Journal of Cellular Biochemistry 19:127-144 (1982)
Tumor Viruses and Differentiation 23-40

# Detection of a Complex of SV40 Large Tumor Antigen and 53K Cellular Protein on the Surface of SV40-Transformed Mouse Cells

**Myriam Santos and Janet S. Butel**

*Department of Virology and Epidemiology, Baylor College of Medicine, Houston, Texas 77030*

The possible interaction between simian virus 40 (SV40) large tumor antigen (T-ag) and cellular proteins in the plasma membrane of SV40-transformed mouse cells was investigated. The presence of SV40 T-ag, 53,000 (53K) cellular protein, and histocompatibility (H-2) antigens on the surface of SV40-transformed cells was demonstrated by immunofluorescence. The use of lactoperoxidase-catalyzed cell surface iodination and a differential immunoprecipitation technique established that large T-ag is associated with the 53K host-coded protein on the surface of the transformed cells. In contrast, no detergent-stable complex between large T-ag and H-2 antigens was detected. Both labeled T-ag and 53K protein were coprecipitated from surface-iodinated SV40-transformed cells by monoclonal antibodies directed against either the viral or the cellular protein. Based on the unique antigenic sites recognized by the anti-T monoclonal antibodies, it appears that both the carboxy and amino termini of the T-ag polypeptide are exposed on the surface of SV40-transformed mouse cells. The nature of the association between surface T-ag and 53K protein, as well as that between the molecular complex and the plasma membrane, remains to be determined. The possible effect of the surface-associated T-ag/53K complex on cellular proliferation is considered.

**Key words: SV40-transformed cells, SV40 large tumor antigen, cellular protein 53K**

Simian virus 40 (SV40)-transformed cells are characterized by the presence of nonstructural viral proteins designated virus-specific tumor antigens. The large tumor antigen (T-ag), a phosphoprotein of about 94,000 (94K) molecular weight, and small tumor antigen (t-ag; 20K) are both encoded by the early region of the SV40 genome [1-6]. Large T-ag is localized predominantly in the nuclei of SV40-transformed cells [7,8]. However, it has recently been demonstrated that a small fraction of the T-ag is also associated with the surface of such cells [9-14].

The presence of new surface antigens, termed tumor-associated antigens (TAA) or tumor-specific transplantation antigens (TSTA), appears to be a common feature

Myriam Santos is on leave from the Department of Cell Biology and Genetics, School of Medicine, University of Chile, Santiago, Chile.

Received May 26, 1982; accepted June 14, 1982.

0730-2312/82/1902-0127$03.50 

of all tumor cells [15–17]. In the SV40 system, the virus-specific TSTA is related to large T-ag [18–21]. The TAA are responsible for inducing host resistance to tumor growth [16,17], and theoretically they could also alter the cell surface. Histocompatibility antigens (mouse H-2 complex), which are involved in cell–cell recognition and interaction [22], have been detected in association with TAA on the surface of some transformed cells [23–27]. Whether such molecular interactions are responsible for changes in cellular physiology is not known. However, it is attractive to postulate that a physical interaction between a virus-induced transforming protein and normal cellular component(s) might induce the myriad of cellular changes that culminate in transformation.

Large T-ag, the SV40 transforming protein, appears to be needed for the initiation and maintenance of the transformed phenotype [28–33], although the mechanisms(s) by which the viral protein exerts its effect is still unknown. A fraction of the population of the nuclear T-ag molecules is tightly associated with a 53,000 (53K) cellular phosphoprotein [12,34–37], originally termed the nonviral tumor antigen (nvT-ag) [38]. The 53K protein has been detected in normal cells, but at much lower levels than in transformed cells [39–43]. Studies addressing a possible correlation between expression of the 53K protein and cell division revealed that only replicating normal cells contained detectable levels of this protein [40–42], prompting suggestions that its expression might be cell cycle-dependent.

We have determined that this 53K cellular protein is present on the surface of SV40-transformed cells, stably complexed with SV40 large T-ag. In contrast, no stable association between histocompatibility antigens and T-ag was detected on the surface of SV40-transformed mouse cells. Finally, a series of monoclonal antibodies directed against large T-ag was employed for preliminary characterization of the conformation of the surface-associated T-ag molecule.

## MATERIALS AND METHODS

### Cell Lines

The following cell lines were used in this study: the transplantable mKSA-Asc line of SV40-transformed BALB/c kidney cells [44,45]; the BALB/WT cell line, derived by the in vitro transformation of BALB/c mouse embryo cells by wild-type SV40 [46]; and the MTV-L/BALB cell line, established from a transplantable spontaneous mammary tumor of BALB/c mice [47]. All cells were cultured as described [48].

### Radioactive Labeling

Cells grown to near confluency were surface-iodinated by the lactoperoxidase-catalyzed reaction as described by Soule et al [46]. To label with [$^{35}$S]methionine or $^{32}P_i$, cells were depleted by incubation for 2 hr in either methionine-free or phosphate-free Eagle's minimal essential medium (MEM), supplemented with 2% dialyzed fetal bovine serum (FBS; Grand Island Biological Co., Grand Island, New York). The cells were then incubated for 2 hr in the same medium containing a total of 100 $\mu$Ci of [$^{35}$S]methionine (>600 Ci/mmol; Amersham, Arlington Heights, Illinois) or 200 $\mu$Ci of carrier-free $^{32}P_i$ (10 mCi/ml, Amersham). Labeled cells were then subjected to differential immunoprecipitation as described below.

## Antisera and Monoclonal Antibodies

Ascitic fluid from hamsters bearing SV40 ascites tumors (HAF) [9], rabbit serum prepared against purified SV40 large T-ag (RαT) [9], and a series of monoclonal antibodies with reactivity against T-ag or 53K protein were used in the immunoprecipitation studies. The hybridomas were kindly provided by E. Gurney (PAb7, PAb412, and PAb122) [49] and by E. Harlow (PAb405, PAb414, PAb416, PAb419, PAb423, PAb430, and PAb421) [50]. The binding sites of the anti-T monoclonal antibodies included in this study are shown in Figure 1, based on data taken from the original papers [49–51]. Antibodies from three of the hybridomas (marked with a star) do not bind sodium dodecyl sulfate (SDS)-denatured T-ag molecules; the authors have concluded that those antibodies recognize antigenic sites determined by the secondary or tertiary structure of the protein. Monoclonal antibodies raised against human immunoglobulins (9N), a gift from G. Dreesman, were used as a negative control.

A mouse serum containing predominantly antibodies against the H-2.4 antigenic specificity was prepared by inoculating B10BR/SnSg female mice (Jackson Laboratories, Bar Harbor, Maine) with spleen cells from B10A/SnSg mice (Jackson Laboratories) following the procedure described previously [52]. This serum (MoαH-2.4) does not contain activity against murine leukemia virus glycoprotein gp70. Antiserum $D^d$-control (anti-H-2.4) was obtained from the Research Resources Branch, National Institute of Allergy and Infectious Diseases, National Institutes of Health (Bethesda, Maryland). In immunoprecipitation experiments of surface-iodinated mKSA-Asc cells, $D^d$-control serum precipitated a protein that migrated with an apparent molecular weight of 70,000 in addition to the heavy and light chains of H-2.4 antigen. Presumably, this extra protein represents recognition of murine leukemia virus glycoprotein, gp70, as described [53–55].

## Differential Immunoprecipitation of Surface and Nuclear T-ag

The differential immunoprecipitation procedure was performed as described by Santos and Butel [48]. The scheme of the technique is shown in Figure 2.

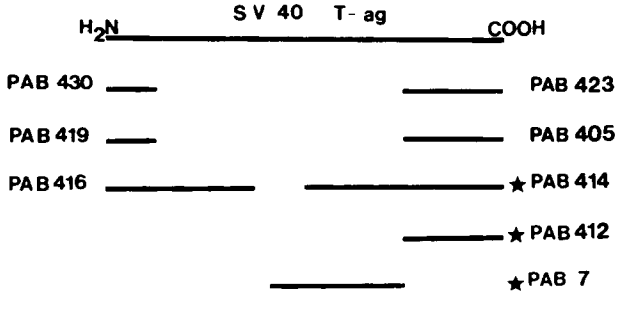


Fig. 1. Regions of large T-ag molecule which contain binding sites for monoclonal antibodies used in this study. Drawing is based on data reported by Gurney et al [49], Harlow et al [50], and Deppert et al [51].

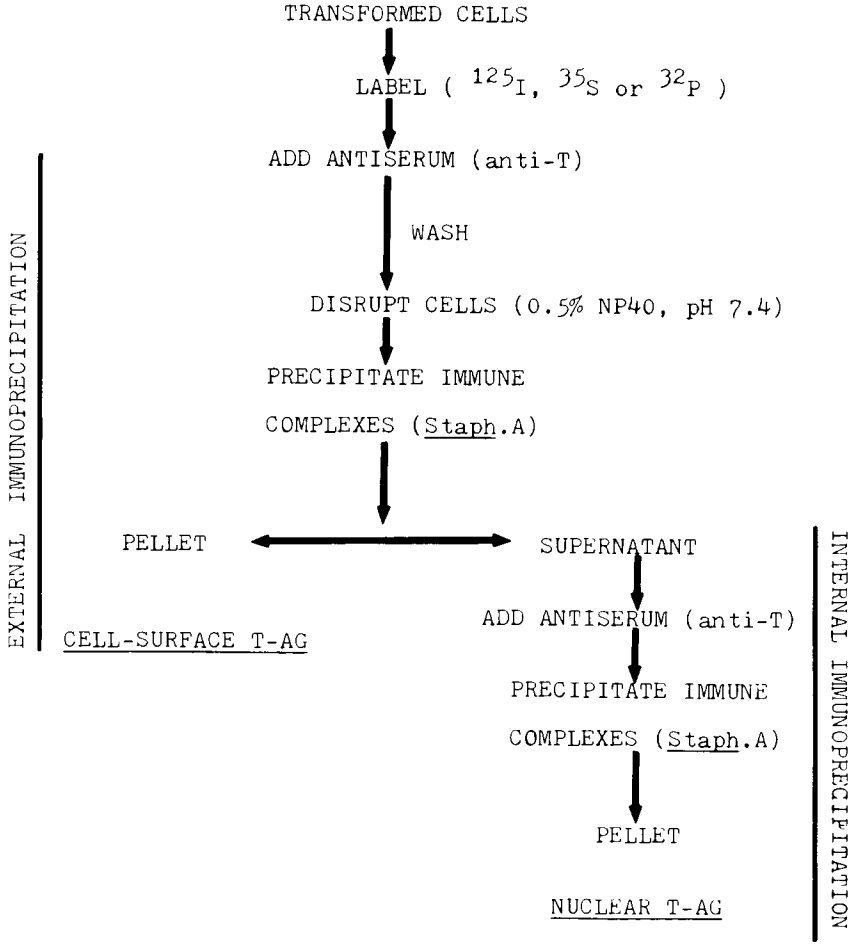


Fig. 2. Scheme of differential immunoprecipitation technique.

## Gel Electrophoresis and Autoradiography

Antigens obtained by immunoprecipitation were analyzed by SDS-polyacrylamide gel electrophoresis (PAGE) in 10% or 12% slab gels [10,11]. $^{14}$C-labeled phosphorylase A (94,000), bovine serum albumin (68,000), ovalbumin (43,000), carbonic anhydrase (30,000), and cytochrome C (11,700), obtained from New England Nuclear (Boston, Massachusetts), were used as molecular weight markers. Autoradiography was performed using Kodak NS5T X-ray film.

### Immunofluorescence Reaction

**Cell surface antigens.** Cell surface T-ag was detected by indirected immunofluorescence on live mKSA-Asc cells in suspension, as described by Lanford and Butel [9]. Cell surface 53K protein and H-2 antigens were detected by indirect immunofluorescence reaction on live mKSA-Asc cells grown on coverslips. Cells were washed three times with Dulbecco's modified Eagle's minimal essential medium (D-MEM; Grand Island Biological Co.) supplemented with 10% newborn calf serum (NCS; Biocell Laboratories, Carson, California), and then incubated for 30 min at room temperature with 50 $\mu$l of the same medium containing 25 $\mu$l of heat-inactivated Mo$\alpha$H-2.4 serum or PAb421 supernatant fluid. After this incubation period, cells were washed three times with D-MEM supplemented with 10% NCS and then reacted with 50 $\mu$l of medium containing 5$\mu$l of heat-inactivated fluorescein-conjugated rabbit IgG antimouse immunoglobulins (Cappell Laboratories, Cochranville, Pennsylvania) for 30 min at room temperature. Cells were washed three times as before, mounted in Elvanol, and observed with a Zeiss fluorescence microscope.

**Nuclear T-ag.** All the hybridoma supernatant fluids were screened for activity against nuclear T-ag by indirect immunofluorescence using acetone-fixed SV40-transformed cells as described [56].

## RESULTS

### Detection of SV40 T-ag, 53K Cellular Protein, and H-2 Antigens on the Surface of SV40-Transformed Mouse Cells by Immunofluorescence

Indirect immunofluorescence reactions were used to determine the presence of T-ag, 53K protein, and H-2 antigens on the surface of SV40-transformed mouse cells. The reaction for surface T-ag utilized about $10^6$ mKSA-Asc cells that had been removed from culture plates by EDTA treatment [9]; the cells were incubated first with HAF and next with fluorescein-conjugated rabbit IgG directed against hamster immunoglobulins (Fig. 3A). A similar reaction was obtained using rabbit antiserum against purified T-ag. To detect the 53K protein and H-2 antigens, mKSA-Asc cells grown on coverslips were incubated with PAb421 hybridoma supernatant fluid or Mo$\alpha$H-2.4 serum, respectively, followed by fluorescein-conjugated rabbit IgG anti-mouse immunoglobulins (Fig. 3B, C). Although quantitative differences among the intensities of the reactions were observed, it was evident that all three antigens were present on the surface of mKSA-Asc cells. It may be noted that the 53K cellular protein was not detectable on the surface of mKSA-Asc cells that had been removed from culture plates by treatment with EDTA.

### Differential Immunoprecipitation of Cell Surface and Nuclear SV40 T-ag

The presence of T-ag on the surface of SV40-transformed cells is well documented. It has been corroborated by a variety of experimental approaches, including indirect immunofluorescence [9,11,13,14], immunoprecipitation from plasma membrane-enriched subcellular fractions [10–12], and iodination of externally exposed molecules followed by immunoprecipitation of surface T-ag from clarified cell extracts [46,57,58]. However, all these procedures involved some manipulation of the cells, such as removal from the substrate by EDTA treatment [9,11], formaldehyde

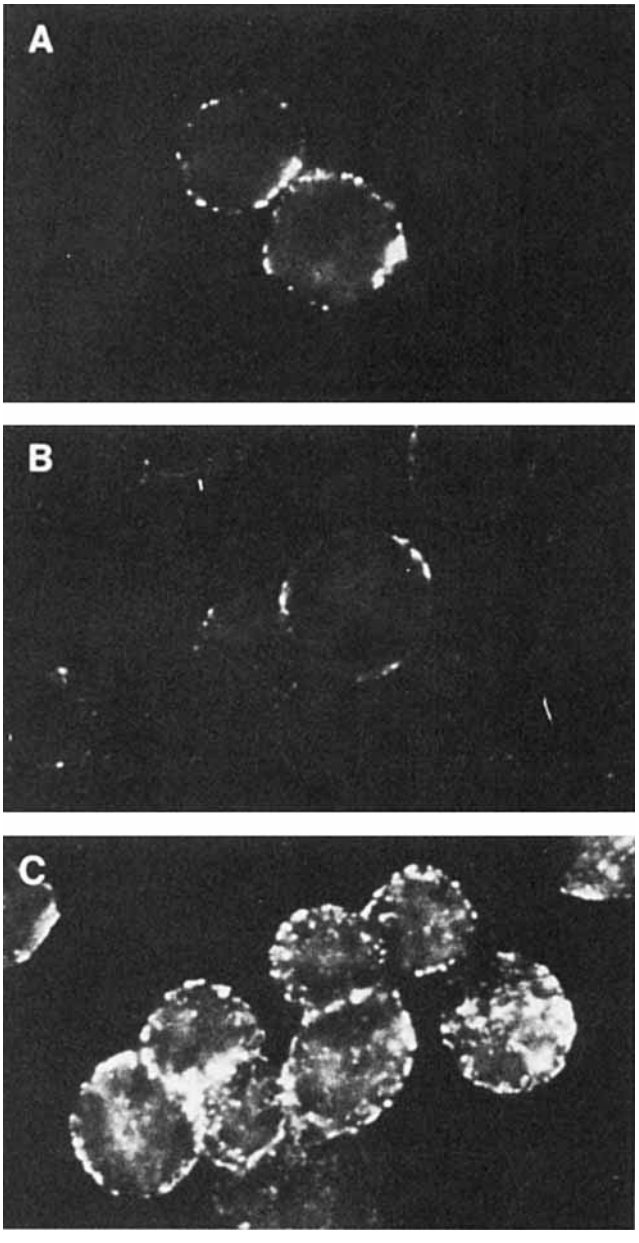


Fig. 3. Detection of large T-ag, 53K protein and H-2 antigens on the surface of SV40-transformed cells by immunofluorescence. To detect T-ag, mKSA-Asc cells were removed from the plates by treatment with 0.05% EDTA and reacted with HAF followed by FITC-labeled rabbit antihamster immunoglobulins (A). To detect 53K protein and H-2 antigens, mKSA-Asc cells were grown on coverslips and stained with either PAb421 monoclonal antibodies (B) or MoαH-2.4 serum (C) followed by FITC-labeled rabbit antimouse immunoglobulins. × 1200.

fixation [13], subcellular fractionation [10,12], or detergent extraction [46,57,58] prior to the specific antigen–antibody interaction. The differential immunoprecipitation technique [48] was devised to permit detection of surface T-ag under conditions in which the anti-T antibodies are bound to surface T-ag while the cells are still living and attached to plastic surfaces (Fig. 2). After the antibodies are externally bound, the cells are disrupted, immune complexes removed, and nuclear T-ag then immunoprecipitated (Fig. 2).

When surface-iodinated mKSA-Asc cells were subjected to differential immunoprecipitation, all the $^{125}$I-labeled T-ag was precipitated by HAF during the external reaction (Fig. 4, lane 2); no labeled T-ag was precipitated during the internal reaction (Fig. 4, lane 4). Also, no iodinated T-ag was precipitated by NHS (Fig. 4, lane 1). The amount of labeled surface T-ag immunoprecipitated was not large enough to be detected by Coomassie blue staining (Fig. 4, lane 3), whereas a large amount of unlabeled nuclear T-ag was present in the internal immunoprecipitate, as revealed by Coomassie blue staining (Fig. 4, lane 5). A second iodinated protein with a molecular weight of about 53,000 (53K) was also specifically immunoprecipitated by HAF during the external reaction (Fig. 4, lane 2). Rabbit serum against purified T-ag also precipitated large T-ag and 53K protein from surface-iodinated mKSA-Asc cells (data not shown). Similar results were obtained when BALB/WT cells were used in place of mKSA-Asc cells [48]. [$^{35}$S]Methionine-labeled mKSA-Asc cells were also sub-

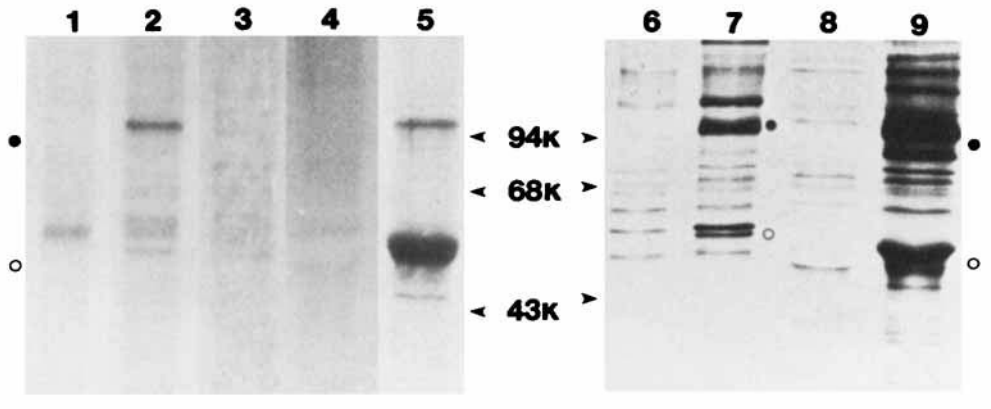


Fig. 4. Independent detection of surface and nuclear T-ag by differential immunoprecipitation. mKSA-Asc cells were surface-iodinated by the lactoperoxidase technique (1–5) or metabolically labeled for 2 hr with [$^{35}$S]methionine (6–9) and subjected to external (1–3,6,7) or internal (4,5,8,9) immunoprecipitation by NHS (1,6,8) or HAF (2–5,7,9). Labeled cells were incubated with the indicated serum for 30 min at 4°C, unattached antibodies were removed by extensive washing, and cells were disrupted in a 0.5% NP40 solution. Immune complexes in the clarified cell lysates were absorbed with heat-inactivated, formalin-fixed Staphylococcus aureus, strain Cowan I (SACI). Bacteria were pelleted and the nuclear T-ag remaining in the supernatant was precipitated by a second incubation with antiserum. Immune complexes were again adsorbed with SACI. Surface and nuclear antigens were eluted from the bacterial pellet with an SDS-containing sample buffer, boiled for 5 min, and analyzed by 12% SDS-polyacrylamide gel electrophoresis. Protein bands were visualized by Coomassie blue staining (3,5) or by autoradiography (1,2,4,6–9). The migration of molecular weight marker proteins is indicated with arrowheads between lanes 5 and 6. ●, large T-ag; ○, 53K protein.

jected to differential immunoprecipitation. Large T-ag and the 53K protein were precipitated by HAF during both the external (Fig. 4, lane 7) and external (Fig. 4, lane 9) reactions.

Appropriate controls have been performed to eliminate the possibility of contamination of surface T-ag by nuclear T-ag and to establish that the two proteins detected during the external reaction of the differential immunoprecipitation technique are bona fide surface antigens [48].

To further characterize the surface T-ag and 53K protein, cells were metabolically labeled with $^{32}P_i$ for 2 hr as described under Materials and Methods and then subjected to differential immunoprecipitation (Fig. 5). Both the externally exposed (Fig. 5, lane 2) and the internally localized (Fig. 5, lane 4) T-ag and 53K protein were revealed to be phosphoproteins.

Based on its molecular weight, its presence in anti-T immunoprecipitates, and the fact it was phosphorylated, the surface-associated 53K protein was hypothesized to correspond to the host-coded 53K cellular protein previously found in association with large T-ag in lysates of both SV40-infected [59] and -transformed cells [10,12,34–36,38,39,49].

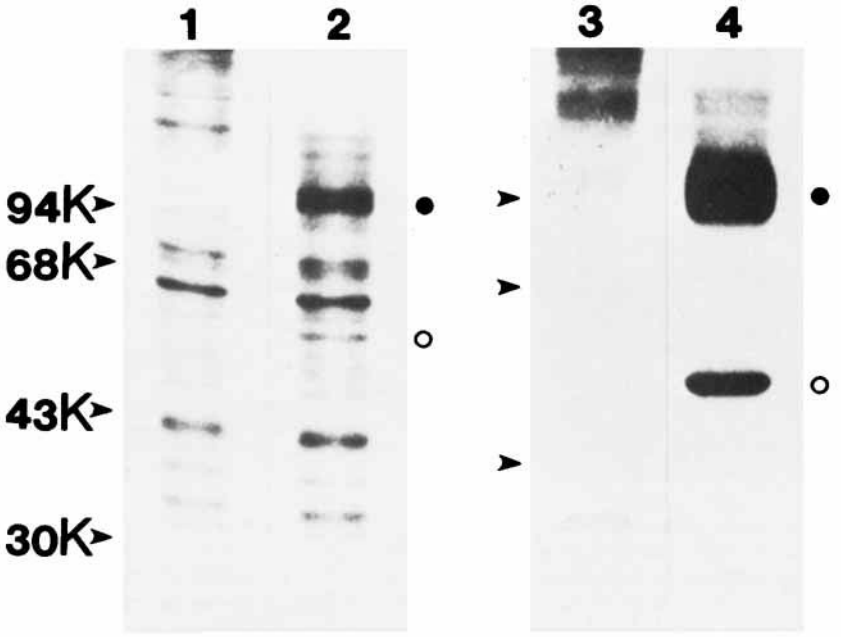


Fig. 5. Demonstration that both external and internal large T-ag and 53K protein are phosphorylated. mKSA-Asc cells were labeled for 2 hr with $^{32}P_i$ (see Materials and Methods) and then subjected to external (1,2) and internal (3,4) immunoprecipitation by NHS (1,3) or HAF (2.4), as described in the legend to Figure 4. Antigens were eluted from the bacterial pellet and analyzed by 12% SDS-polyacrylamide gel electrophoresis and autoradiography. Only 10% of the total internal immunoprecipitates were loaded in lanes 3 and 4. The positions of marker proteins are indicated with arrowheads. ●, large T-ag; ○, 53K protein.

## Immunoprecipitation of Surface SV40 T-ag and 53K Protein by Monoclonal Antibodies Against the 53K Mouse Cellular Protein

To determine whether the surface-associated 53K protein was, in fact, the described 53K cellular protein, monoclonal antibodies (PAb122) directed against the mouse 53K protein [49] were used to immunoprecipitate surface antigens from surface-iodinated mKSA-Asc cells. Large T-ag and the 53K protein were coprecipitated by the PAb122 monoclonal antibodies during the external reaction (Fig. 6, lane 3); the proteins were not precipitated by control monoclonal antibodies directed against human immunoglobulins (Fig. 6, lane 2). The iodinated molecules comigrated with large T-ag and 53K protein coprecipitated by PAb122 monoclonal antibodies from whole cell lysates of $^{32}P_i$-labeled mKSA-Asc or BALB/WT cells (Fig. 6, lanes 5 and 7, respectively). These data suggest that the surface-associated 53K phosphoprotein and the previously described 53K cellular protein are the same molecule.

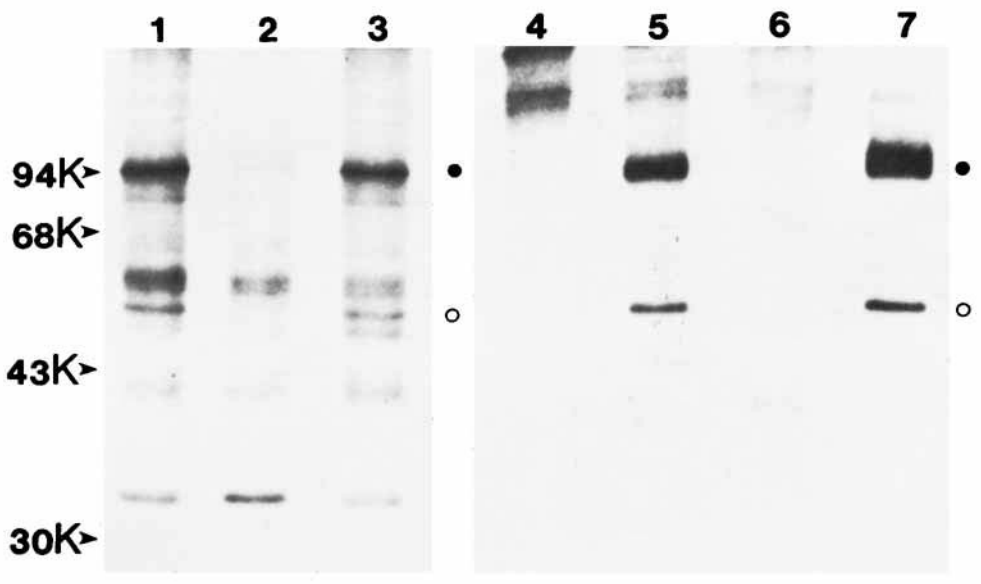


Fig. 6. Immunoprecipitation of large T-ag and 53K protein by monoclonal antibodies against the 53K mouse cellular protein. mKSA-Asc cells were surface-iodinated by the lactoperoxidase technique (1-3) or metabolically labeled for 2 hr with $^{32}P_i$ (4,5). BALB/WT cells were also labeled for 2 hr with $^{32}P_i$ (6,7). Iodinated cells were subjected to external immunoprecipitation using 50 μl of HAF (1), 500 μl of control 9N supernatant fluid (2), or 500 μl of PAb122 supernatant fluid (3). Incubations with 9N or PAb122 monoclonal antibodies were followed by incubations with goat antimouse immunoglobulins. The cells were disrupted with an NP40 solution and the immune complexes were adsorbed with SACI as described in the legend to Figure 4. $^{32}P_i$-labeled cells were disrupted with an NP40 solution and the antigens present in the clarified cell lysate were precipitated by overnight incubation at 4°C with 100 μl of 9N (4,6) or PAb122 medium (5,7), followed by a second incubation with goat antimouse immunoglobulins. Immune complexes were again adsorbed with SACI as indicated. Eluted antigens were analyzed by 12% SDS-polyacrylamide gel electrophoresis and autoradiography. Only 10% of the total immunoprecipitates was loaded on lanes 4–7. Molecular weight markers are indicated on the left. ●, large T-ag; ○, 53K protein.

Two obvious explanations for the observation that surface large T-ag and the 53K cellular protein were coprecipitated by both anti-T and anti-53K antibodies were that (i) both molecules possessed some common antigenic determinant(s) or (ii) the two proteins were physically associated on the cell surface.

To rule out the possibility of antigenic cross-reactivity between large T-ag and the surface-associated 53K cellular protein, it was demonstrated that RαT serum does not recognize free (uncomplexed) 53K cellular protein and, conversely, that PAb122 antibodies do not recognize free large T-ag molecules. Mouse mammary tumor cells (MTV-L/BALB cells) express the host-coded 53K protein (Slagle and Butel, unpublished observations), but not SV40 large T-ag. [$^{35}$S]Methionine-labeled 53K protein was specifically immunoprecipitated from MTV-L/BALB cell lysate by PAb122 antibodies (Fig. 7, lane 2), but not by RαT serum (Fig. 7, lane 4). However, both 53K and large T-ag were precipitated from [$^{35}$S]methionine-labeled mKSA-Asc cell lysates by PAb122 antibodies (Fig. 7, lane 6) and RαT serum (Fig. 7, lane 8). The band of 53K cellular protein precipitated by RαT serum appears more diffuse because of the effect of a large amount of rabbit immunoglobulins that migrate faster than the 53K protein [59]. After complete removal of 53K/T-ag complexes from an

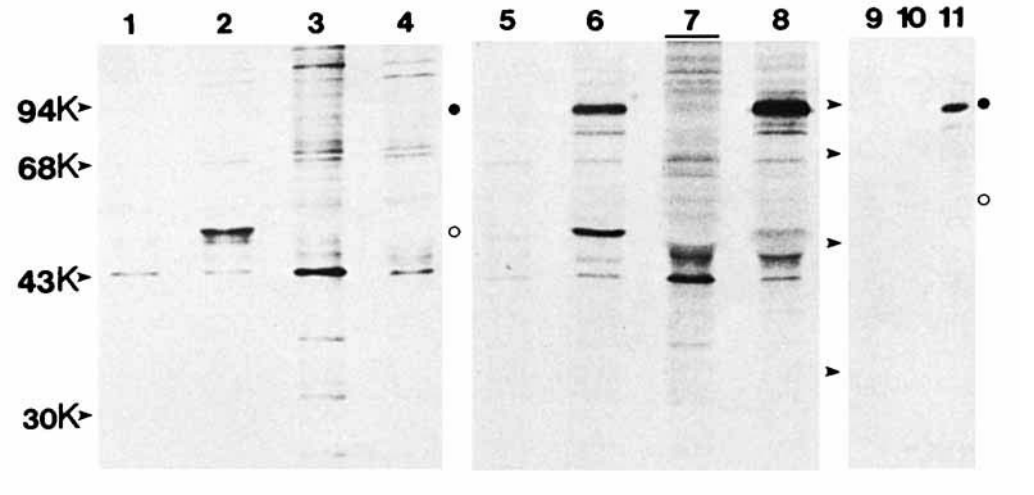


Fig. 7. Lack of antigenic cross-reactivity between surface-associated T-ag and 53K protein. MTV-L/BALB cells (1–4) and mKSA-Asc cells (5–8) were metabolically labeled for 3 hr with [$^{35}$S]methionine (see Materials and Methods), were disrupted with an NP40 solution, and then incubated overnight at 4°C with 100 μl of 9N medium (1,5), 100 μl of PAb122 medium (2,6), 15 μl of NRS (3,7), or 15 μl of RαT serum (4,8). Incubations with 9N or PAb122 medium were followed by incubations with goat antimouse immunoglobulins for 30 min on ice and the immune complexes adsorbed with SACI. mKSA-Asc cells were also labeled for 15 min and then disrupted with an NP40 solution. Each of three 100-μl aliquots of clarified cell lysate (≈ 5 × $10^5$ cells) was subjected to four sequential immunoprecipitations with 10 μl of PAb122 medium for 1 hr at 4°C, followed by incubation with 2 μl of goat antimouse immunoglobulins for 30 min at 4°C. Immune complexes were adsorbed with SACI each time; a fifth adsorption with SACI was included to remove any remaining immune complexes. The final adsorbed lysates were then reacted with 10 μl of 9N medium (9), PAb122 medium (10), or RαT serum (11) by incubation at 4°C for 1 hr. Incubations with 9N or PAb122 were again followed by incubation with a second antibody. Antigens were eluted from the bacterial pellets and analyzed by 12% SDS-polyacrylamide gel electrophoresis and autoradiography. Molecular weight markers are indicated with arrowheads. ●, large T-ag; ○, 53K protein.

[$^{35}$S]methionine-labeled mKSA-Asc cell lysate by four successive immunoprecipitations with PAb122 antibodies [49,59], the remaining free (unbound) T-ag was precipitated by R$\alpha$T serum (Fig. 7, lane 11), but not by monoclonal antibodies against 53K (Fig. 7, lane 10). The results from this experiment demonstrate that PAb122 antibodies do not recognize any antigenic determinant on the T-ag molecule and that antibodies against purified T-ag do not recognize 53K protein. Therefore, in the absence of antigenic cross-reactivity, coprecipitation of T-ag and 53K cellular protein by either type of antibody suggests that the molecules are physically associated on the surface of SV40-transformed mouse cells.

It can be concluded from these studies that the 53K cellular phosphoprotein is exposed on the surface of SV40-transformed mouse cells such that it can be iodinated and recognized by specific antibodies and that the 53K host-coded protein is associated with SV40 large T-ag both internally and on the surface of SV40-transformed mouse cells.

## Characterization of Surface SV40 T-ag and 53K Cellular Protein Complex by Monoclonal Antibodies Against SV40 Large T-ag

Monoclonal antibodies are ideal immunological tools with which to analyze the structure and function(s) of SV40 large T-ag. They were utilized to further characterize the association of T-ag and 53K protein on the surface of SV40-transformed cells.

Independent series of monoclonal antibodies with reactivity against SV40 large T-ag or 53K host protein have been described by Gurney et al [49] and Harlow et al [50]. The binding sites of the anti-T monoclonal antibodies available for this study were shown under Materials and Methods (Fig. 1).

Supernatants from the various anti-T hybridomas were tested for reactivity against nuclear T-ag by immunofluorescence using acetone-fixed transformed cells (Table 1). Despite some quantitative differences, all the anti-T monoclonals reacted

**TABLE I. Reactivity of Anti-T Monoclonal Antibodies With Nuclear SV40 T-ag by Immunofluorescence***

| Hybridoma | Antibody titer[a] |
|---|---|
| PAb416 | 32 |
| PAb419 | 32 |
| PAb430 | 16 |
| PAb7 | 16 |
| PAb412 | 32 |
| PAb405 | 64 |
| PAb414 | 16 |
| PAb423 | 64 |

*SV40-transformed cells were grown on coverslips, fixed with acetone for 5 min, and reacted with different dilutions of hybridoma supernatant fluids for 30 min at room temperature. The cells were washed and then incubated with FITC-conjugated rabbit antimouse immunoglobulins for 30 min at room temperature.

[a]Antibody titers are expressed as the reciprocal of the highest dilution at which positive nuclear staining was evident.

with nuclear T-ag. Supernatants from PAb421 and PAb122, which recognize antigenic determinants on the 53K cellular protein, also had a positive nuclear reactivity. The reagents were then tested for the ability to immunoprecipitate surface T-ag and 53K protein from surface-iodinated mKSA-Asc cells (Fig. 8). The same volume (200 μl) of hybridoma supernatant fluid was employed with each sample. All the monoclonals, except PAb430 (Fig. 8, lane 3), precipitated both T-ag and 53K protein during the external reaction. However, both proteins were weakly precipitated by PAb430 when a 30-fold concentrate of supernatant fluid was used (data not shown). Interestingly, the same amount of PAb430 concentrate was able to precipitate a relatively large amount of internal T-ag and 53K protein (data not shown).

These results obtained with anti-T monoclonal antibodies indicate that, regardless of the antigenic site on the T-ag molecule recognized by specific antibodies, coprecipitation of the 53K protein occurs. Furthermore, it is apparent that both the amino and carboxy termini of the T-ag molecule are exposed on the cell surface. The tertiary configuration of the protein in the membrane with respect to internal portions of the molecule remains to be determined, however, when additional monoclonal antibodies become available.

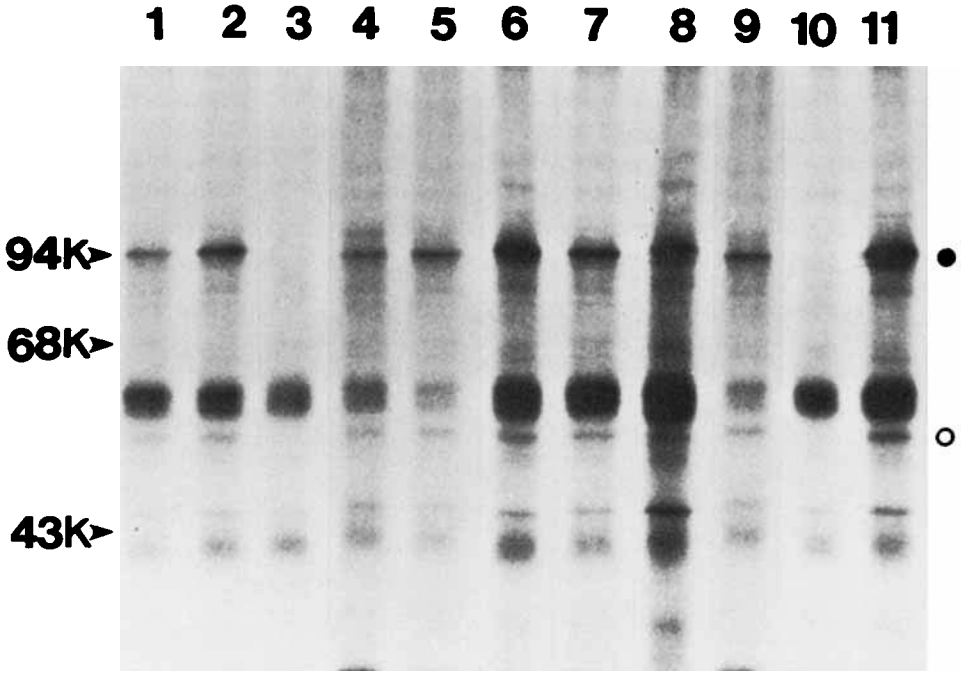


Fig. 8. Detection of complex between T-ag and 53K protein on the surface of SV40-transformed cells by the use of monoclonal antibodies. mKSA-Asc cells were surface-iodinated by the lactoperoxidase technique and subjected to external immunoprecipitation using 200 μl of supernatant fluids from PAb416 (1), PAb419 (2), PAb430 (3), PAb7 (4), PAb412 (5), PAb405 (6), PAb414(7), PAb423 (8), PAb122(9), or 9N (10) hybridomas or 50 μl of HAF (11). After a second incubation with goat antimouse immunoglobulins, cells were disrupted with an NP40 solution, and the immune complexes in the clarified cell lysates were adsorbed with SACI. Antigens were eluted from the bacterial pellets and analyzed by 12% SDS-polyacrylamide gel electrophoresis and autoradiography. Molecular weight markers are indicated on the left. ●, large T-ag; ○, 53K protein.

The exposure of surface T-ag could be influenced by a relatively unstable association of T-ag with the membrane. Soule et al [46] have shown that T-ag appears to be completely replaced on the surface of SV40-transformed mouse or hamster cells during a period of 2.5 hr. The disappearance of $^{125}$I-labeled T-ag from the cell surface is illustrated in Figure 9. Surface-iodinated mKSA-Asc cells were subjected to external immunoprecipitation by PAb405 (Fig. 9A) or by PAb419 (Fig. 9B), either immediately after iodination or after different chase periods (incubation in fresh medium at 37°C). A significant decrease in the amount of $^{125}$I-labeled surface T-ag was observed as soon as 5–10 min after iodination (Fig. 9A, lanes 3 and 4; Fig. 9B, lanes 3 and 4), and iodinated T-ag was not detectable after a 60-min chase (Fig. 9A, lane 7; Fig 9B, lane 7). This relatively rapid loss of surface T-ag appears to be specific for that molecule rather than representative of a general plasma membrane phenomenon (Santos and Butel, in preparation).

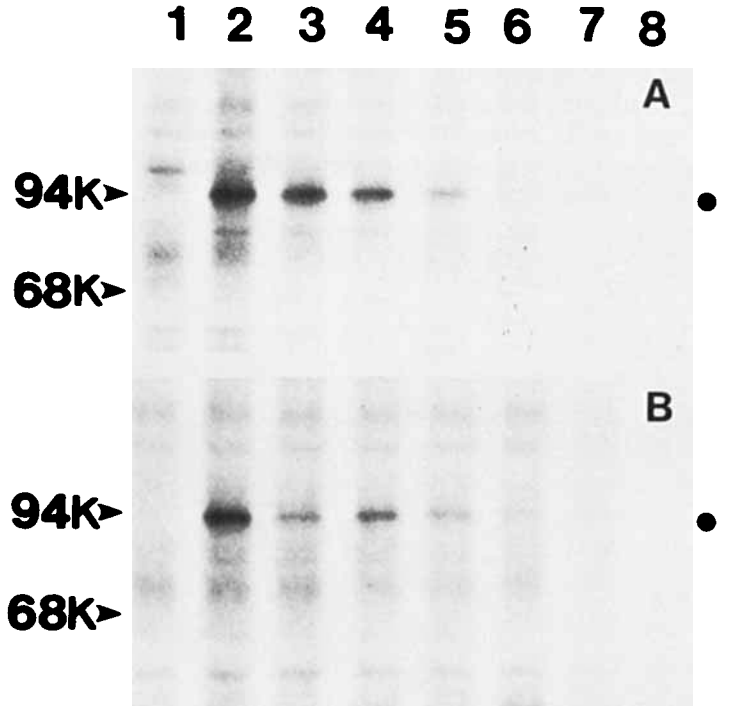


Fig. 9. Disappearance of $^{125}$I-labeled T-ag and 53K protein from the surface of SV40-transformed cells. mKSA-Asc cells were surface-iodinated by the lactoperoxidase technique and subjected to external immunoprecipitation immediately after labeling (A1,2;B1,2) or after 5 min (A3,B3); 10 min (A4,B4), 15 min (A5,B5), 30 min (A6,B6), 60 min (A7,B7), or 120 min (A8,B8) of incubation in fresh medium at 37°C (chase period). At each time point, cells in a 100-mm petri dish were incubated first for 30 min at 4°C with 1 ml of DMEM containing 50 $\mu$l of a 30-fold concentrate of supernatant fluids from PAb405 (A) or PAb419 (B) hybridomas. Cells were then incubated with goat antimouse immunoglobulins for 30 min at 4°C, were disrupted with an NP40 solution, and immune complexes were adsorbed with SACI. Eluted antigens were analyzed by 12% SDS-polyacrylamide gel electrophoresis and autoradiography. Lanes A1 and B1 correspond to the external immunoprecipitate obtained with control 9N hybridoma. Molecular weight markers are indicated on the left. ●, large T-ag.

## Histocompatibility Antigens on the Surface of SV40-Transformed Cells

H-2 antigens are glycoproteins of 45,000 (45K) molecular weight that are inserted in the plasma membrane and noncovalently associated with β-2-microglobulin (12K) [60]. Neither one of these proteins was specifically precipitated by anti-T antibodies (eg, Figs. 4–6). It has been shown that H-2 antigens bind to specific antibodies more efficiently at 37°C than at 4°C [61]. Since T-ag is routinely immunoprecipitated at 4°C, it was necessary to determine whether H-2 antigens could also be detected under these conditions. mKSA-Asc cells were surface-iodinated by the lactoperoxidase catalyzed reaction and then subjected to external immunoprecipitation by incubation with NMS (Fig. 10, lanes 1 and 2) and with $D^d$ antiserum (Fig. 10, lanes 3 and 4) for 30 min at 4°C (Fig. 10, lanes 1 and 3) or at 37°C (Fig. 10, lanes 2 and 4). The heavy chain of H-2.4 antigen was specifically immunoprecipitated at both temperatures. β-2-microglobulin was also precipitated (data not shown). Similar results were obtained when BALB/WT cells were tested (data not shown).

Adsorption of $D^d$ antiserum with BALB/c ($H\text{-}2^d$) lymphocytes (Fig. 10, lane 6) completely abolished the anti-H-2.4 reactivity, whereas adsorption with C3H ($H\text{-}2^k$) lymphocytes had no effect (Fig. 10, lane 8). Therefore, the H-2 antigen precipitation described above is due to a specific reaction. No large T-ag was precipitated by anti-H-2.4 serum, confirming those results obtained through the use of different anti-T antibodies. These results indicate that no detergent-stable complex between large T-ag and H-2 antigens is present on the surface of SV40-transformed mouse cells.

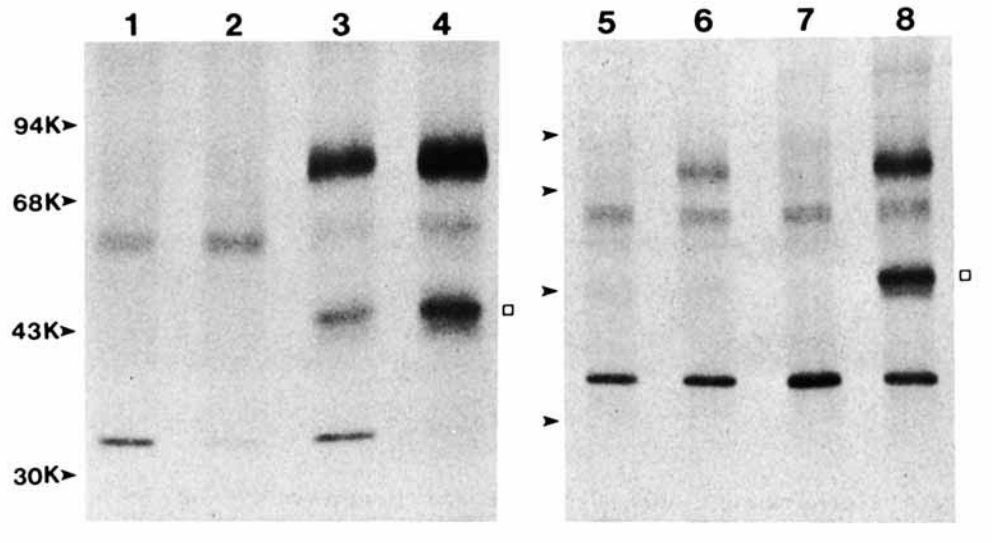


Fig. 10. Immunoprecipitation of H-2 antigens from the surface of SV40-transformed cells. mKSA-Asc cells were surface-iodinated by the lactoperoxidase technique and subjected to external immunoprecipitation by incubation with NMS (1,2,5,7) or anti-H-2, $D^d$ serum (3,4,6,8) for 30 min at 4° C (1,3) or 37°C (2,4–8). The antisera were adsorbed with lymphocytes from BALB/c (5,6) or C3H (7,8) mice prior to incubations with the cells. Cells were disrupted in an NP40 solution, cell lysates clarified by centrifugation, and immune complexes adsorbed with SACI. Eluted antigens were analyzed by 10% SDS-polyacrylamide gel electrophoresis and autoradiography. The positions of molecular weight marker proteins are indicated by arrowheads. □, H-2 antigens (heavy chain).

## DISCUSSION

A complex composed of large T-ag and the 53K cellular protein appears to be present on the surface of SV40-transformed mouse cells. This conclusion is supported by the fact that monoclonal antibodies directed against large T-ag as well as those directed against the 53K protein are able to coprecipitate both proteins from the surface of SV40-transformed cells. No antigenic cross-reactivity between the viral and cellular proteins was detected. Therefore, coprecipitation must be due to a physical interaction between large T-ag and 53K cellular protein.

The data presented here constitute further evidence for the presence of large T-ag on the surface of SV40-transformed cells and confirm that the 53K cellular protein is also located at that subcellular level. The 53K protein had been previously detected in plasma membrane-enriched subcellular fractions prepared from SV40-transformed cells [10,12] and has been labeled by the lactoperoxidase-catalyzed radioiodination procedure [58], suggesting that the molecule is exposed on the cell surface.

Partial characterization of the surface-associated large T-ag has been accomplished through the use of a series of T-reactive monoclonal antibodies. The series includes antibodies directed against unique antigenic sites located near the amino or carboxy terminus of the T-ag molecule. All the antibody preparations tested were able to precipitate external T-ag, suggesting that each correspondent antigenic determinant is exposed on the cell surface. The observed variability in the amount of surface T-ag recovered from immunoprecipitates by different monoclonal antibodies may reflect different degrees of exposure of the individual antigenic sites. The carboxy terminus might be more accessible for recognition and binding by specific antibodies than the amino terminus. The exposure of the carboxy terminus of T-ag on the cell surface was suggested first by the fact that SV40-specific proteins that are coded for by the nondefective adenovirus type 2-simian virus 40 hybrid viruses ($Ad2^+ND1$ and $Ad2^+ND2$) are present on the surface of cells infected with these hybrid viruses [62,63]. The truncated SV40-related proteins induced by the hybrid viruses have been mapped to the COOH-terminal portion of the SV40 T-ag molecule [64,65].

Different extents of exposure of individual domains of the T-ag molecule might be the result of its tertiary conformation in the membrane or a consequence of association with other cell surface components, such as the 53K protein. Figure 11 is a schematic representation of the cell surface complex between T-ag and the 53K protein. The precise nature of the interaction between the two proteins is unknown at this time, as is their configuration in the plasma membrane. However, results with the monoclonal antibodies suggest that a relatively large part of the T-ag molecule is exposed on the exterior of the cell surface. A note of caution must be emphasized regarding that interpretation, however, since as a consequence of the shedding process T-ag molecules might be differentially exposed on the cell surface. In a population of membrane-associated molecules, most, if not all, antigenic sites would be represented and accessible to antibody binding.

The presence of the complex between large T-ag and 53K cellular protein on the surface of the SV40-transformed cells might play a role in maintaining the transformed phenotype. An integration between cell surface events and nuclear events, throughout the cell cycle, has been shown [66–68]. Extensive change of the cell surface, as occurs in transformed cells, must surely alter control of cellular

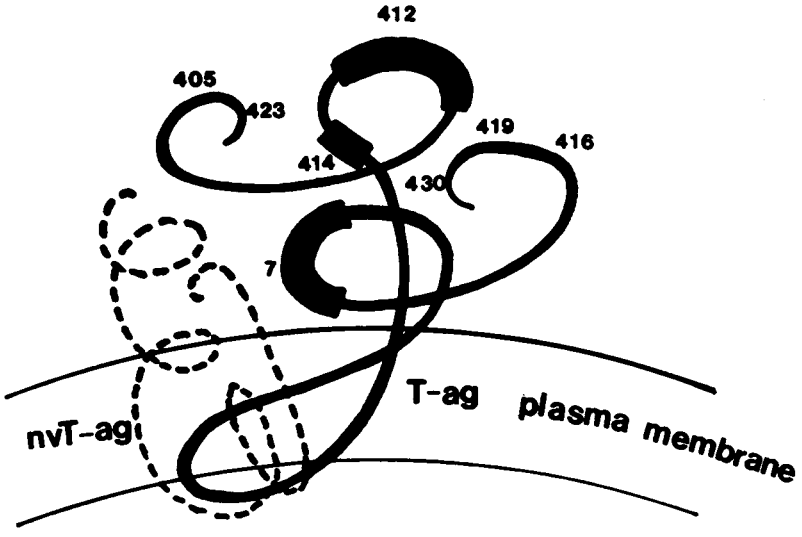


Fig. 11. Schematic representation of the complex between large T-ag and 53K protein on the surface of SV40-transformed mouse cells. The numbers represent the approximate positions of the antigenic sites that are recognized on T-ag by the indicated monoclonal antibodies. The conformation of the molecule has been drawn to reflect the relative surface reactivity of the respective monoclonal antibodies in external immunoprecipitation assays. The thickened bars represent conformational antigenic sites. The 53K cellular protein is designated as nvT-ag. Neither the precise nature of the interaction between T-ag and 53K nor the configuration of the complex in the plasma membrane is known at this time.

physiology. Normal cell growth control mechanisms are lost as a result of transformation; the majority of transformed cells will remain in the proliferative cycle, while normal cell counterparts will be predominantly in a resting state.

SV40 large T-ag is able to induce cell DNA synthesis [69–72], and the 53K protein has been postulated to be involved in the normal regulation of cell division [40–42]. Therefore, the presence of the complex between large T-ag and 53K protein in the nuclei of SV40-transformed cells might constitute a continuous stimulus for cell proliferation. A direct effect at the nuclear level might be potentiated by the constant presence of the complex on the cell surface, where it might constitute a permanent signal that instructs the nucleus to keep the cell in the division cycle.

The molecular identity of TSTA in SV40-transformed cells is still not known [for a review, see 16,17]. However, there have been suggestions that either large T-ag and TSTA are the same molecular entity or that TSTA could be the result of an association between T-ag and some cellular protein [18–21,73]. Therefore, it is possible that the complex between large T-ag and 53K protein on the surface of SV40-transformed cells could represent the TSTA. Histocompatibility antigens have been found in association with tumor antigens [23–27,74], and they have been postulated to be part of TSTA. We have not detected any association between large T-ag and H-2 antigens on the surface of SV40-transformed cells, but such a putative complex might be unstable under the experimental conditions employed. Studies are in progress to further analyze this point.

## ACKNOWLEDGMENTS

The authors gratefully acknowledge the excellent technical assistance of Mary Ann Hrovat and the receipt of hybridomas from E. Gurney, E. Harlow, and G. Dreesman. This study was supported in part by research grant CA 22555 from the National Cancer Institute.

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## NOTE ADDED IN PROOF

PAb7 and PAb412 have been designated recently PAb100 and PAb101, respectively.