND4), essentially as described by Grodzicker et al. (7). Suspension cultures were infected by addition of virus stocks to a cell suspension at 6×10^5 to 8×10^5 cells/ml. After incubation for 1 h at 37 C, the culture was diluted with an equal volume of fresh medium containing 5% horse serum. Monolayers were infected by removal of the medium and addition of 0.5 ml of virus stock (SV40) or virus stock diluted in phosphate-buffered saline (PBS) (adenoviruses). After adsorption at 37 C for 1 h, the medium containing unadsorbed virus (adenoviruses only) was removed, and 10 ml of fresh medium containing 10% serum was added per 10-cm plate.

Isolation of nucleic acids and cell-free protein synthesis. SV40 DNA was isolated from purified virions as described by Keller (manuscript in preparation). Ad2 DNA and Ad2+ND1 DNA were isolated from purified virions after Pronase digestion and phenol extraction as described by Pettersson and Sambrook (21). Cytoplasmic RNA was isolated from Nonidet P-40-disrupted KB cells after removal of nuclei as previously described (2). Monolayers of CV-1 cells were scraped into ice-cold PBS containing 1 mM EDTA, washed in PBS, and processed as described for KB cells. The hybridization technique used to obtain purified RNA for translation in vitro has been described (15), as has the fractionated mammalian cell-free translation system (2, 3). Hybridized RNA was recovered by adsorption on oligo(dT)-cellulose columns (15).

Radioactive labeling of cultures and sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis. Cultures were labeled with [^{35}S]methionine at 25 to 100 μCi/ml in Dulbecco modified Eagle medium (2.0 ml/6-cm plate) containing 1.5 μg of methionine per ml and 5% serum; suspension cultures were centrifuged at room temperature and resuspended in the above medium at 2×10^6 to 5×10^6 cells/ml. Cell extracts were prepared for SDS-polyacrylamide gel electrophoresis as previously described (1).

RESULTS

In vivo synthesis. Lewis et al. (13) reported that the early SV40 functions contained within the Ad2-SV40 hybrids (e.g., Ad2+ND1 and Ad2+ND4) are expressed late in infection. Since Ad2 infection of human cells inhibits host protein synthesis late in infection, permitting ready detection of most late viral products by electrophoresis of radioactively labeled infected-cell extracts in SDS-polyacrylamide gels, one might expect to be able to detect the protein product(s) corresponding to the SV40 region inserted in the Ad2-SV40 hybrids by examining extracts of infected cells labeled with a radioactive amino acid late in the infection.

HeLa or KB cells were infected with Ad2, Ad2+ND1, or Ad2+ND4 and subsequently pulse labeled with [^{35}S]methionine for 1 h late in infection (see legend to Fig. 1). The labeled cells were harvested, disrupted by heating in SDS and dithiothreitol, and subjected to electrophoresis through SDS-polyacrylamide gels. Ad2+ND4-infected cells contained a major polypeptide of 92K that was not present in Ad2- or Ad2+ND1-infected cells, nor was there a major polypeptide of this molecular weight in uninfected cells (Fig. 1). Also observed in Ad2+ND4- (Fig. 1c, d) but not in Ad2-infected cells (Fig. 1b) was a polypeptide of 60K (band immediately below IV in Fig. 1c, d), the same apparent molecular weight as a prominent host polypeptide. At this point we cannot be certain whether this polypeptide originated from the SV40 insertion in Ad2+ND4, from enhanced synthesis of the minor Ad2 virion component IVa_1 (1), from an altered (or processed) Ad2 protein, or from the host. However, a 60K polypeptide was translated from SV40-specific Ad2+ND4 mRNA (see below), and Walter and Martin have recently described a 56K protein specific for Ad2+ND4-infected cells (28).

It is apparent from Fig. 1 that one major Ad2-coded viral polypeptide, the fiber (IV), was present in significantly reduced amounts as compared with Ad2-infected cells. Reduced amounts of fiber polypeptide in Ad2+ND4-infected cells was apparent from both the autoradiogram and the Coomassie blue-stained gel pattern (not shown). The fiber protein has recently been shown to map just right of the SV40 insertion in the Ad2+ND4 hybrid as the Ad2 map is customarily drawn (15, 18; Grodzicker, Sambrook, and Anderson, manuscript in preparation).

Ad2+ND1-infected cells also contained a virus-induced polypeptide (30K) that was not found in Ad2- or Ad2+ND4-infected cells (Fig. 1); however, uninfected HeLa cells contained a prominent polypeptide band of the same molecular weight. Previously it was reported that this Ad2+ND1-induced polypeptide was not observed in cells infected with a host-range mutant of Ad2+ND1 that is unable to grow in African green monkey kidney cells and is also deficient in the induction of SV40 U antigen (7). Ad2+ND1-infected cells exhibited normal amounts of fiber synthesis, and no other late

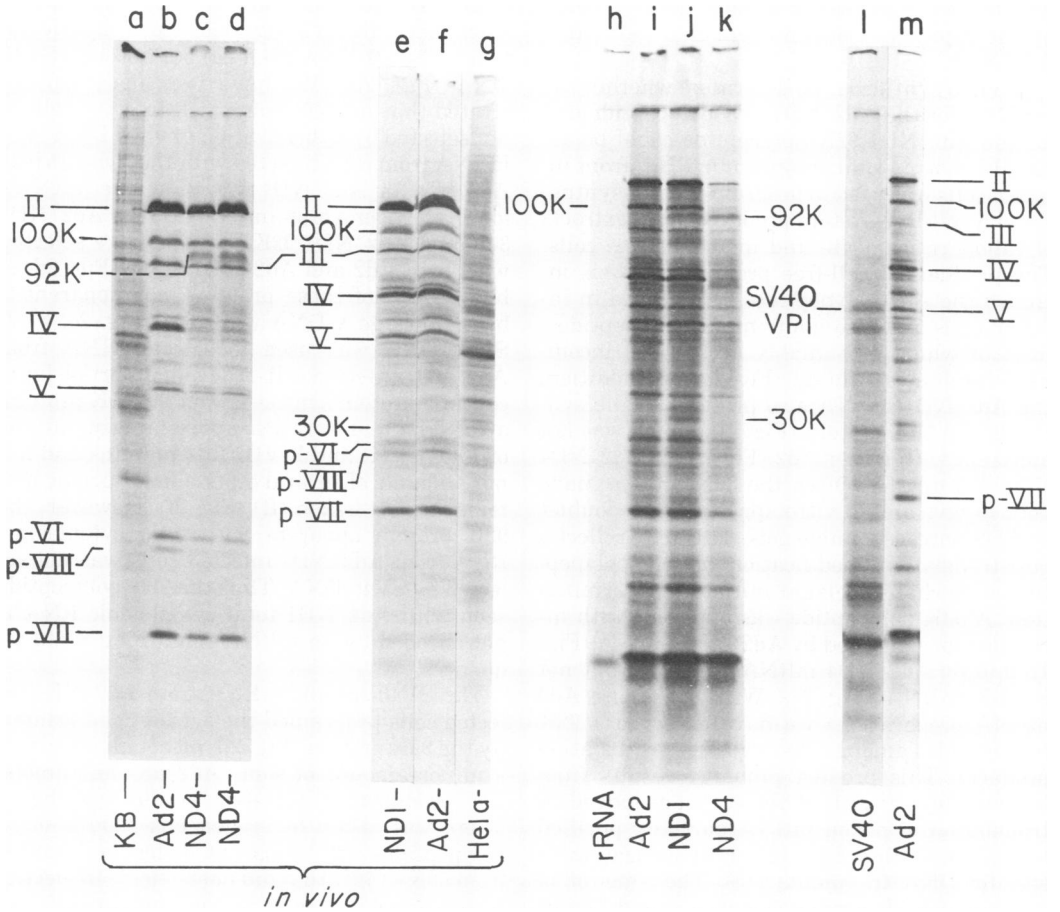

FIG. 1. *Synthesis of Ad2, Ad2+ND1, and Ad2+ND4 proteins in vivo and in vitro. Extracts of cells labeled in vivo for 1 h with [35S]methionine were analyzed by electrophoresis on 17.5% polyacrylamide gels (a-g), as described by Anderson et al. (1). The pattern of polypeptide synthesis is shown for uninfected KB cells (a); uninfected HeLa cells (g); KB cells labeled 30 h after infection with 37 PFU of Ad2 per cell (b) or 33 h after infection with 2 PFU of Ad2+ND4 per cell in two different experiments (c, d); and HeLa cells labeled 24 h after infection with 100 PFU of Ad2 (f) or of Ad2+ND1 (e) per cell. RNA isolated from infected KB cells grown in spinner cultures was used to program cell-free protein synthesis in extracts of mammalian cells (h-m), as previously described by Anderson et al. (2) and Atkins et al. (3). A 25-μl reaction mixture for cell-free protein synthesis contained 0.125 A_{260} units of ribosomal subunits, 2 μl of pH 5 enzyme (containing 34 μg of protein and 2 μg of RNA), 0.375 μl of rabbit reticulocyte ribosome wash precipitating at 30 to 40% $(NH_4)_2SO_4$ (8 μg of protein), and 1 μl of reticulocyte ribosomal wash precipitating between 40 and 70% $(NH_4)_2SO_4$ (22 μg of protein). The other ingredients were 1.0 mM ATP, 0.4 mM GTP, 10 mM creatine phosphate, 20 μg of creatine kinase per ml, 30 mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid), pH 7.2, 20 mM NH_4Cl, 87 mM KCl, 2.0 mM $Mg(OAc)_2$, 0.6 mM spermidine, 0.5 mM dithiothreitol, 30 μM each of 19 amino acids (minus methionine), and 5 to 15 μCi of [35S]methionine at greater than 100-Ci/mmol specific activity. Incubation was for 90 min at 37 C. Cell-free protein synthesis was programed by 2 μg of Escherichia coli rRNA (h), 6 μg of total cytoplasmic RNA isolated from KB cells 23 h after infection with Ad2 (i), 12 μg of total cytoplasmic RNA from KB cells 26 h after infection with Ad2+ND1 (j), 11 μg of cytoplasmic RNA from KB cells 32 h after infection with Ad2+ND4 (k), and, in a separate experiment, 5.2 μg of total cytoplasmic RNA from CV-1 cells 28 h after infection with SV40 (l) and 6 μg of total cytoplasmic RNA from KB cells 23 h after infection with Ad2 (m). The incorporation of [35S]methionine into trichloroacetic acid-insoluble radioactivity was 1.1% (h), 6.1% (i), 5.1% (j), and 3.9% (k). The incorporation was 5.2% (l) and 17.5% (m) versus 2.2% for rRNA in that experiment. A 3-μl amount of each reaction mixture was loaded onto an SDS-17.5% polyacrylamide gel.*

Ad2 functions appeared to have been affected by the deletion insertion event that generated Ad2$^+$ND1.

In vitro synthesis. To determine whether the mRNA's for the Ad2$^+$ND1 30K polypeptide and for the Ad2$^+$ND4 92K polypeptide could be assayed in vitro, total cytoplasmic RNA from infected cells was used to program protein synthesis in a cell-free system prepared from extracts of rabbit reticulocyte and mouse ascites cells. The products of cell-free protein synthesis included the Ad2 polypeptides synthesized in infected cells and also many minor polypeptides, most of which presumably arose from incomplete protein synthesis (Fig. 1i). In addition, the Ad2$^+$ND1 mRNA also programed the synthesis of a 30K protein (Fig. 1j), which was not apparent with either Ad2 (Fig. 1i) or Ad2$^+$ND4 (Fig. 1k) mRNA. Often the 30K protein made both in vivo and in vitro appeared as a doublet on SDS-polyacrylamide gels. This may reflect a post-translation modification of the polypeptide, or perhaps aberrant initiation or termination. A 92K polypeptide was apparent with incorporations directed by Ad2$^+$ND1 mRNA (Fig. 1j) and by Ad2$^+$ND4 mRNA (Fig. 1k), but not by Ad2 mRNA (Fig. 1i). With other late Ad2 mRNA preparations we have observed a 92K polypeptide among the cell-free translation products. This product probably results from incomplete synthesis of hexon (II), as judged by translation of hexon mRNA partially purified by sedimentation rate (2) or hybridization to specific DNA fragments (15). The amount of 92K polypeptide synthesized varied in different preparations of Ad2 or Ad2$^+$ND1 mRNA, but was always substantially less than the hexon. By contrast, the 92K product was synthesized in comparatively large amounts in response to Ad2$^+$ND4 RNA. Selection of SV40-specific mRNA (see below) was used to differentiate between incomplete synthesis of hexon and Ad2$^+$ND4 92K. The generally lower level of protein synthesis with Ad2$^+$ND4 RNA (Fig. 1k) compared with Ad2$^+$ND1 and Ad2 RNA may be either a characteristic of the preparation of Ad2$^+$ND4 RNA used in these experiments or a general characteristic of Ad2$^+$ND4 infection. Total cytoplasmic RNA from SV40-infected CV-1 cells programed the synthesis in vitro of SV40 VP1 (Fig. 1l), but other SV40-specific products were not apparent above the background of host-coded proteins.

Purification of SV40-specific mRNA. To determine which virus-specific proteins are indeed virus coded, mRNA was first selected by hybridization to DNA and then used to program cell-free protein synthesis. By this procedure the Ad2 proteins hexon (II), 100K, penton base (III), etc., were shown to be virus coded, since their mRNA's were selected by hybridization with Ad2 DNA (Fig. 2a) but not with SV40 DNA (Fig. 2b). We have previously demonstrated that mRNA from mock-infected cells is not selected by hybridization to viral DNA (15). If RNA from cells infected with the hybrid virus Ad2$^+$ND1 or Ad2$^+$ND4 was used, the mRNA's for all the adeno proteins and also the Ad2$^+$ND1 30K and Ad2$^+$ND4 92K proteins were selected with both Ad2 and Ad2$^+$ND1 DNA (Fig. 2d, e, h, i). None of these proteins was apparent if bacteriophage λ DNA was used (Fig. 2c, g). If SV40 DNA was used to select mRNA from Ad2$^+$ND1-infected cells, the major part by far of cell-free protein synthesis was the 30K protein, although there were also appreciable quantities of 100K, 38K, and P-VIII Ad2 proteins and minor amounts of several other adeno-specific proteins such as hexon (II) (Fig. 2f). However, the 92K protein apparent with total cytoplasmic RNA from Ad2$^+$ND1-infected cells was not selected by SV40 DNA. Thus the 92K polypeptide seen with Ad2$^+$ND1 total cytoplasmic RNA is not encoded by an mRNA containing SV40 sequences.

The SV40-specific mRNA from Ad2$^+$ND4-infected cells programed the synthesis of primarily the 92K protein (Fig. 2j), although there was a minor amount of some Ad2 proteins among the products of cell-free protein synthesis. There was also a heterogeneous population of polypeptides with apparent molecular weights from 60K to 70K that did not coincide in electrophoretic mobility with any prominent Ad2 proteins. These polypeptides may correspond to the polypeptides of similar size found by Prives et al. (22) and Smith et al. (26) to be among the SV40-coded early proteins immunoprecipitated by anti-T sera. These polypeptides could represent incomplete translation of the 92K mRNA, translation of a fragment of this mRNA, or post-translational cleavage of the polypeptide. The prominent band at 60K could be related to the 60K polypeptide seen in vivo (Fig. 1c, d; 28).

If mRNA from SV40-infected cells was first hybridized to SV40 DNA and then used to program cell-free synthesis, the predominant product was VP1, but there were additional polypeptides of 40K, 43K, and 92K (Fig. 2l, m). A small amount of these polypeptides was also synthesized with mRNA selected with Ad2 DNA (Fig. 2k), but this was probably due to a small quantity of SV40 DNA contaminating the RNA preparation, since these polypeptides were not seen after hybridization to Ad2 DNA if mRNA was first purified by affinity to oligo(dT)-cellulose (Fig. 2n).

A more precise comparison of the 92K pro-

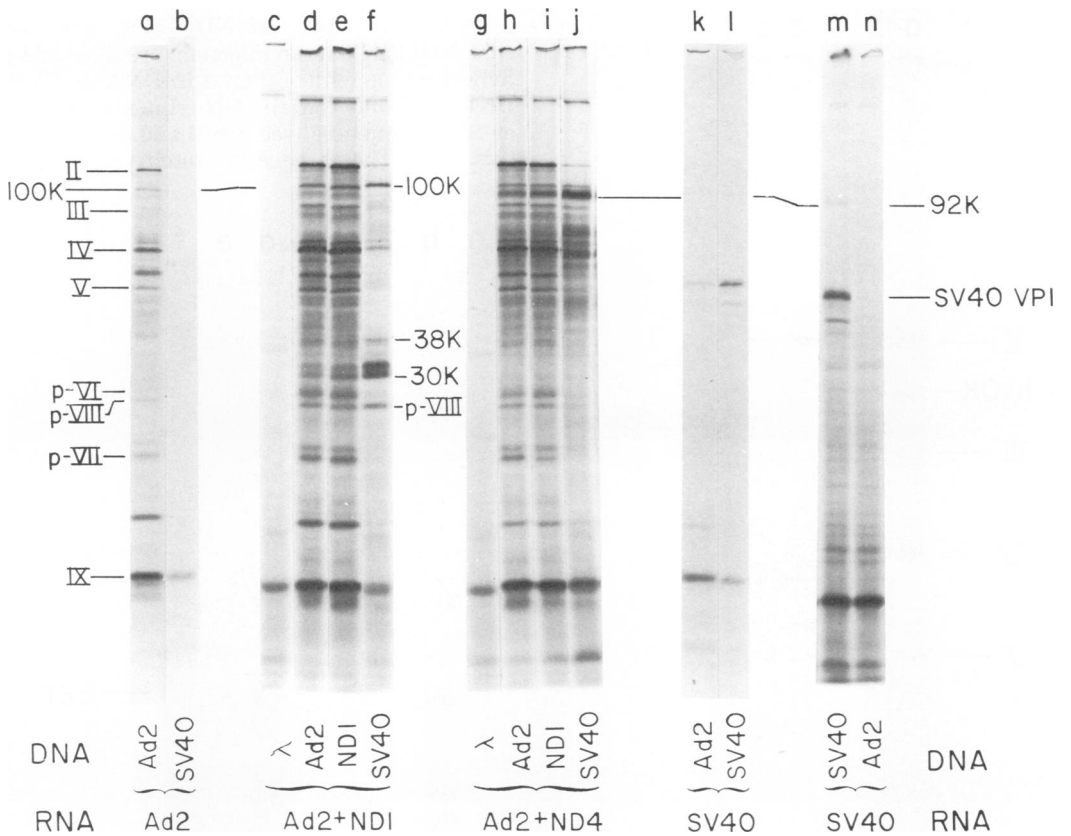

FIG. 2. *Products of cell-free protein synthesis programed by hybridization-selected RNA. A 100-μg amount of Ad2 DNA (a, d, h, k, n), 100 μg of Ad2+ND1 DNA (e, i), 100 μg of bacteriophage λ DNA (c, g), or 16 μg of SV40 DNA (b, f, j, l, m) was used to select complementary mRNA species by nucleic acid hybridization. A 960-μg amount of total cytoplasmic RNA from Ad2-infected cells (a, b), 1,220 μg of RNA from Ad2+ND1-infected cells (c–f), 1,030 μg of RNA from Ad2+ND4-infected cells (g–j), or 945 μg of RNA from SV40-infected cells (k, l) was used for each hybridization. These preparations of cytoplasmic RNA are the ones described in the legend to Fig. 1. Poly(A)-containing RNA isolated by oligo(dT)-cellulose columns starting with 650 μg of RNA from SV40-infected CV-1 cells was used in each of the hybridizations in (m, n). Sixteen percent (a, b) or 8% (c–m) of the hybridization-selected RNA was used to program cell-free protein synthesis as described in the legend to Fig. 1. The incorporation of [^{35}S]methionine into trichloroacetic acid-insoluble radioactivity was (a) 2.8%, (b) 0.9%, (c) 1.5%, (d) 4.0%, (e) 4.2%, (f) 2.6%, (g) 1.4%, (h) 4.8%, (i) 5.0%, (j) 4.3%, (k) 1.4%, (l) 1.8%, (m) 3.7%, and (n) 4.4%. If E. coli rRNA was added to an aliquot of the protein synthesis mixture instead of exogenous mRNA, the incorporation of [^{35}S]methionine was 2.2% (a, b, k, l), 1.1% (c–j), and 2.8% (m, n). With saturating amounts of different preparations of total cytoplasmic RNA as exogenous mRNA, the incorporation was 5 to 18% (a, b, k, l), 4 to 6% (c–j), and 20% (m, n). A 3-μl amount of each reaction mixture was loaded onto an SDS-17.5% polyacrylamide gel for electrophoresis, and the dried gel was exposed for autoradiography for 1 day (a, b, k, l), 3 days (m, n), or 5 days (c–j).*

teins on 12.5% polyacrylamide gels (Fig. 3) showed co-migration of the in vitro and in vivo counterparts.

The 92K product synthesized in vitro with SV40-specific RNA from Ad2+ND4-infected cells (Fig. 4b, d) also had the same electrophoretic mobility as the product of cell-free protein synthesis programed with SV40-specific mRNA from SV40-infected CV-1 cells (Fig. 4f). Both the Ad2+ND4 and SV40 in vitro-synthesized 92K polypeptides migrated very slightly ahead of the polypeptide precipitated from SV40-infected cells with SV40 anti-T serum. This polypeptide, which Tegtmeyer (27) has characterized as 100K, has an apparent molecular weight of 94K with our gel system by comparison with the Ad2 proteins II, 100K, and III.

DISCUSSION

Ad2+ND1 contains a small segment of the SV40 early region inserted into the Ad2 genome

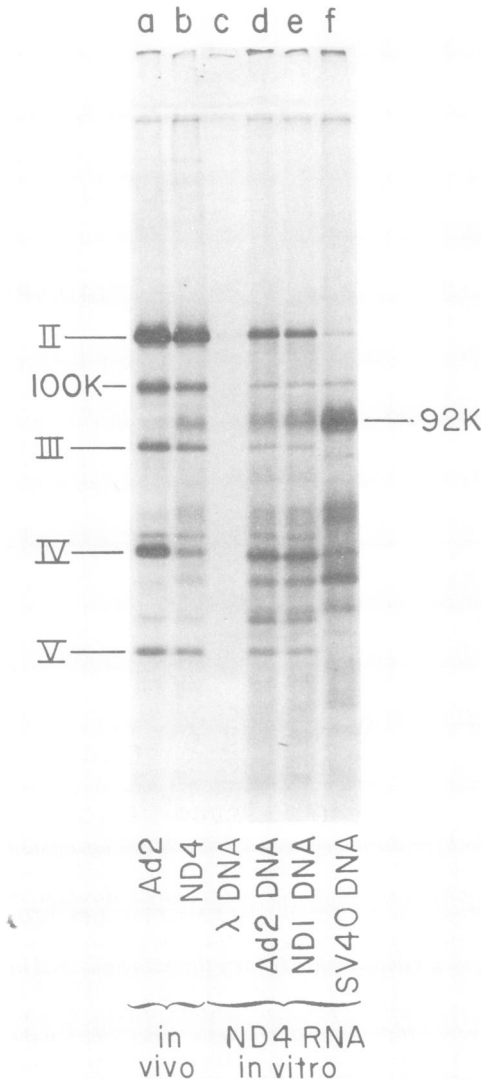

FIG. 3. *Comparison on a 12.5% polyacrylamide gel of Ad2⁺ND4 92K polypeptide synthesized in vivo and in vitro. Extracts of cells infected with Ad2 (a) and Ad2⁺ND4 (b) were prepared as described in the legend to Fig. 1. (c–f) Aliquots of the same samples used for the 17.5% polyacrylamide gel in Fig. 2g–j. The autoradiogram was exposed for 5 days.*

near position 80 to 87 on the standard Ad2 map, just to the left of the fiber gene. The direction of transcription in this region of the Ad2 genome is from left to right, and the orientation of the SV40 insertion is such that the sense strand of the SV40 early region would also be transcribed in this direction (8). Ad2 transcription may proceed without interruption into the SV40 insertion, producing an RNA molecule with both SV40 and Ad2 sequences. The fact that the mRNA's for the Ad2⁺ND1 30K and for Ad2⁺ND4 92K (late in infection) are selected by hybridization with either Ad2 DNA or SV40 DNA, and thus contain Ad2 sequences as well as SV40 sequences (see Fig. 2 and 3), supports this view. The three Ad2 proteins present in

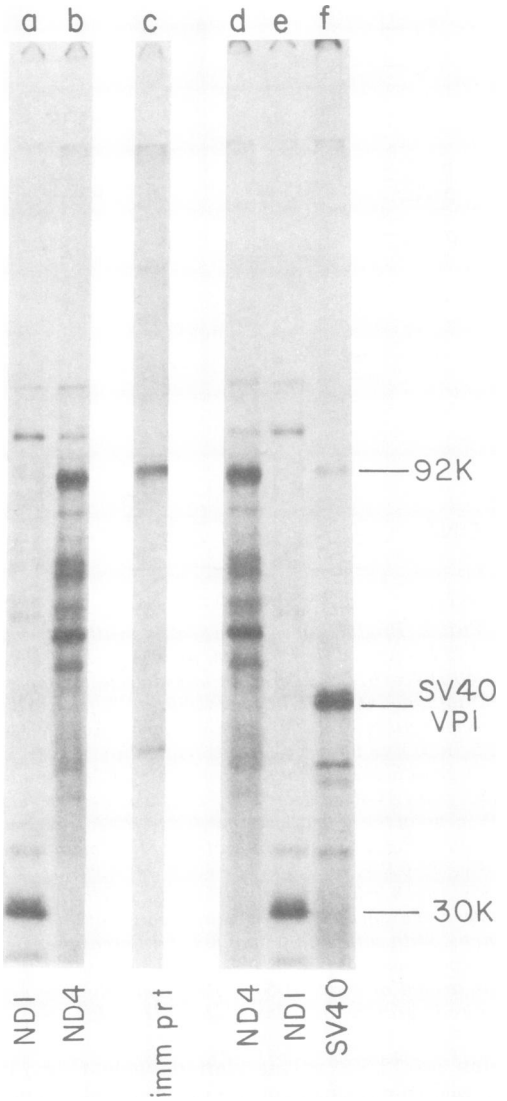

FIG. 4. *Comparison on a 10% polyacrylamide gel of products of cell-free protein synthesis with protein precipitated from extracts of SV40-infected CV-1 cells by anti-T serum. The sample of the immune precipitate (c) was a generous gift of Peter Tegtmeyer. The products of cell-free protein synthesis shown in tracks a and e were aliquots of the samples used for the 17.5% gel in Fig. 2f; the products in tracks b and d were aliquots of the sample in Fig. 2j; the product in f was an aliquot of the sample in Fig. 2m.*

largest quantity among the translation products of SV40-specific Ad2+ND1 mRNA (100K, 38K, and P-VIII) all map immediately to the left of the SV40 insertion (15). The co-purification of this subset of Ad2 mRNA's and the 30K mRNA suggests that at least some of these Ad2 mRNA molecules have sequences extending into the SV40 region of Ad2+ND1. Clearly, the presence of the inserted SV40 sequences does alter the metabolism of Ad2-specific RNA in infected cells. Flint et al. (6) have shown that early in Ad2+ND1 and Ad2+ND3 infections there are present in the cytoplasm RNA molecules complementary to about 1,000 base pairs of Ad2 DNA immediately left of the insertion, whereas these sequences are not present in cytoplasmic RNA early in Ad2 infection.

If infection by the Ad2-SV40 hybrids induces the synthesis of hybrid mRNA species, then the observed polypeptides may also be hybrids. Indeed, we have no direct evidence that any of the peptide sequences in 30K are SV40 encoded. That Ad2+ND1 30K contains at least some Ad2-specified sequences is suggested by the fact that the early region of SV40 inserted into this hybrid has a coding capacity of only about 16,000 to 20,000 daltons. However, since SV40 anti-late sequences are also present in small amounts in the cytoplasm of Ad2+ND1-infected cells late in infection (6), it is also possible that part of the peptide sequence of 30K derives from SV40 anti-late genetic information. This question should be resolved by tryptic peptide analysis of these polypeptides. Alternatively one could study the virus-specified polypeptides present early in Ad2+ND1-infected cells, at which time the SV40 anti-late sequences are not present in the cytoplasm (6).

SV40 has a coding capacity of approximately 180,000 daltons of proteins. Since RNA sequences complementary to one-half of the SV40 genome are found in the cytoplasm early in infection (9, 10, 25), the SV40 early proteins could have a total molecular weight of about 90,000. The facts that (i) only one early SV40 cistron has been identified genetically (4) and (ii) an early SV40-specific RNA can be found in infected cells that is of sufficient size (19S) to represent a transcript of the entire early region of SV40 (30) have led to speculation that SV40 may encode a single early polypeptide of approximately 90K.

The precipitation of a 100K protein from SV40-infected cells demonstrated by Tegtmeyer (27) adds support to this hypothesis, as does the recent finding by Prives et al. (22) and Smith et al. (26) that an 85–90K polypeptide that is precipitable with SV40 anti-T serum can be synthesized in vitro by using SV40-specific RNA from infected cells. Our demonstration that a 92K protein is synthesized in Ad2+ND4-infected cells and that a protein of similar size can be synthesized in vitro with the use of RNA selected by hybridization to SV40 DNA further confirms this hypothesis. Although SV40-specific mRNA from SV40-infected cells also programs the synthesis of a 92K polypeptide, we have not shown that the SV40 and Ad2+ND4 92K are the same polypeptide. Since the Ad2+ND4 92K mRNA contains some Ad2 sequences, it remains possible that this polypeptide contains both SV40 and Ad2 sequences, so that co-migration of SV40 92K and Ad2+ND4 92K could be coincidental. The failure of the in vitro-synthesized product to co-migrate exactly with what we postulate is its in vivo counterpart (the immunoprecipitated polypeptide) might be explained by a report that the SV40 T antigen is phosphorylated (P. Tegtmeyer, personal communication). Modifications to the polypeptide chain, e.g., phosphorylation or glycosylation, would tend to increase its apparent molecular weight estimated by this technique (29). The fact that the Ad2+ND4 product is of nearly the same size as the in vivo-immunoprecipitated product is consistent with the hypothesis that the early SV40 region can be expressed in its entirety and that the resulting polypeptide forms all or part of the immunologically identified entity known as T antigen. We are currently attempting to analyze the in vivo and in vitro products of the Ad2-SV40 hybrids further with specific SV40 antisera and by tryptic peptide analysis. Preliminary results obtained by G. Fey indicate that the Ad2+ND1 30K polypeptide sequence is contained within the sequence of the Ad2+ND4 92K polypeptide.*

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