Proc. Natl. Acad. Sci. USA
Vol. 73, No. 7, pp. 2505–2509, July 1976
Microbiology

Simian virus 40 (SV40) tumor-specific proteins in nucleus and plasma membrane of HeLa cells infected by adenovirus 2-SV40 hybrid virus Ad2$^+$ND2

(immunoprecipitation/peptide mapping/T antigen/tumor specific transplantation antigen/U antigen)

WOLFGANG DEPPERT* AND GERNOT WALTER

Tumor Virology Laboratory, The Salk Institute, San Diego, California 92112

Communicated by Robert W. Holley, May 5, 1976

ABSTRACT **HeLa cells infected with the nondefective adenovirus 2-SV40 hybrid virus (Ad2$^+$ND2) have previously been shown to synthesize two SV40-specific proteins with molecular weights of 56,000 (56 K protein) and 42,000 (42 K protein). The present study demonstrates that these proteins are immunoprecipitable with serum from hamsters bearing SV40 tumors. By peptide analysis, it is shown that the 56 K and 42 K proteins share common amino acid sequences. Both proteins are metabolically stable in nuclei and plasma membranes but unstable in the cytoplasm, as shown by pulse-chase experiments and cell fractionation studies.**

The nondefective adenovirus (Ad) 2-simian virus 40 (SV40) hybrid viruses (1, 2) are useful tools for the study of early SV40 proteins, the proteins which are probably involved in the establishment and maintenance of the transformed state in SV40-induced tumors and in SV40-transformed cells (3–7). Recently, several SV40-specific proteins in HeLa cells infected by the nondefective hybrid viruses, Ad2$^+$ND1, Ad2$^+$ND2, Ad2$^+$ND4, and Ad2$^+$ND5, have been identified (8–10). Ad2$^+$ND2 contains a segment of 32–36% of the SV40 DNA covalently inserted in the Ad2 genome (11, 12). HeLa cells infected by Ad2$^+$ND2 synthesize two SV40-specific proteins of 56,000 daltons (56 K protein) and 42,000 daltons (42 K protein) that are metabolically unstable (9). If both of these proteins are coded for by the SV40 genome, they must have amino acid sequences in common since the segment of SV40 DNA in Ad2$^+$ND2 can code for only approximately 60,000 daltons of protein. One might also expect that they share common antigenic determinants.

Ad2$^+$ND2 induces the synthesis of two SV40-specific antigens, the tumor-specific transplantation antigen (TSTA) and U antigen (13, 2). U antigen can be detected in the nucleus of SV40-transformed and SV40-infected cells (14), whereas TSTA appears to be located in the plasma membrane (15). An important point to be considered is the possible structural relationship between U antigen and TSTA, and the 56 K and 42 K proteins. A study of the intracellular localization may give a first indication whether such a relationship exists.

The present paper demonstrates by peptide analysis that the 56 K and 42 K proteins are related. Serum from hamsters bearing SV40-induced tumors specifically precipitates both the 56 K and 42 K proteins. By cell fractionation, we show that both proteins are present in the nucleus, cytoplasm, and plasma membrane of infected cells. They are metabolically unstable in the cytoplasmic fraction but stable in the nucleus and the plasma membrane.

Abbreviations: SV40, simian virus 40; TSTA, tumor-specific transplantation antigen.

* Present address: Max-Planck-Institut für biophysikalische Chemie, D34 Göttingen, Postfach 968, West Germany.

MATERIALS AND METHODS

Virus and cells

Seed stocks of Ad2$^+$ND2 were obtained from A. M. Lewis, Jr. High titer stocks were prepared in HeLa S3 cells grown in Eagle's minimum essential medium supplemented with 5% calf serum (growth medium), and plaque titered on HeLa cells as described by Walter and Martin (9). The Ad2$^+$ND2 stocks used in the experiments described had a titer of 10^9 plaque-forming units (PFU)/ml on HeLa cells.

Infection and labeling of cells

HeLa S3 cells (5×10^7) in suspension were infected with 5 ml of undiluted Ad2$^+$ND2 virus stock. After an adsorption period of 20 min, the cells were diluted with 300 ml of growth medium. Thirty-five hours post-infection, the cells were washed twice with 40 ml of Eagle's medium minus amino acids and labeled in 5 ml of Eagle's medium minus amino acids that contained 40 μCi/ml of a ^{14}C-labeled L-amino acid mixture (NEN, specific activity 10 mCi/mmol) and 5% calf serum. After 30 min, the cells were split into two equal aliquots and washed once with 20 ml of Eagle's minimum essential medium. The cells of one aliquot (pulse) were immediately subjected to cell fractionation (see below). The cells of the other aliquot (pulse-chase) were diluted with 150 ml of growth medium and kept in this medium for 5.5 hr. Then they were washed once with 20 ml of Eagle's medium before being subjected to cell fractionation.

For immunoprecipitation and peptide mapping, cells were infected with Ad2$^+$ND2 or Ad2 at a multiplicity of 100 PFU per cell and labeled 37 hr post-infection with 165 μCi/ml of [^{35}S]methionine (specific activity 512 Ci/mmol) for 2 hr in methionine-free Eagle's medium.

Cell fractionation

(*a*) ***Membrane Isolation.*** Cells were washed twice with 20 ml of 0.15 M NaCl and suspended in 2 ml of cold lysis buffer composed of 5 mM $MgCl_2$, 2 mM $CaCl_2$, buffered with 10 mM 2-(*N*-morpholino)ethane sulfonic acid (MES) at pH 6.5. After swelling for 5 min in the cold, the cells were homogenized in a stainless steel Dounce-homogenizer (clearance 0.05 mm). The homogenization procedure was monitored by examining the cell lysate under a phase-contrast microscope. Douncing was stopped after about 80% of the cells were broken (10 to 15 strokes). The cell lysate was spun at 2000 rpm for 15 min in an LEC centrifuge, model PR-2, rotor head 269 ($800 \times g$). The supernatant (cytoplasm) was further fractionated (see below). The pellet of the low-speed centrifugation, containing membrane bags, unbroken cells and nuclei, was subjected to centrifugation in a two-phase system containing polyethylene

glycol 6000 and dextran 500 as described by Brunette and Till (16). Plasma membranes were found at the interface; nuclei and unbroken cells in the pellet. Plasma membranes were further purified as described by Brunette and Till (16). Phase-contrast microscopy of the purified plasma membranes suspended in water showed about 60% of the membrane material as bags. The rest was present as large sheets. No contamination by nuclei could be detected. It should be pointed out that the cells were dounced in lysis buffer not containing $ZnCl_2$ since we found that $ZnCl_2$ causes aggregation of cytoplasmic proteins which subsequently pellet during a 60 min centrifugation at 100,000 $\times g$. However, $ZnCl_2$ was present in the two-phase system.

(*b*) ***Subfractionation of Cytoplasm.*** The cytoplasm from the low-speed centrifugation of the cell lysate (see above) was separated into a 100,000 $\times g$ cytoplasmic supernatant and 100,000 $\times g$ cytoplasmic pellet by centrifugation at 100,000 $\times g$ for 60 min at 4°.

(*c*) ***Purification of Nuclei.*** The pellet collected after the two-phase spin, consisting of unbroken cells and nuclei, was resuspended in isotonic Tris-buffer (0.14 M NaCl, 5 mM KCl, 0.5 mM $MgCl_2$, 1 mM $CaCl_2$, 25 mM Tris·HCl, pH 7.4). Nonidet P40 (NP40) was added to a final concentration of 0.5%. After 10 min on ice, the nuclei were pelleted 2 min at 800 $\times g$ and washed three times with isotonic Tris-buffer.

Preparation of samples for gel electrophoresis

Aliquots of the cell lysate ("homogenate") and of the cytoplasm were diluted 1:1 with electrophoresis sample buffer (0.0625 M Tris·HCl, pH 6.8, 3% sodium dodecyl sulfate, and 5% 2-mercaptoethanol). The 100,000 $\times g$ cytoplasmic supernatant was precipitated with ice-cold trichloroacetic acid [10% (vol/vol) final concentration], as was the 100,000 $\times g$ cytoplasmic pellet after resuspension in 1 ml of lysis buffer. The precipitates were washed three times with cold acetone, air dried, and dissolved in electrophoresis sample buffer. Nuclei and plasma membranes were pelleted and then dissolved in electrophoresis sample buffer. Immediately after the addition of sample buffer, the samples were sonicated with a Branson sonifier equipped with a microtip (position 4, three times for 10 sec) and heated for 3 min in a boiling-water bath. The protein content of the samples was determined with the method of Lowry *et al.* (17) and adjusted to approximately 15 $\mu g/10\ \mu l$.

Polyacrylamide gel electrophoresis and fluorography

The polyacrylamide gel system of Laemmli and Maizel as described by Laemmli (18) was employed. Details have been described elsewhere (9). Fifteen micrograms of protein in approximately 10 μl samples were loaded per slot. Electrophoresis was performed at a constant current of 10 mA. After electrophoresis, the gels were prepared for fluorography as described by Bonner and Laskey (19). In order to obtain a linear response of the film to the radioactivity, we pre-exposed the x-ray film with a light flash (20). Scanning of the fluorographs was performed with a Gilford recording spectrophotometer as described (21).

Immunoprecipitation

Cytoplasmic extracts from Ad2 and Ad2$^+$ND2 infected cells labeled with [^{35}S]methionine were prepared by lysing the cells in buffer containing 0.14 M NaCl, 3 mM $MgCl_2$, 10 mM Tris·HCl, pH 7.4, 0.5% NP40, and 1% phenylmethylsulfonyl fluoride. Nuclei and debris were sedimented at 800 $\times g$ rpm for 10 min. Twenty microliters of the supernatant were incubated with either 5 μl of normal hamster serum (Flow Laboratories) or 5 μl of serum from hamsters bearing SV40 tumors (received from Dr. Jack Gruber, Chief, Office of Program Resources and Logistics, National Cancer Institute, National Institutes of Health) for 60 min at 30°. Then 0.4 ml of rabbit antiserum prepared against hamster gamma globulin (Cappel Laboratories) containing 0.1% NP-40 was added at equivalence. After 15 hr at 4°, the precipitate was collected by centrifugation, washed, and dissolved in 50 μl of sample buffer. Ten microliters of this sample were applied on a slab gel. Prior to use, all sera were absorbed with adenovirus 2-infected HeLa cells and decomplemented by heating to 56° for 30 min.

Peptide mapping

Ad2$^+$ND2-infected cells were labeled with [^{35}S]methionine as described above. After dissolving the cells in electrophoresis sample buffer, aliquots containing 1 mg of protein were subjected to electrophoresis on preparative polyacrylamide slab gels. After electrophoresis, the gels were dried and labeled proteins were visualized by autoradiography. Sections of the gel containing the 56 K and 42 K proteins were cut out and eluted with 0.05 M ammonium bicarbonate containing 0.1% sodium dodecyl sulfate and 10% 2-mercaptoethanol. The eluted samples were further processed and subjected to peptide analysis as described by Gibson (22), except that the buffer used to suspend tryptic peptide digests and for their subsequent electrophoretic separation on thin-layer cellulose plates contained butanol:pyridine:acetic acid:H_2O at volume ratios of 2:1:1:36 instead of 2:1:1:18 (W. Gibson, personal communication).

RESULTS

Immune precipitation

A cytoplasmic extract of Ad2$^+$ND2-infected cells labeled with [^{35}S]methionine was first exposed to serum from hamsters bearing SV40 tumors (antitumor serum) and then to rabbit antihamster serum (*Materials and Methods*). The precipitated proteins were analyzed by sodium dodecyl sulfate gel electrophoresis. As a control, normal hamster serum was used instead of antitumor serum in the immune precipitation. As an additional control, the immune precipitation was performed with extracts from Ad2-infected cells. Fig. 1 demonstrates that both 56 K and 42 K proteins specifically reacted with the antitumor serum and not with normal hamster serum. A small amount of hexon (the major capsid protein of Ad2) was precipitated by both sera from extracts of Ad2- and Ad2$^+$ND2-infected cells.

Comparison of 56 K and 42 K proteins by peptide analysis

[^{35}S]Methionine labeled 56 K and 42 K proteins were purified by preparative sodium dodecyl sulfate gel electrophoresis and digested with trypsin. The tryptic digests were analyzed by two-dimensional separation on thin-layer cellulose plates, as described in *Materials and Methods*. The peptide maps shown in Fig. 2 demonstrate that all ^{35}S-labeled tryptic peptides from the 42 K protein are also present in the digest of the 56 K protein. The map of the 56 K protein shows several peptides not detected in the 42 K protein. We conclude that the 56 K and 42 K proteins share common amino acid sequences.

Subcellular distribution of 56 K and 42 K proteins

Ad2$^+$ND2-infected cells were labeled with a mixture of ^{14}C-amino acids for 30 min at 36 hr post-infection and either harvested immediately (pulse) or chased for 5.5 hr in the presence of a large excess of unlabeled amino acids (chase). The cells were fractionated into nuclei, cytoplasm, and plasma membranes.

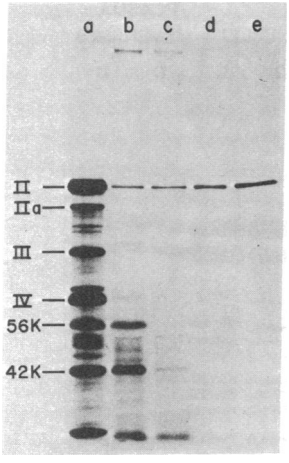

FIG. 1. Sodium dodecyl sulfate-polyacrylamide gel fluorogram of immunoprecipitates from extracts of cells infected with Ad2+ND2 and Ad2. Cell extracts of Ad2+ND2- and Ad2-infected HeLa cells were immunoprecipitated as described in *Materials and Methods*. Aliquots of the immunoprecipitates were applied to a 7.5% acrylamide slab gel and run for 5 hr at 10 mA. The sample order is (a) cell extract of Ad2+ND2-infected cells; (b) same, immunoprecipitated with SV40 antitumor serum; (c) same, immunoprecipitated with control serum; (d) cell extract of Ad2-infected cells, immunoprecipitated with SV40 antitumor serum; and (e) same, immunoprecipitated with control serum.

The cytoplasm was further fractionated into a 100,000 × *g* pellet and 100,000 × *g* supernatant (*Materials and Methods*). Fig. 3 shows the distribution of the SV40-specific 56 K and 42 K proteins in these different cell fractions as analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. After a 30 min pulse, both proteins are present in all cell fractions. After a 5.5 hr chase, the amount of radioactivity in the 56 K and 42 K proteins is markedly decreased in the homogenate, unfractionated cytoplasm, 100,000 × *g* cytoplasmic supernatant, and 100,000 × *g* cytoplasmic pellet. In nuclei and plasma membranes, on the other hand, the amount of radioactivity in these two proteins is approximately the same after the chase as after the pulse. To obtain a quantitative estimate of the relative amounts of radioactive 56 K and 42 K proteins in the different cell fractions, we analyzed fluorograms of the acrylamide gel shown in Fig. 3 by densitometry. The cytoplasmic radioactivity of the 56 K protein decreases to 14% and of the 42 K protein to 2% during the chase (Table 1). In contrast, the amount of radioactive 56 K protein remains constant in nuclei and shows a slight increase in plasma membranes. The 42 K protein decreases only to 81% in nuclei and to 49% in plasma membranes.

Table 2 demonstrates the relative quantities of radioactively labeled 56 K protein and 42 K protein in different cell fractions after the pulse as compared to the chase. After a 30 min pulse, the cytoplasm contains the major portion of the radioactively labeled 56 K and 42 K proteins, i.e., 67% and 79%, respectively, of the total amount present in homogenates. Nuclei and plasma membranes contain a minor fraction. In comparison, after a 5.5 hr chase, the cytoplasm contains only 28% of the 56 K protein and 17% of the 42 K protein, whereas nuclei and plasma membranes together contain the bulk of the radioactive 56 K and 42 K proteins.

The pulse-chase experiment shown in Fig. 3 reveals interesting features concerning the distribution of several Ad2-specific proteins in various cell fractions. Two Ad2 virus capsid proteins, III (penton base) and IV (fiber), are predominantly found in the cytoplasm and are barely detectable in nuclei and

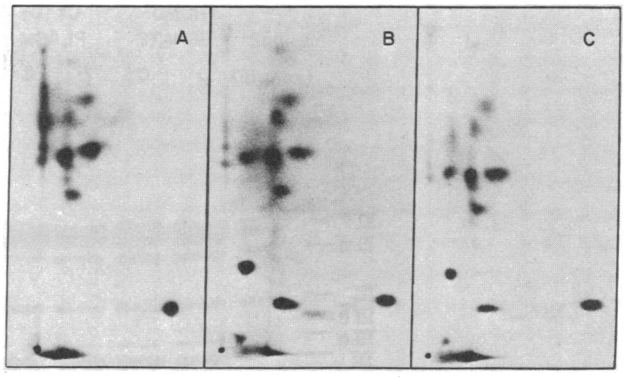

FIG. 2. Peptide maps of [35S]methionine-labeled 56 K and 42 K proteins. Tryptic digests of 56 K and 42 K proteins were prepared and analyzed by two-dimensional separation on thin-layer cellulose plates as described in *Materials and Methods*. Electrophoresis was toward the cathode, i.e., from left to right. Chromatographic development was from the bottom to the top. [35S]Methionine-labeled tryptic peptides were visualized by autoradiography. (A) Peptide pattern of 42 K protein; (B) peptide pattern of 56 K protein; and (C) peptide pattern of a 1:1 mixture of 42 K and 56 K proteins.

plasma membranes. This finding indicates that the nuclei and plasma membrane fractions are essentially free of cytoplasmic proteins. The proteins IIIb, V (core), and Vc, are predominantly found in isolated nuclei and are essentially absent in cytoplasm (100,000 × *g* supernatant and 100,000 × *g* pellet) and in plasma membranes; this suggests that the cytoplasmic and the membrane fractions are not contaminated significantly by nuclear proteins. A significant amount of protein II and IIa in the plasma membrane fraction is probably due to nonspecific binding occurring during or after cell homogenization. This was shown in reconstitution experiments by mixing unlabeled plasma membranes with labeled cytoplasmic extracts. In contrast, these reconstitution experiments also showed that the binding of the 56 K and 42 K proteins to the plasma membranes must occur before cell lysis (W. Deppert and G. Walter, unpublished).

A change in the distribution of proteins III, IIIa, and IV was observed after the chase as compared to the pulse. Proteins III and IV are enriched in the 100,000 × *g* pellet and protein IIIa is more prominent in nuclei of chased cells. The apparent increase in molecular weight of proteins IIIb and Vc during the chase has been described previously (9).

DISCUSSION

The present study has revealed three properties of the SV40-specific 56 K and 42 K proteins synthesized in Ad2+ND2-infected cells. First, they are immune precipitable with serum from hamsters bearing SV40 tumors; second, they share common amino acid sequences; third, they are metabolically stable in nuclei and plasma membranes but unstable in the cytoplasm. In addition, recent experiments demonstrate that both proteins are at least in part coded for by the SV40 genome. This was shown by protein synthesis *in vitro* in which purified SV40-specific RNA from Ad2+ND2-infected cells was used (G. Walter and T. Hunter, in preparation).

Are the 56 K and 42 K proteins true plasma membrane proteins or are they simply adsorbed from the cytoplasmic pool by plasma membranes during the isolation procedure? One can assume that the cytoplasmic pool of these proteins does not change dramatically throughout the labeling and chase periods. Because of rapid turnover, this pool has a higher specific radioactivity at the end of the pulse than at the end of the chase.

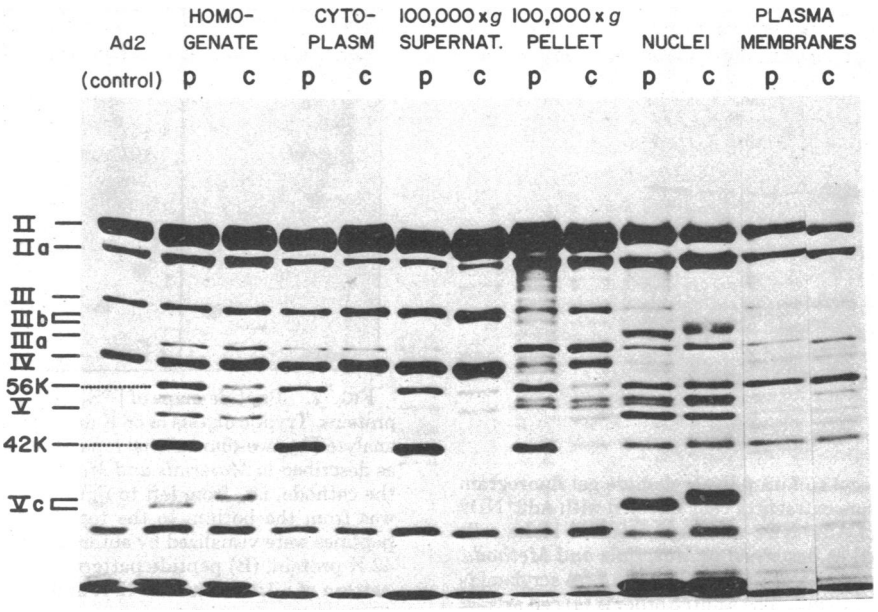

FIG. 3. Sodium dodecyl sulfate-polyacrylamide gel fluorograms of pulsed and pulse-chased proteins in different cell fractions of Ad2+ND2-infected HeLa cells. Pulse labeled (p) and pulse-chase labeled (c) Ad2+ND2-infected HeLa cells were subfractionated as described in *Materials and Methods*. Aliquots of each fraction containing 15 μg of protein were applied to a 7.5% slab gel and run for 5 hr at 10 mA. Ad2 (control) represents homogenate of Ad2-infected cells.

Therefore, if the 56 K and 42 K proteins were predominantly bound to membranes after cell lysis, the amounts of radioactively labeled 56 K and 42 K proteins in the isolated membranes should be higher after the pulse than at the end of the chase. Since we find approximately the same amount of radioactively labeled 56 K and 42 K proteins in membranes after a long chase as after the 30 min pulse, we conclude that the location of these proteins in the plasma membrane is not an artifact of membrane isolation, and that they are an integral part of plasma membranes of intact cells. Using the same argument, we further conclude that the 56 K and 42 K proteins are also present in the nuclei of intact cells in a metabolically stable form. Preliminary experiments indicate that the location of these proteins in the nucleus is the inner nuclear membrane since a crude preparation of nuclear membranes contains both proteins in similar quantities to unfractionated nuclei and removal of the outer nuclear membrane from isolated nuclei with a mixture of Tween 40 and deoxycholate (23) does not remove the 56 K and 42 K proteins (W. Deppert and G. Walter, unpublished data).

The finding that serum from SV40 tumor-bearing hamsters precipitates the 56 K and 42 K proteins implies that proteins structurally related to the 56 K and 42 K proteins are expressed in SV40 tumors. An interesting question, therefore, is the relationship between the 56 K and 42 K proteins and SV40 T antigen. Del Villano and Defendi identified a 70,000 dalton protein in cells transformed or acutely infected by SV40 which bound specifically to immunosorbents reacting with T antigen (24). Tegtmeyer *et al.* (25) were able to immunoprecipitate a protein of 100,000 daltons present in SV40-infected monkey cells and in SV40-transformed cells with antitumor serum. They suggested that this protein is T antigen, coded for by the entire early region of the SV40 genome. Recent experiments by Graessmann *et al.* (26) have provided direct evidence that the SV40 genome codes for SV40 T antigen. In view of these findings, it seems likely that both the 56 K and 42 K proteins, immunoprecipitable and virus coded, share common amino

Table 1. Amount of radioactive 56 K protein and 42 K protein after the chase

Cell fraction	Radioactivity after chase (% of pulse)	
	56 K	42 K
Homogenate	29	9
Cytoplasm	14	2
Nuclei	98	81
Plasma membranes	120	49

The amount of radioactivity present in the 56 K and 42 K proteins was measured by scanning the fluorograms of Fig. 3 with a Gilford spectrophotometer. From the amount of protein per slot and the total amount of protein in each subcellular fraction, the total amount of radioactivity in the 56 K and 42 K proteins in each subcellular fraction was calculated. The amounts of 56 K and 42 K proteins in homogenate were used as 100% values. The recovery of protein in purified plasma membranes was about 3–4% of the total protein in homogenate. The calculation of the amount of 56 K and 42 K proteins in plasma membranes was based on a value of 5% protein for 100% recovery of plasma membranes (27).

Table 2. Intracellular distribution of 56 K and 42 K proteins after the pulse and after the chase

Cell fraction	% Radioactivity			
	Pulse		Chase	
	56 K	42 K	56 K	42 K
Cytoplasm	67	79	28	17
Nuclei	12	6	43	53
Plasma membranes	4	2	16	8

Fluorograms of Fig. 3 were evaluated as described in the legend to Table 1. The amounts of radioactivity of 56 K and 42 K proteins in homogenates after the pulse and after the chase were used as 100% values. The per cent values in the different cell fractions do not add up to 100% due to loss of material during cell fractionation.

acid sequences and antigenic determinants with T antigen. If such a relationship exists, it is puzzling that cells infected by Ad2$^+$ND2 do not exhibit the bright nuclear fluorescence characteristic for T antigen (2), despite the fact that the 56 K and 42 K proteins are found in nuclei. It is possible, however, that the 56 K and 42 K proteins do not carry the strong antigenic determinants of T antigen but contain only weaker determinants sufficient for immunoprecipitation but not for the fluorescent antibody staining reaction. Another possible explanation is that, due to their location in the nuclear membrane, the 56 K and 42 K proteins may be sterically hindered to react with antibody or that they are extracted from the nuclear membrane during the fixation procedure.

Ad2$^+$ND2 induces the synthesis of SV40 U antigen and SV40 TSTA (2). U antigen can be detected in the nucleus of SV40-infected and SV40-transformed cells whereas TSTA appears to be located in the plasma membrane (14, 15). The observation that the 56 K protein and the 42 K protein are present in nuclei and in plasma membranes of Ad2$^+$ND2-infected cells raises the possibility that either one or both proteins could be structurally related to these antigens. If such a relationship could be demonstrated, then this would be proof that SV40 U antigen and TSTA are virus-coded proteins.

How is it possible that the 56 K and 42 K proteins could be structurally related to both SV40 T antigen and SV40 U antigen in the nucleus and to SV40 TSTA in the plasma membrane? Our hypothesis is that T antigen has an affinity for the nuclear membrane. The 56 K protein and the 42 K protein may contain that part of T antigen which has a strong affinity for the nuclear membrane and also for the plasma membrane. Similar proteins may occur in plasma membranes of SV40-transformed cells or in SV40 tumors, possibly as fragments of T antigen, and could be recognized by the immune system as TSTA.

We thank Hawley Martin for excellent technical assistance and Wade Gibson for introducing us to the technique of two-dimensional peptide analysis. We thank A. M. Lewis, Jr. for providing us with seed stocks of the nondefective Ad2-SV40 hybrid viruses. This investigation was supported by Public Health Service Grant no. 5-RO1 CA-15365-01 A1 from the National Cancer Institute; Grant no. GB-41879 from the National Science Foundation; Special Grant no. 688 from the American Cancer Society, California Division; and Core Grant no. 14195 from the National Cancer Institute. W. Deppert was a recipient of a postdoctoral fellowship from the European Molecular Biology Organization.

1. Lewis, A. M., Jr., Levin, M. J., Wiese, W. H., Crumpacker, C. S. & Henry, P. H. (1969) "A non-defective (component) adenovirus-SV40 hybrid isolated from the Ad2-SV40 hybrid population," *Proc. Natl. Acad. Sci. USA* **63**, 1128–1135.
2. Lewis, A. M., Jr., Levine, A. S., Crumpacker, C. S., Levin, M. J. & Henry, P. H. (1973) "Studies of nondefective adenovirus 2-simian virus 40 hybrid viruses. V. Isolation of additional hybrids which differ in their simian virus 40-specific biological properties," *J. Virol.* **11**, 655–664.
3. Brugge, J. S. & Butel, J. S. (1975) "Involvement of the simian virus 40 gene A function in the maintenance of transformation," *J. Virol.* **15**, 619–635.
4. Kimura, G. & Itagaki, A. (1975) "Initiation and maintenance of cell transformation by simian virus 40: A viral genetic property," *Proc. Natl. Acad. Sci. USA* **72**, 673–677.
5. Martin, R. G. & Chou, J. Y. (1975) "Simian virus 40 functions required for the establishment and maintenance of malignant transformation," *J. Virol.* **15**, 599–612.
6. Osborn, M. & Weber, K. (1975) "Simian virus 40 gene A function and maintenance of transformation," *J. Virol.* **15**, 636–644.
7. Tegtmeyer, P. (1975) "Function of simian virus 40 gene A in transforming infection," *J. Virol.* **15**, 613–618.
8. Lopez-Revilla, R. & Walter, G. (1973) "Polypeptide specific for cells with adenovirus 2-SV40 hybrid Ad2$^+$ND1," *Nature New Biol.* **244**, 165–167.
9. Walter, G. & Martin, H. (1976) "Simian virus 40-specific proteins in HeLa cells infected with nondefective adenovirus 2-simian virus 40 hybrid viruses," *J. Virol.* **16**, 1236–1247.
10. Grodzicker, T., Anderson, C., Sharp, P. A. & Sambrook, J. (1974) "Conditional lethal mutants of adenovirus 2-SV40 hybrids. I. Host range mutants of Ad2$^+$ND1," *J. Virol.* **13**, 1237–1244.
11. Kelly, T. J., Jr. & Lewis, A. M., Jr. (1973) "Use of nondefective adenovirus-simian virus 40 hybrids for mapping the simian virus 40 genome," *J. Virol.* **12**, 643–652.
12. Lebowitz, P., Kelly, T. J., Jr., Nathans, D., Lee, T. N. H. & Lewis, A. M., Jr. (1974) "A colinear map relating the simian virus 40 (SV40) DNA segments of six adenovirus-SV40 hybrids to the DNA fragments produced by restriction endonuclease cleavage of SV40 DNA," *Proc. Natl. Acad. Sci. USA* **71**, 441–445.
13. Lewis, A. M., Jr. & Rowe, W. P. (1973) "Studies of nondefective adenovirus 2-simian virus 40 hybrid viruses. VIII. Association of simian virus 40 transplantation antigen with a specified region of the early viral genome," *J. Virol.* **12**, 836–840.
14. Lewis, A. M., Jr. & Rowe, W. P. (1971) "Studies on nondefective adenovirus 2-simian virus 40 hybrid viruses. I. A newly characterized simian virus 40 antigen induced by the Ad2$^+$ND$_1$ virus," *J. Virol.* **7**, 189–197.
15. Tevethia, S. S. & Rapp, F. (1966) "Prevention and interruption of SV40 induced transplantation immunity with tumor cell extracts," *Proc. Soc. Exp. Biol. Med.* **123**, 612–615.
16. Brunette, D. M. & Till, J. E. (1971) "A rapid method for the isolation of L-cell surface membranes using an aqueous two-phase polymer system," *J. Membr. Biol.* **5**, 215–224.
17. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) "Protein measurement with the Folin phenol reagent," *J. Biol. Chem.* **193**, 265–275.
18. Laemmli, U. K. (1970) "Cleavage of structural proteins during the assembly of the head of bacteriophage T4," *Nature* **227**, 680–685.
19. Bonner, W. M. & Laskey, R. A. (1974) "A film detection method for tritium-labelled proteins and nucleic acids in polyacrylamide gels," *Eur. J. Biochem.* **46**, 83–88.
20. Laskey, R. A. & Mills, A. D. (1975) "Quantitative film detection of ^{3}H and ^{14}C in polyacrylamide gels by fluorography," *Eur. J. Biochem.* **56**, 335–341.
21. Walter, G., Roblin, R. & Dulbecco, R. (1972) "Protein synthesis in simian virus 40-infected monkey cells," *Proc. Natl. Acad. Sci. USA* **69**, 921–924.
22. Gibson, W. (1974) "Polyoma virus proteins: a description of the structural proteins of the virion based on polyacrylamide gel electrophoresis and peptide analysis," *Virology* **62**, 319–336.
23. Penman, S. (1969) in *Fundamental Techniques in Virology*, eds. Habel, K. & Salzman, N. P. (Academic Press, New York and London), pp. 35–48.
24. Del Villano, B. C. & Defendi, V. (1973) "Characterization of SV40 T antigen," *Virology* **51**, 34–46.
25. Tegtmeyer, P., Schwartz, M., Collins, J. K. & Rundell, K. (1975) "Regulation of tumor antigen synthesis by simian virus 40 gene A," *J. Virol.* **16**, 168–178.
26. Graessmann, A., Graessmann, M., Hoffmann, H., Niebel, J., Brandner, G. & Mueller, N. (1974) "Inhibition by interferon of SV40 tumor antigen formation in cells injected with SV40 cRNA transcribed *in vitro*," *FEBS Lett.* **39**, 249–251.
27. Atkinson, P. H. & Summers, D. F. (1971) "Purification and properties of HeLa cell plasma membranes," *J. Biol. Chem.* **246**, 5162–5175.