JOURNAL OF VIROLOGY, Feb. 1977, p. 636-646

Vol. 21, No. 2
Printed in U.S.A.

Identification of Simian Virus 40 Protein A

KATHLEEN RUNDELL,[1] JOHN K. COLLINS, PETER TEGTMEYER,* HARVEY L. OZER, CHING-JUH LAI, AND DANIEL NATHANS

Department of Microbiology, State University of New York, Stony Brook, New York 11794; Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts 01545; Department of Microbiology, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205*

Received for publication 6 July 1976

A large simian virus 40 (SV40)-specific protein can be efficiently immunoprecipitated from infected cell extracts with antisera obtained from hamsters bearing SV40-induced tumors. The protein has an apparent molecular weight of 88,000 to 100,000 with respect to markers with known molecular weights, but behaves anomalously on sodium dodecyl sulfate (SDS)-polyacrylamide gels. Cell lines infected by two different strains of SV40 synthesize immunoreactive proteins that differ slightly in mobility during SDS-polyacrylamide gel electrophoresis, evidence that the protein is coded for by the virus. These differences in protein size correlate with differences in the electrophoretic mobility of viral DNA fragments obtained by digestion with *Hin*dII and III restriction enzymes. The size of the viral capsid proteins VP2 and VP3 also varies with the strain of virus. *dl*-1001, a constructed deletion mutant that lacks part of the SV40 *A* gene, directs the synthesis of a 33,000-dalton polypeptide that is not detected in cells infected with wild-type virus. The deletion fragment, like the larger protein, is phosphorylated. Maps of tryptic peptides from the 88,000- to 100,000-dalton protein and the 33,000-dalton fragment show common peptides and provide strong direct evidence that the proteins are products of the SV40 *A* gene. The deletion fragment reacts with antitumor sera and binds to double-stranded DNA in the presence of the complete A protein.

Simian virus 40 (SV40)-specific tumor (T) antigen can be detected in productively infected or transformed cells using serum from hamsters bearing tumors induced by virus-free transformed cells. It has been difficult to determine whether these antigens are viral gene products or induced cellular proteins, because host macromolecular synthesis is stimulated after infection and several new enzymes appear in infected cells. However, the presence of immunologically cross-reacting T antigens in the nuclei of SV40-infected cells of various species (4, 19, 21, 24) and the lack of cross-reactivity with the nuclear T antigen induced by other viruses (4) originally suggested that T antigen is a viral protein. More recently, related papovaviruses of human origin have been shown to induce T antigen that cross-reacts with SV40 T antigen (27, 35).

Investigations with SV40 *ts*A mutants revealed the presence of an 88,000- to 100,000-dalton protein in extracts of infected cells (29, 33). The efficient immunoprecipitation of the protein with antitumor (anti-T) serum showed that it was either the T antigen or a protein associated with T antigen and allowed the identification of the protein in wild-type (WT)-infected, but not uninfected, cells. Using the immunoprecipitation technique, it was shown that the protein is overproduced in all *ts*A mutant-infected cells and that the protein is less tightly associated with the cell nucleus than the WT antigen. The altered behavior of the protein suggested that it might be a mutant protein and, therefore, the product of the *A* gene. More direct evidence concerning the identification of T antigen and the large-molecular-weight protein as the *A* gene product is presented in this paper.

[1] Present address: Department of Microbiology/Immunology, Northwestern University-McGaw Medical Center, Chicago, IL 60611.

MATERIALS AND METHODS

Cells and virus. The experiments described in this paper were performed using the TC7 clone of CV-1 cells (22). Cells were grown in Eagle basal medium containing 5% fetal bovine serum and were infected at confluence. Infections were carried out using a multiplicity of infection of 20 to 50 PFU/cell.

The WT viruses studied were the SV-S clone of Takemoto (17, 26) and a clone of strain VA 45-54 (30). The deletion mutant *dl*-1001, a mutant constructed from SV-S and lacking only *Hin* fragments H and I (14), was grown at 41°C in the presence of

the helper temperature-sensitive (*ts*) mutant B4 (31). The *ts* mutant was derived from the WT clone VA 45-54.

Labeling and extraction of infected cells. Infected or uninfected cells were labeled with 0.2 to 1 mCi of [^{35}S]methionine per ml (New England Nuclear Corp., Boston, Mass.) in methionine-free medium containing 2% fetal bovine serum for 1 to 2 h. In most experiments, the cells were labeled at 32 h postinfection at 41°C, a time during which the rate of synthesis of the 100K protein is maximal (unpublished data). The cells were then washed twice with cold Tris-buffered saline at pH 8.0 and extracted for 10 min with 1.0 ml of 1% Nonidet P-40 (NP-40) in Tris-buffered saline, pH 8.0, containing $CaCl_2$ and $MgCl_2$ (extraction buffer) as described previously (33). The extracts were centrifuged at 2,000 × *g* to separate "nuclear" and "cytoplasmic" fractions.

To label the cells with [^{32}P]phosphoric acid, newly infected cells were washed once and maintained in phosphate-free medium containing 2% fetal bovine serum throughout the infection at 41°C. At 32 h postinfection, the cells were labeled with [^{32}P]phosphoric acid (500 μCi/ml) for 1 to 2 h. The extractions were done as described above.

Immunoprecipitation and gel electrophoresis. The cytoplasmic extracts, containing approximately 500 μg of protein in 300 to 500 μl, were immunoprecipitated using 10 to 15 μl of anti-T serum and 150 to 200 μl of rabbit anti-hamster globulin as described previously (33). The immunoprecipitated proteins were dissolved in the appropriate sample buffer containing 5% 2-mercaptoethanol and analyzed using either of two sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis systems. System I has been described previously (33). Briefly, separating slab gels contained 20% acrylamide, 0.1% bisacrylamide, and a buffer of 0.375 M Tris-sulfate at pH 8.3. The stacking gel (6% acrylamide, 0.12% bisacrylamide) contained the same buffer at 0.075 M. The samples were applied to the gels in 0.075 M Tris-sulfate, and the gels were subjected to electrophoresis using 0.065 M Tris-borate buffer, pH 8.3. System II is essentially that described by Ahmad-Zadeh et al. (1) and Maizel (16). Separating gels contained 7.5% acrylamide, 0.2% bisacrylamide, and 0.1 M sodium phosphate buffer, pH 7.0. Samples were dissolved in a sample buffer containing 0.01 M sodium phosphate buffer, pH 7.0, and were loaded onto the separating gel without a stacking gel. The electrophoresis buffer contained 0.1 M sodium phosphate buffer, pH 7.0.

DNA-cellulose chromatography. Proteins extracted with NP-40 at pH 8.0 were dialyzed to adjust the pH to 6.0 and applied to columns of double-stranded calf-thymus DNA-cellulose prepared according to standard procedures (2, 5). After the columns were washed with pH 6.0 buffer (5 mM potassium phosphate, 0.1 M NaCl, 1 mM dithioerythritol, 10% glycerol, 0.5% NP-40), bound proteins were eluted at pH 9.0 (50 mM Tris-hydrochloride, 0.1 M NaCl, 1 mM dithioerythritol, 10% glycerol, 0.5% NP-40). Finally, the column was washed with the pH 9.0 buffer containing 0.6 M NaCl to remove more tightly bound proteins. The column fractions were dialyzed to adjust the pH to 8.0, immunoprecipitated, and analyzed on SDS-polyacrylamide gels.

Purification of radioactive proteins. Radioactive proteins were purified by immunoprecipitation and SDS-polyacrylamide gel electrophoresis. Gels were dried immediately without staining, because the recovery of the immunoreactive protein was drastically decreased by acid fixation. After autoradiography, the protein bands were cut from the gel, rehydrated in 2% SDS, and eluted by electrophoresis through agarose into dialysis bags. The electrophoresis buffer contained 0.05 M Tris-hydrochloride, pH 8.0, and 0.2% SDS. The protein and SDS were precipitated after the addition of 0.25 volume of 2% KCl and centrifuged at 27,000 × *g* for 15 min. The pellet was vigorously washed twice in acetone to free the protein of potassium dodecyl sulfate. The precipitated protein was dissolved in 5 mM Tris-acetate, pH 7.8, dialyzed overnight in the same buffer, and lyophilized.

Peptide mapping. The purified protein was oxidized for 2 h at 4°C in 100 μl of formic acid and 5 μl of performic acid prepared by incubating formic acid and H_2O_2 (9:1) for 2 h at room temperature. After lyophilization, the oxidized protein was dissolved in 0.1 ml of 0.1 M NH_4HCO_3, and 4 μg of trypsin-TPCK (tolylsulfonyl phenylalanyl chloromethyl ketone) (Worthington, Freehold, N.J.) was added for 12 to 16 h. An additional 4 μg of trypsin was added for 8 h and the mixture was lyophilized. Salts were removed from the peptide mixture by passing the solution in 0.1 N HCl through a 0.5-ml column of AG50W-X2 (Bio-Rad) in the acid form. The column was washed with 4 volumes of water, and the peptides were eluted with 2 N NH_4OH. After lyophilization tryptic peptides were dissolved in water and separated by thin-layer electrophoresis and chromatography on cellulose MN (Brinkmann, Westbury, N.Y.; CEL 300) plates (20 by 20 cm). Electrophoresis in the first dimension was performed using pyridine-acetic acid-water (289:111:1,600) as solvent. The plates were saturated with the solvent and subjected to electrophoresis at 500 V for 1 h. The solvent for the chromatography in the second dimension was *n*-butanol-pyridine-acetic acid-water (40:28.9:11.1:40).

DNA fragment analysis. Restriction endonuclease cleavage of viral DNA and electrophoresis of *Hin*dII/III fragments on 4% polyacrylamide gels or 1.4% agarose were carried out as described previously (12, 14).

RESULTS

Behavior of the immunoreactive protein on SDS-gels. Shortly after the infection of permissive or nonpermissive cell lines by SV40, a large-molecular-weight protein is synthesized by the infected cells. The protein can be efficiently immunoprecipitated by anti-T serum and analyzed using SDS-polyacrylamide gel electrophoresis. The migration of the protein on SDS-gels is somewhat anomalous relative to markers with known molecular weights (Fig. 1). After analysis of the protein on two different

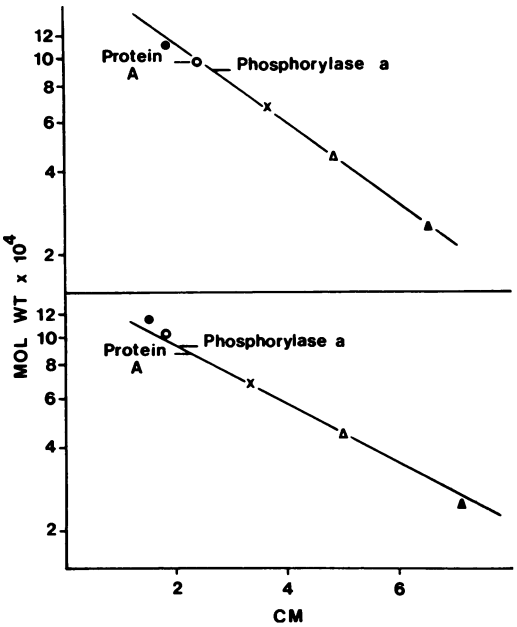

FIG. 1. *Migration of the SV40 protein A and molecular weight standards on SDS-polyacrylamide gels. The migration of the SV40 immunoreactive protein relative to protein markers with known molecular weights was determined using the two SDS-gel systems described in Materials and Methods. [^{35}S]methionine-labeled protein was prepared by immunoprecipitation of cytoplasmic extracts of infected cells, and the immunoprecipitated protein was applied to slab gels along with [^{35}S]methionine-labeled adenovirus 5-infected cell extracts and 1 to 2 μg of standard molecular weight markers. The markers used were chymotrypsinogen, 25K (▲); ovalbumin, 45K (△); bovine serum albumin, 68K (×); adenovirus 100K protein (○); adenovirus 5 hexon, 115K (●). Phosphorylase a has a molecular weight of 94K. The plots were obtained using gel electrophoresis system I (upper panel) and system II (lower panel).*

gel electrophoresis systems, apparent molecular weights of 100,000 (system I) and 88,000 (system II) can be calculated. In addition, the protein migrates more slowly than phosphorylase a (94,000 daltons) in system I, but more rapidly than phosphorylase a in system II. Phosphorylase a migrates normally with respect to the other molecular weight markers in these systems. The reason for the altered mobility of the SV40 immunoreactive protein in different electrophoretic systems is unknown. Gel analyses discussed in this paper were performed using system I and, for convenience, the immunoreactive protein will be referred to as the 100K protein. However, it must be recognized that the actual molecular weight of the protein is open to question.

Strain difference in SV40 proteins. Comparative studies of two widely used strains of SV40 have shown small size differences in at least three of the virus-specific proteins. The capsid proteins VP2 and VP3 isolated from purified virions of strain SV-S display a slightly lower mobility on SDS-polyacrylamide gels relative to the proteins from VA 45-54 (Fig. 2). The difference in the protein VP3 is quite distinct, but the difference in VP2 is more difficult to demonstrate because VP1 obscures VP2 in the SV-S strain. VP3 from the two viral strains can be resolved during coelectrophoresis (Fig. 3f).

In addition to the differences in the capsid proteins, analysis of the proteins precipitated from cytoplasmic extracts with anti-T serum revealed a slight electrophoretic difference in the predominant immunoreactive protein. Cells infected with strain SV-S synthesize a protein with a slightly greater mobility than the 100K protein from cells infected with the *ts* mutant B4 (parent strain VA 45-54) (Fig. 3). During coelectrophoresis these proteins migrate together as a widened band, but it has not been possible to resolve them using gels of varying concentrations of acrylamide. However, the difference is highly reproducible using the two WT strains or mutant viruses isolated from either strain. Furthermore, the same differences are observed when extracts from cells transformed by the two viral strains or derived mutants are compared.

Strain differences in SV40 DNA. As previously reported (12, 15), endo R·*Hin*dII/III digests of DNA from strains SV-S and VA 45-54 show differences in electrophoretic mobility of several *Hin* fragments (Fig. 4). Specifically, the two large *Hin* fragments (A and B) from VA 45-54 DNA, which are derived from the early genomic region, have slightly reduced mobility compared to *Hin*-A and -B from SV-S DNA. On the basis of mobility differences, one can estimate that the early reigon of VA 45-54 DNA is about 40 nucleotide pairs longer than the early region of SV-S DNA. Within the late genomic segment the *Hin*-C fragment of VA 45-54 DNA has reduced mobility relative to the SV-S *Hin*-C fragment (equivalent to about 30 nucleotide pairs), and the *Hin*-F fragment of VA 45-54 DNA has a greater mobility than the SV-S *Hin*-F (equivalent to about 20 nucleotide pairs). Differences other than fragment lengths are, of course, not excluded by these tests.

Identification of the A protein as T antigen. Although unlikely, it could have been argued that the 100K protein was not T antigen but was found in immunoprecipitates because it was bound to T antigen present in undetectable

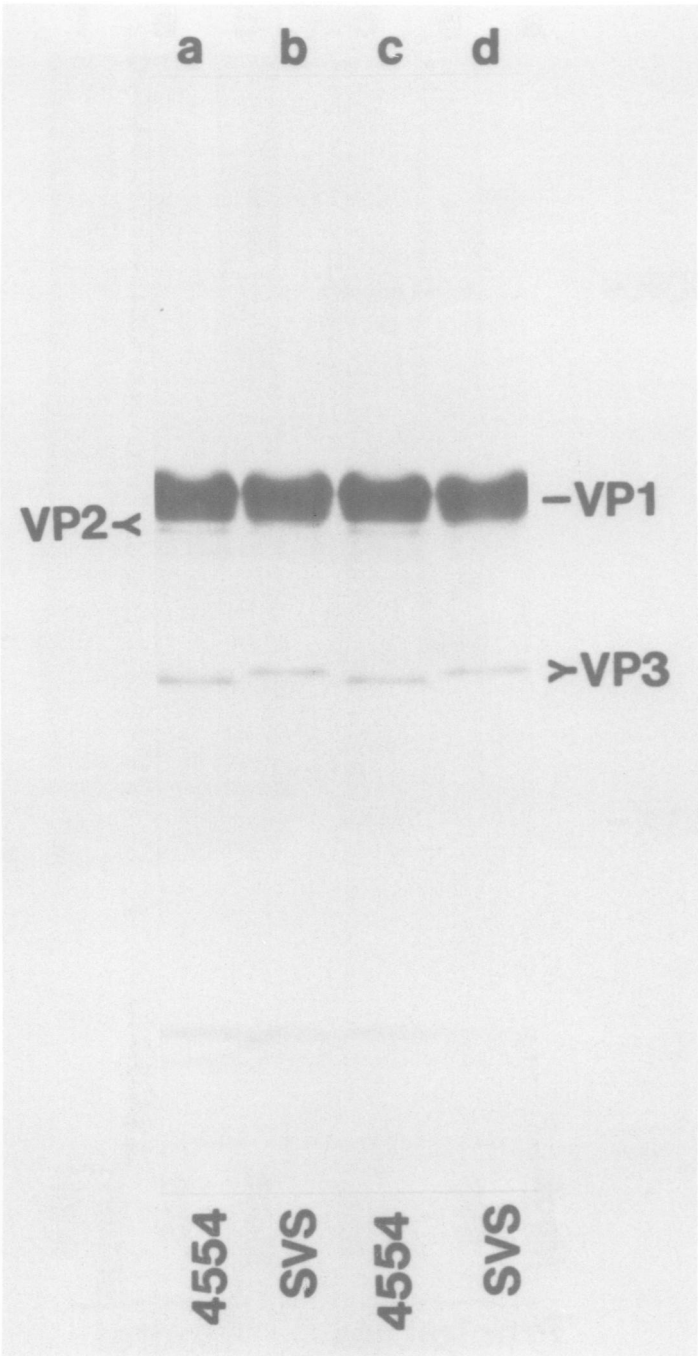

FIG. 2. *SDS-polyacrylamide gel electrophoresis of virion proteins from SV40 SV-S and VA 45-54. Virus particles produced in medium containing [^{14}C]arginine were recovered from lysates of infected cells and purified as described by Ozer (18). Virions were disrupted in buffer containing SDS and 2-mercaptoethanol, and the proteins were resolved by polyacrylamide gel electrophoresis. (a) VA 45-54; (b) SV-S; (c) VA 45-54; (d) SV-S.*

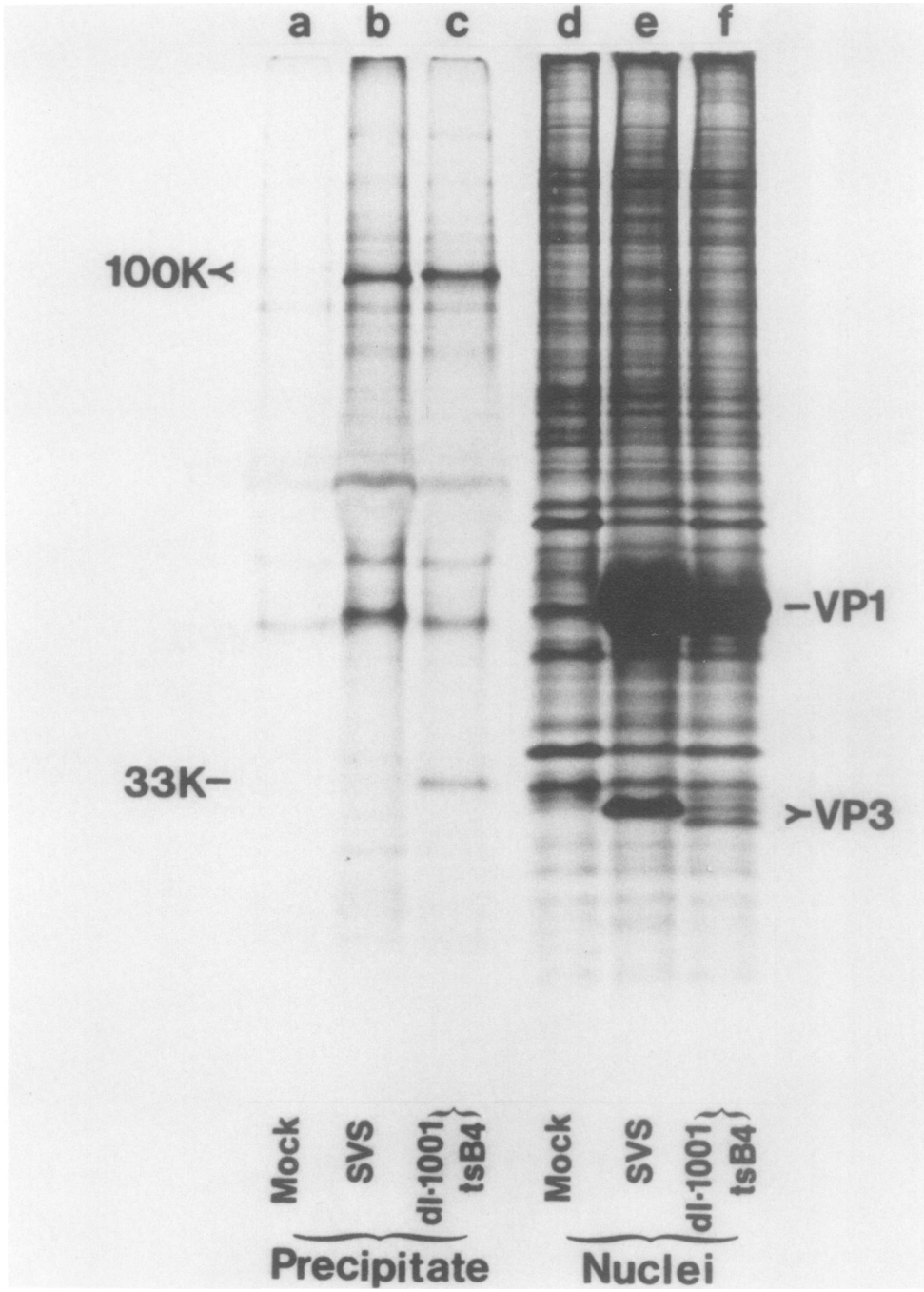

FIG. 3. *Comparison of viral proteins present in cells infected by SV40 SV-S or by dl-1001 (parent strain SV-S) in association with helper mutant B4 (VA 45-54). Cultures in 8-ounce (ca. 0.24-liter) bottles were infected with appropriate virus and incubated at 41°C for 48 h. [^{35}S]methionine in methionine-free medium was added for 1 h, and then the cells were extracted with NP-40. The nuclear fractions were suspended directly in SDS electrophoresis sample buffer. Portions (300 μl) of the NP-40 extracts were immunoprecipitated and analyzed by SDS-gel electrophoresis as described in Materials and Methods. (a) Uninfected cells, immunoprecipitated proteins; (b) cells infected with SV40 SV-S, immunoprecipitated proteins; (c) cells infected with dl-1001 and B4, immunoprecipitated proteins; (d) uninfected cells, nuclear proteins; (e) cells infected with SV40 SV-S, nuclear proteins; (f) cells infected with dl-1001 and B4, nuclear proteins.*

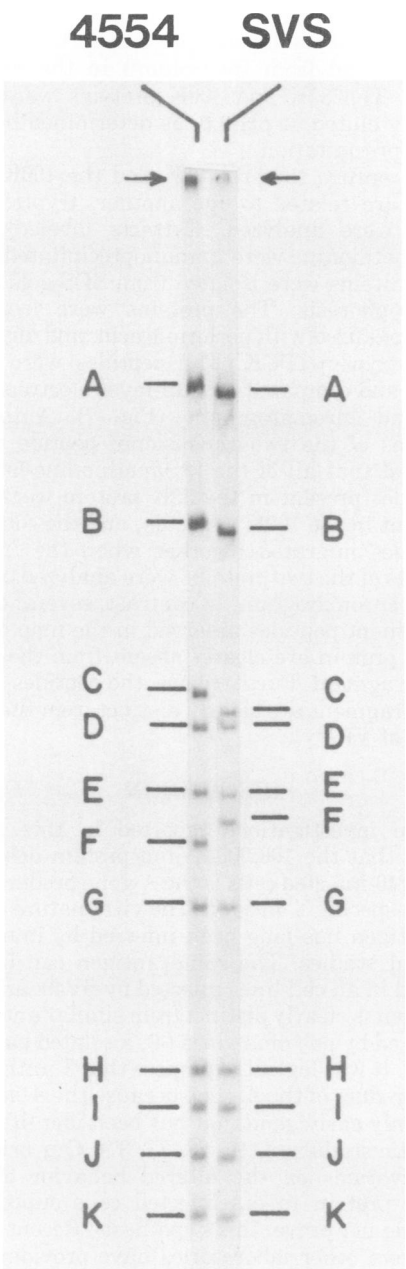

FIG. 4. *Autoradiogram of ^{32}P-labeled HindII/III fragments of SV40 VA 45-54 and SV-S DNAs after electrophoresis in a 4% acrylamide slab gel. The arrows at the top indicate the origin; A, B, C, . . . indicate individual fragments. The actual distance of Hin-K from the origin was 26 cm.*

amounts. To exclude this possibility, the antigenicity of the 100K protein was tested after purification. The immunoprecipitated, radioactive, 100K protein was isolated from SDS-gels, freed of SDS, dialyzed, and lyophilized as described in Materials and Methods. The purified protein was dissolved in extraction buffer and exposed to control or anti-T sera. The results shown in Table 1 confirm that the 100K protein reacts directly with anti-T serum.

TABLE 1. *Immunoprecipitation of purified 100K protein[a] by anti-T serum*

Sera[b]	cpm		% of total precipitated
	Soluble fraction	Precipitated fraction	
Control	2,320	420	15
Anti-T	360	1,420	85

[a] The 100K protein labeled with [^{35}S]methionine was purified and prepared for immunoprecipitation as described in Materials and Methods.

[b] Anti-T serum was obtained from hamsters bearing tumors induced by SV40-transformed hamster cells; control serum was obtained from normal hamsters.

Protein fragment specified by *dl*-1001. The mutant *dl*-1001 is a constructed "excisional" deletion mutant that lacks only the *Hin*dII/III fragments H and I (approximately 20% of the early region of the genome) (14). Mutant *dl*-1001 can be grown in the presence of late *ts* mutants but cannot be grown with the A mutants as helper virus (13). Cells infected with *dl*-1001 synthesize a 33,000-dalton protein that is easily detected in immunoprecipitates of infected cell extracts and is clearly distinct from the virion proteins in nuclear extracts of the same cells (Fig. 3). The 100K protein present in the immunoprecipitates can be accounted for by the presence of helper virus *ts*B4 in the inoculum. The presence of a small protein in immunoprecipitates of cells infected with the deletion mutant strongly suggests that the protein is the translation product of the *dl*-1001 early mRNA and represents a fragment of the 100K T antigen found in cells infected with WT SV40.

In addition to the interaction with anti-T serum, the 33K protein displays two other properties expressed by the 100K molecule. First, the 33K protein is phosphorylated (Fig. 5). Phosphorylation of the deletion fragment was detected by labeling infected cells in the presence of [^{32}P]phosphoric acid and immunoprecipitating the proteins extracted with NP-40. The 100K protein observed in the figure is the protein synthesized by the *ts* helper virus *ts*B4. Second, the fragment binds to double-stranded DNA cellulose columns with the same characteristics as the 100K protein from the helper virus (Fig. 6). To examine the ability of the fragment to bind DNA in vitro, infected cultures were labeled with [^{35}S]methionine at 41°C, and the NP-40-extracted proteins were

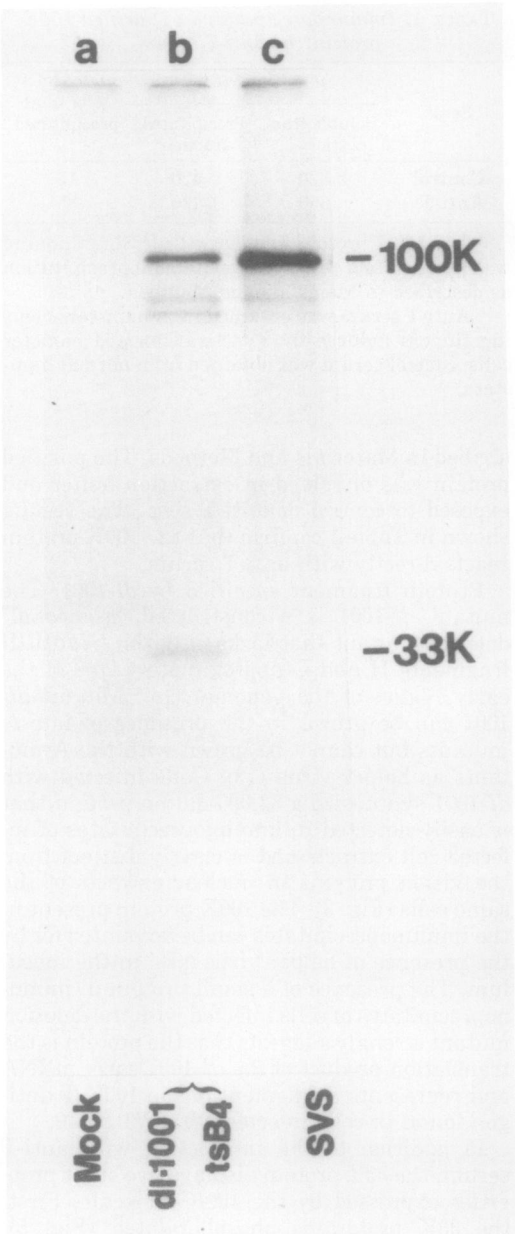

FIG. 5. *Phosphorylation of immunoreactive proteins during infection with SV40 VA 45-54 or with dl-1001 and B4. Monolayer cultures in 8-ounce bottles were infected with appropriate virus. After 32 h at 41°C, infected cells were pulsed with [^{32}P]phosphoric acid for 2 h, extracted with NP-40, and immunoprecipitated. Immunoreactive proteins were analyzed by SDS-polyacrylamide gel electrophoresis and autoradiography. (a) Uninfected cells, immunoprecipitated proteins; (b) cells infected with dl-1001 and B4, immunoprecipitated proteins; (c) cells infected with SV40 SV-S, immunoprecipitated proteins.*

applied to the DNA cellulose columns at pH 6.0. Over 95% of the labeled proteins in the extract were eluted from the column in the pH 6.0 wash. The 33K and 100K proteins were efficiently eluted at pH 9.0, as determined by immunoprecipitation.

To confirm that the 33K and the 100K proteins are related to one another, tryptic peptides were analyzed. Extracts labeled with [^{35}S]methionine were immunoprecipitated, and the proteins were isolated from SDS-gels after electrophoresis. The proteins were freed of SDS, oxidized with performic acid, and digested with trypsin-TPCK. The peptides were analyzed and compared by thin-layer electrophoresis and chromatography (Fig. 7). Autoradiograms of the two-dimensional peptide maps showed that all of the [^{35}S]methionine-labeled peptides present in the 33K protein were also present in the 100K molecule, and the common peptides migrated together when the tryptic digests of the two proteins were analyzed on the same chromatogram. In contrast, several of the prominent peptides observed in the map of the 100K protein are clearly absent from the deletion fragment. Furthermore, the peptides of the 33K fragment are clearly distinct from the peptides of VP1.

DISCUSSION

The investigations reported in this paper show that the 100,000-dalton protein observed in SV40-infected cells is the *A* gene product, the virus-specific T antigen. The viral nature of the T antigen has long been inferred by immunological studies. The same antigen can be detected in all cell lines infected by SV40, and the antigen is clearly distinct from similar antigens induced by polyoma virus (4), a related papovavirus. It was logical to propose that T antigen is the product of the *A* gene because the *A* gene is the only early gene that has been identified by genetic studies (6, 10, 28, 31, 37). Our original observations on the altered behavior of the 100K protein in *ts*A-infected cells supported, but did not prove, this hypothesis. Recent studies from other laboratories have provided further evidence that T antigen is the product of the SV40 *A* gene, without identification of the specific proteins involved. The T antigen from A mutant-infected cells is more thermolabile than the WT antigen, both in its ability to fix complement (3, 11) and in its ability to bind DNA (34). Furthermore, synthesis of immunoreactive proteins from SV40-specific RNA has been accomplished both *in vivo*, by microinjection of individual actinomycin-treated cells (8), and *in vitro*, using protein-synthesizing sys-

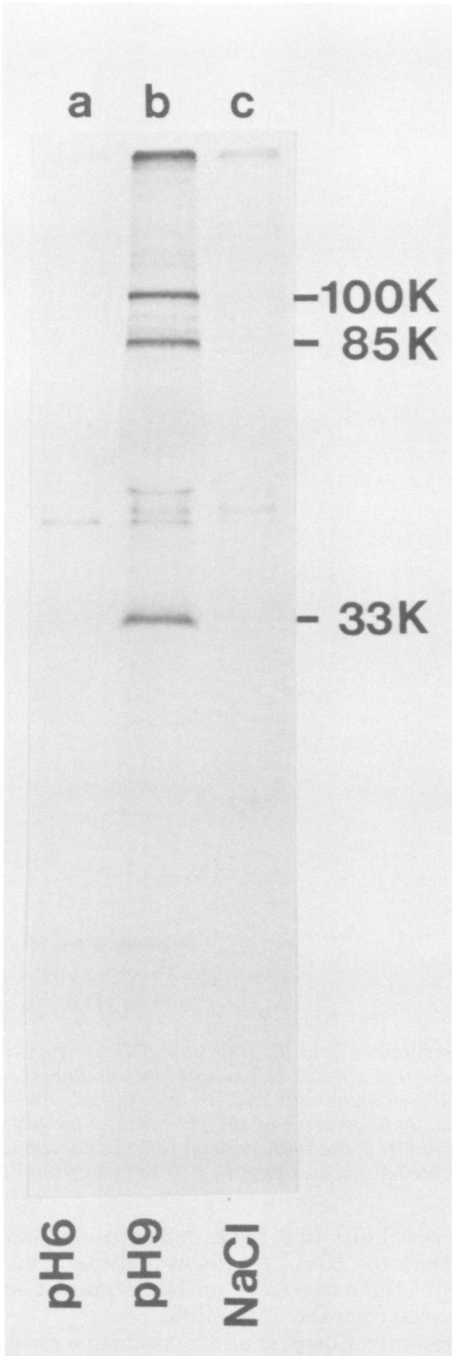

FIG. 6. *Binding of immunoreactive proteins to double-stranded DNA cellulose columns. Monolayer cultures were infected with dl-1001 and B4 at 41°C for 48 h and then labeled with [^{35}S]methionine for 1 h. The cells were extracted at pH 8.0 with NP-40 containing 1 mM dithioerythritol, and cytoplasmic extracts were analyzed on DNA-cellulose columns as described in Materials and Methods. The fractions were adjusted to contain equivalent amounts of radioactivity, and 300-μl samples were analyzed by immunoprecipitation, gel electrophoresis, and autoradiography. (a) Immunoreactive proteins eluted at pH 6.0; (b) immunoreactive proteins eluted at pH 9.0; (c) immunoreactive proteins eluted with 0.6 M NaCl.*

tems (20, 23). These results leave little doubt that the virus codes for the SV40 T antigen.

Confirmation of the identity of the 100K protein and the T antigen was necessary, because it was possible that the 100K protein was not T antigen itself but interacted with T antigen during immunoprecipitation and DNA binding in vitro. We have found that the 100K protein is immunoreactive after isolation of the protein from SDS-gels. Therefore, the protein is recognized by the antiserum and is not simply interacting with other immunoreactive proteins in cell extracts.

We have also found consistent differences in the size of the T antigen and of VP2 and VP3 related to the strain of SV40 examined. These observations support the conclusion that T antigen (as well as VP2 and VP3) is virus coded. Although strain differences in the size of T antigen are correlated with differences in the size of *Hin* fragments from the early genomic segment, the relationship between the sizes of VP2 and VP3 from the two SV40 strains examined and differences in fragments from the late regions of their genomes is not clear. Further studies, particularly with recombinant molecules, may clarify this relationship.

The relatedness of the 100K protein and the 33K deletion fragment synthesized during infection with *dl*-1001 provides independent and direct evidence that the 100K protein is the product of the *A* gene. The deletion mutant lacks only *Hin* fragments H and I, fragments that are known to be included in the early region of the SV40 genome. The deleted region includes that portion of the genome in which nearly all *ts*A mutants map (12, 15), evidence that the deletion is within the *A* gene. Thus, the deletion in the *A* gene results in the synthesis of a protein fragment related to the 100K molecule, the product of the intact *A* gene.

Results with *dl*-1001 raise questions concerning the size of the A protein and the location of initiation and termination signals of translation in the early SV40 mRNA. The early region of SV40 encompasses approximately 2,500 nucleotide pairs, 48% of the viral genome (9). This region is large enough to code for a protein of 100,000 daltons. The deletion mutant contains intact *Hin*-A and *Hin*-B fragments, but lacks 20% of the early DNA, *Hin* fragments H and I

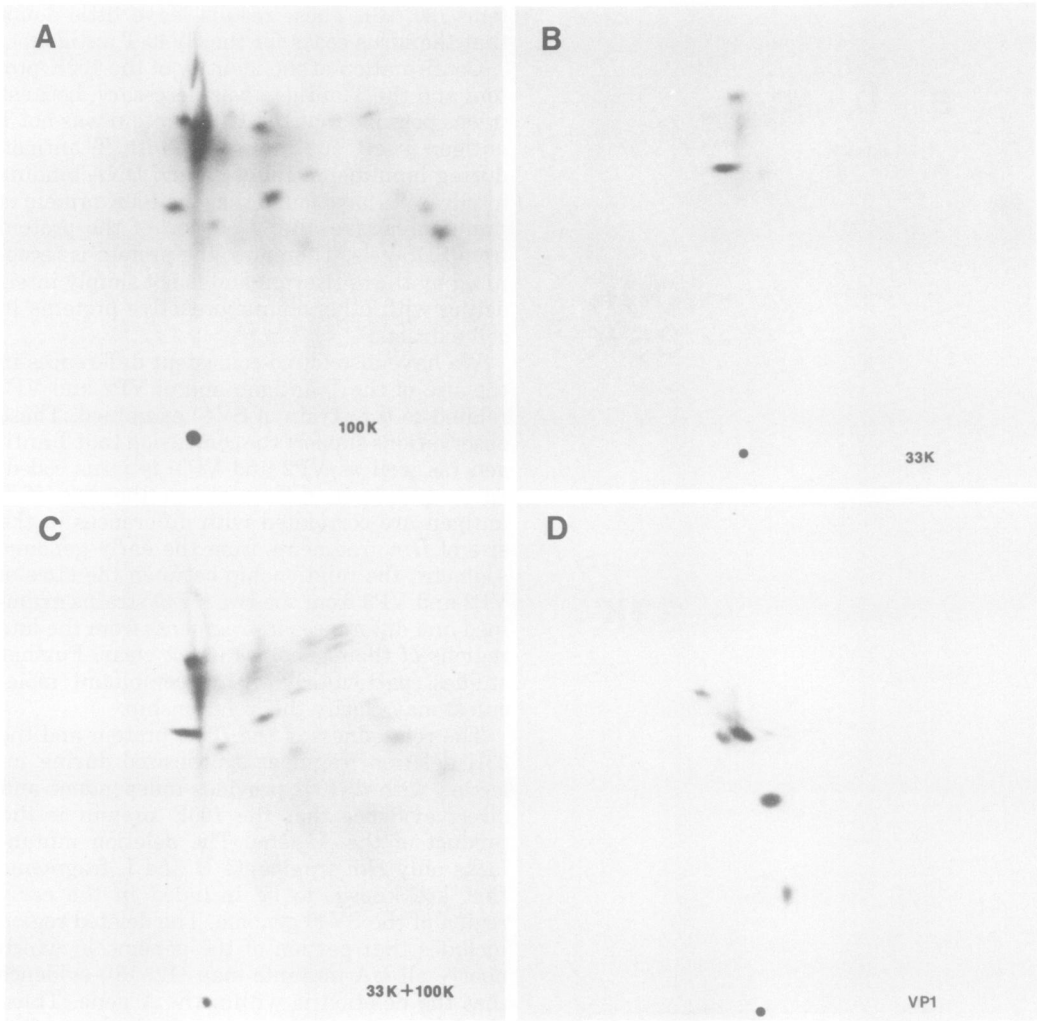

FIG. 7. *Tryptic fingerprints of [^{35}S]methionine containing peptides from the 100K and 33K proteins. The 100K and 33K proteins were isolated from [^{35}S]methionine-labeled infected cell extracts by immunoprecipitation and separated by polyacrylamide gel electrophoresis. The proteins were isolated from the gels, freed of SDS, and oxidized as described in Materials and Methods. A fingerprint of the [^{35}S]methionine-labeled peptides from VP1 is included as a negative control. (a) Peptides from the 100K protein; (b) peptides from the 33K deletion fragment; (c) peptides from the 100K and 33K proteins analyzed together; (d) peptides from VP1.*

(Fig. 8). The early region of DNA included in the mutant is sufficient to produce a protein as large as 80,000 daltons. Smaller products would be expected if the deletion created a nonsense codon somewhere in the *Hin*-B fragment by shifting the translation frame of the mRNA. In this case, the smallest protein expected would be 45,000 daltons, a product of the intact *Hin*-A fragment. However, the 33K protein is the only new protein observed in immunoprecipitates of infected cell extracts. Although several models can be evoked to explain this difference, the observed size of the deletion fragment raises the possibility that the *A* gene does not extend through the *Hin*-A fragment and that translation of the early SV40 mRNA begins at some distance from the 5′ terminus.

Recently, Shenk et al. (25) isolated a series of viable deletion mutants that lack portions of the viral DNA between 0.54 and 0.57 map units (Fig. 8). The observation that the size of the SV40 T antigen on SDS-polyacrylamide gels is unaltered by the deletions has been confirmed (Tegtmeyer and Rundell, unpublished data). The anomalous behavior of the SV40 A protein in SDS-gels makes this observation difficult to

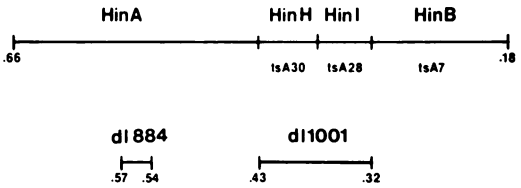

FIG. 8. *Location of the A gene within the SV40 genome. The early region of the SV40 DNA is diagrammed with the HindII/III DNA fragments as reference points. The numbers represent SV40 map units according to standard nomenclature. Representative tsA mutants can be rescued by Hin-H, -I, and -B DNA fragments (12, 15). The viable deletion mutant dl-884 specifies an A protein of apparently normal size (25), whereas the nonviable dl-1001 specifies an incomplete A protein.*

interpret, but the most likely explanation is that the *A* gene extends no further than 0.54 on the SV40 genome. Assuming that the SV40 *A* gene is located between 0.54 and 0.18, the 3′ end of the stable early 19S RNA (9, 36), the size of the protein produced from the gene should be smaller than the 88,000- to 100,000-dalton protein observed in infected cells. This discrepancy cannot be adequately explained at present. It seems unlikely, however, that modifications of the protein can account for the large molecular weight because proteins of similar size have been synthesized in vitro (20).

The phosphorylated deletion fragment reacts with anti-T sera and binds to double-stranded DNA in the presence of the complete A protein provided by the helper virus but has not been shown to have either property independently. These properties of the deletion fragment could be inherent to the structure of the fragment or could be indirectly mediated by a binding between the fragment and the complete protein. In either case, the binding of the deletion fragment to DNA or to the complete A protein points to the conclusion that the phosphorylation site and at least one functional binding site are located within the same third of the 100K molecule. These findings demonstrate the feasibility of localizing specific functions to different domains of the A protein.

ACKNOWLEDGMENTS

This investigation was supported by grants PRA-113 and VC-132 from the American Cancer Society; grants CA 16497, CA 05346, CA 14670, CA 12708, and CA 16519 from the Public Health Service, National Cancer Institute; and grant 1256 from the Damon Runyon Fund.

We are grateful for the skillful technical assistance of Judith Kohout and Lie Ping Li. We also thank Carl Anderson for the protein extracts from adenovirus 5-infected cells.

LITERATURE CITED

1. **Ahmad-Zadeh, C., B. Allet, J. Greenblatt, and R. Weil.** 1976. Two forms of simian-virus-40 specific T-antigen in abortive and lytic infection. Proc. Natl. Acad. Sci. U.S.A. 73:1097-1101.
2. **Alberts, B. M., F. J. Amodio, M. Jenkins, E. D. Gutman, and F. L. Ferris.** 1968. Studies with DNA-cellulose chromatography. I. DNA-binding proteins from *Escherichia coli*. Cold Spring Harbor Symp. Quant. Biol. 33:289-305.
3. **Alwine, J. C., S. I. Reed, J. Ferguson, and G. R. Stark.** 1975. Properties of T antigens induced by wild-type SV40 and tsA mutants in lytic infection. Cell 6:529-533.
4. **Black, P. H., W. P. Rowe, H. C. Turner, and R. J. Huebner.** 1963. A specific complement-fixing antigen present in SV40 tumor and transformed cells. Proc. Natl. Acad. Sci. U.S.A. 50:1148-1156.
5. **Carroll, R. B., L. Hager, and R. Dulbecco.** 1974. SV40 T antigen binds to DNA. Proc. Natl. Acad. Sci. U.S.A. 71:3754-3757.
6. **Chou, J. Y., and R. G. Martin.** 1974. Complementation analysis of simian virus 40 mutants. J. Virol. 13:1101-1109.
7. **Danna, K. J., and D. Nathans.** 1971. Specific cleavage of simian virus 40 DNA by restriction endonuclease of *Haemophilus influenzae*. Proc. Natl. Acad. Sci. U.S.A. 68:2913-2917.
8. **Graessmann, M., and A. Graessmann.** 1976. "Early" simian virus 40-specific RNA contains information for tumor antigen formation and chromatin replication. Proc. Natl. Acad. Sci. U.S.A. 73:366-370.
9. **Khoury, G., P. Howley, D. Nathans, and M. Martin.** 1975. Post-transcriptional selection of simian virus 40-specific RNA. J. Virol. 15:433-437.
10. **Kimura, G., and R. Dulbecco.** 1973. A temperature-sensitive mutant of simian virus 40 affecting transforming activity. Virology 52:529-534.
11. **Kuchino, T., and N. Yamaguchi.** 1975. Characterization of T antigen in cells infected with a temperature-sensitive mutant of simian virus 40. J. Virol. 15:1302-1307.
12. **Lai, C.-J., and D. Nathans.** 1974. Mapping temperature-sensitive mutants of simian virus 40: rescue of mutants by fragments of viral DNA. Virology 60:466-475.
13. **Lai, C.-J., and D. Nathans.** 1974. Mapping the genes of simian virus 40. Cold Spring Harbor Symp. Quant. Biol. 39:53-60.
14. **Lai, C.-J., and D. Nathans.** 1974. Deletion mutants of simian virus 40 generated by enzymatic excision of DNA segments from the viral genome. J. Mol. Biol. 89:179-193.
15. **Lai, C.-J., and D. Nathans.** 1975. A map of temperature-sensitive mutants of simian virus 40. Virology 66:70-81.
16. **Maizel, J. V.** 1969. Acrylamide gel electrophoresis of proteins and nucleic acids, 334-362. *In* K. Habel and N. P. Salzman (ed.), Fundamental techniques in virology. Academic Press Inc., New York.
17. **Nathans, D., and K. Danna.** 1972. Studies of SV40 DNA. IV. Differences in DNA from various strains of SV40. J. Mol. Biol. 64:515-518.
18. **Ozer, H.** 1972. Synthesis and assembly of simian virus 40. I. Differential synthesis of intact virions and empty shells. J. Virol. 9:41-51.
19. **Pope, J. H., and W. P. Rowe.** 1964. Detection of specific antigen in SV40 transformed cells by immunofluorescence. J. Exp. Med. 120:121-128.
20. **Prives, C., H. Aviv, E. Gilboa, E. Winocour, and M. Revel.** 1975. The cell-free translation of early and late classes of SV40 messenger RNA. Colloq. Inst. Nat. Sante R. Med. 47:305-312.
21. **Rapp, F., J. S. Butel, and J. L. Melnick.** 1964. Virus-induced intranuclear antigen in cells transformed by papovavirus SV40. Proc. Soc. Exp. Med. 116:1131-1142.

22. **Robb, J. A., and K. Huebner.** 1973. Effect of cell chromosome number on simian virus 40 replication. Exp. Cell Res. 81:120-126.
23. **Roberts, B. E., M. Gorecki, R. C. Mulligan, K. J. Danna, S. Rozenblatt, and A. Rich.** 1975. Simian virus 40 DNA directs the synthesis of authentic viral polypeptides in a linked transcription-translation cell-free system. Proc. Natl. Acad. Sci. U.S.A. 72:1922-1926.
24. **Sabin, A. B., and M. A. Koch.** 1964. Sources of genetic information for specific complement-fixing antigens in SV40 virus-induced tumors. Proc. Natl. Acad. Sci. U.S.A. 52:1131-1138.
25. **Shenk, T. E., J. Carbon, and P. Berg.** 1976. Construction and analysis of viable deletion mutants of simian virus 40. J. Virol. 18:664-671.
26. **Takemoto, K. K., R. L. Kirschstein, and K. Habel.** 1966. Mutants of simian virus 40 differing in plaque size, oncogenicity, and heat sensitivity. J. Bacteriol. 92:990-994.
27. **Takemoto, K. K., and M. F. Mullarkey.** 1973. Human papovavirus, BK strain: biological studies including antigenic relationship to simian virus 40. J. Virol. 12:625-631.
28. **Tegtmeyer, P.** 1972. Simian virus 40 deoxyribonucleic acid synthesis: the viral replicon. J. Virol. 10:591-598.
29. **Tegtmeyer, P.** 1974. Altered patterns of protein synthesis in infection by SV40 mutants. Cold Spring Harbor Symp. Quant. Biol. 39:9-16.
30. **Tegtmeyer, P., C. Dohan, and C. Reznikoff.** 1970. Inactivating and mutagenic effects of nitrosoguanidine on simian virus 40. Proc. Natl. Acad. Sci. U.S.A. 66:745-752.
31. **Tegtmeyer, P., and H. L. Ozer.** 1971. Temperature-sensitive mutants of simian virus 40: infection of permissive cells. J. Virol. 8:516-524.
32. **Tegtmeyer, P., K. Rundell, and J. K. Collins.** 1977. Modification of simian virus 40 protein A. J. Virol. 21:647-657.
33. **Tegtmeyer, P., M. Schwartz, J. K. Collins, and K. Rundell.** 1975. Regulation of tumor antigen synthesis by simian virus 40 gene *A*. J. Virol. 16:168-178.
34. **Tenen, D. G., P. Baygell, and D. Livingston.** 1975. Thermolabile T (tumor) antigen from cells transformed by a temperature-sensitive mutant of simian virus 40. Proc. Natl. Acad. Sci. U.S.A. 72:4351-4355.
35. **Walker, D. L., B. L. Padgett, G. M. ZuRhein, A. E. Albert, and R. F. Marsh.** 1973. Human papovavirus (JC): induction of brain tumors in hamsters. Science 181:674-676.
36. **Weinberg, R. A., and J. E. Newbold.** 1974. Mapping of SV40 mRNA species on the viral genome. Cold Spring Harbor Symp. Quant. Biol. 39:161-164.
37. **Yamaguchi, N., and T. Kuchino.** 1975. Temperature-sensitive mutants of simian virus 40 selected by transforming ability. J. Virol. 15:1297-1301.