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# Antigenic Structure of Simian Virus 40 Large Tumor Antigen and Association with Cellular Protein p53 on the Surfaces of Simian Virus 40-Infected and -Transformed Cells

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**The antigenic structure of simian virus 40 (SV40) large tumor antigen (T-ag) in the plasma membranes of SV40-transformed mouse cells and SV40-infected monkey cells was characterized as a step toward defining possible biological function(s). Wild-type SV40, as well as a deletion mutant of SV40 (*dl*1263) which codes for a truncated T-ag with an altered carboxy terminus, was used to infect permissive cells. Members of a series of monoclonal antibodies directed against antigenic determinants on either the amino or the carboxy terminus of the T-ag polypeptide were able to precipitate surface T-ag (as well as nuclear T-ag) from both SV40-transformed and SV40-infected cells. Cellular protein p53 was coprecipitated with T-ag by all T-ag-reactive reagents from the surface and nucleus of SV40-transformed cells. In contrast, T-ag, but not T-ag–p53 complex, was recovered from the surface of SV40-infected cells. These results confirm that nuclear T-ag and surface T-ag are highly related molecules and that a complex of SV40 T-ag and p53 is present at the surface of SV40-transformed cells. Detectable levels of such a complex do not appear to be present on SV40-infected cells. Both the carboxy and amino termini of T-ag are exposed on the surfaces of SV40-transformed and -infected cells. The possible relevance of the presence of a T-ag-p53 complex on the surface of SV40-transformed cells and its absence from SV40-infected cells is considered.**

Many biochemical, physiological, and morphological changes take place after cell transformation induced by a variety of agents, including viruses (for a review, see reference 38). Viruses are ideal models with which to dissect the molecular mechanisms involved in cell transformation since their genetic content is small, well defined, and amenable to manipulation. Nonstructural viral proteins, termed tumor antigens (T-ag), are synthesized by simian virus 40 (SV40)-infected and -transformed cells (for reviews, see references 35, 63, and 65). Large T-ag, a phosphoprotein with an apparent molecular weight of 94,000 (94K), appears to be responsible for initiation and maintenance of the transformed phenotype (2, 4, 29, 37, 41, 59).

Large T-ag may be considered a multifunctional molecule because of the many functions that it mediates in both SV40-transformed and -infected cells. In productively infected cells, large T-ag is required for initiation of viral DNA replication (7, 58) and is involved in autoregulation of transcription of the early region of the viral genome (1, 28, 43, 45). Induction of cellular DNA and rRNA synthesis in SV40-transformed cells also appears to be dependent on the presence of a functional large T-ag (3, 8, 19–21, 26, 50–53, 62, 66).

Several of the recognized biological activities of T-ag have been assigned to relatively small, specific coding sequences in the *A* gene of SV40 and, by extrapolation, localized to defined regions of the T-ag molecule (44). Therefore, the T-ag molecule can be visualized as being composed of different functional domains which can be related to the structure of the polypeptide.

The majority of the large T-ag in the cell is concentrated in the nucleus, but a small amount is also present on the surface of SV40-transformed cells (6, 15, 18, 25, 27, 32, 34, 36, 46, 47, 49, 54–56). The biological significance of this surface-associated subset of T-ag is still unknown, but the possibility exists that those molecules may also be multifunctional. To better understand the function(s) of surface-associated T-ag, it is necessary to know which structural domains of the molecule are exposed on the cell surface, as well as the disposition of the T-ag molecule in the plasma membrane. Monoclonal antibodies with specificities against defined, unique portions of the T-ag molecule are powerful tools which can be exploited to obtain that information. Further, to establish whether surface T-ag has a transformation-related role, similarities and dissimilarities between surface T-ag from SV40-transformed and -infected cells must be determined.

We have analyzed the reactivities of cell surface-associated T-ag and nuclear T-ag with a series of immunological reagents, including monoclonal antibodies directed against antigenic sites located on either the amino or the carboxy third of the molecule. SV40-transformed cells and permissive cells infected with either wild-type SV40 (WT SV40) or a deletion mutant of SV40 were compared. The results obtained indicate that both the amino and the carboxy termini of the T-ag polypeptide are exposed on the exterior of the cell surface in both SV40-transformed and -infected cells. The anti-T-ag monoclonal antibodies coprecipitated the 53K cellular protein (p53) from the surface of SV40-transformed cells, confirming previous indications that a complex between large T-ag and p53 polypeptide is present on the surface of such cells (46, 47). However, detectable levels of such a complex did not appear to be present on the surface of SV40-infected cells. These observations may suggest a potential role for the cell surface-associated T-ag–p53 complex in transformation.

## MATERIALS AND METHODS

**Cells, viruses, and radioactive labeling.** The transplantable mKSA-Asc line of SV40-transformed BALB/c mouse kidney cells (30, 67) was cultured as described previously (46). TC7 cells, a stable clonal line of African green monkey kidney

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cells derived from the CV-1 line, were routinely propagated as previously described (39). The Baylor reference strain of WT SV40 (40) and *dl*1263, a viable deletion mutant of SV40 (11), were used. TC7 cells were infected with either WT SV40 or *dl*1263 at a multiplicity of infection of 10 PFU per cell as described previously (54). Metabolic labeling with [$^{35}$S]methionine and lactoperoxidase-catalyzed cell surface iodination were accomplished with procedures previously described (46, 54, 56).

**Antisera and monoclonal antibodies.** Ascitic fluid from hamsters bearing SV40 ascites tumors (HAF) (32), sera from mice bearing tumors induced by SV40-transformed cells (MoαT) (55), sera from mice which had rejected transplants of SV40 tumor cells (MoαS) (55), sera from mice which had been inoculated with plasma membrane- or nucleus-enriched fractions obtained from SV40 tumor cells (MoαPM and MoαNuc, respectively) (55), and a series of monoclonal antibodies with reactivity against purified SV40 large T-ag or mouse p53 protein were used. The hybridomas were generously provided by E. Gurney (PAb100, PAb101, PAb122) (22), E. Harlow (PAb405, PAb414, PAb416, PAb419, PAb421, PAb423, PAb430) (23), and D. Lane and R. Tjian (PAb204) (9). The hybridoma nomenclature is as proposed by Crawford and Harlow (12). Supernatant fluids from the hybridomas were concentrated 30-fold by 18% $Na_2SO_4$ precipitation. The unique antigenic sites recognized by the anti-T-ag monoclonal antibodies have been mapped to the amino third or the carboxy third of the T-ag polypeptide (9, 14, 23). Those binding sites are indicated in Table 1. The antigenic determinant which is recognized by PAb204 has been mapped to 0.29 to 0.37 map units (9). Monoclonal antibodies directed against human immunoglobulins (9N), a gift from G. Dreesman, were used as a negative control.

**Differential immunoprecipitation and electrophoresis.** Cell surface T-ag and nuclear T-ag were selectively detected by the differential immunoprecipitation technique described by Santos and Butel (46, 47). Briefly, to detect surface T-ag, cells were first incubated with specific antibodies and then extracted with buffer containing nonionic detergent (1% Nonidet P-40 [NP-40]), pH 7.5. Solubilized immune complexes were removed from the cell lysate by adsorption with Formalin-fixed *Staphylococcus aureus* Cowan I (SACI). Nuclear T-ag which remained free in the supernatant after the bacteria had been removed was then immunoprecipitated by a second incubation with specific antibodies, followed by a second adsorption with SACI.

Adsorbed proteins were eluted from bacterial pellets and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in 12% slab gels (46, 54). [$^{14}$C]phenylisothiocyanate (Amersham Corp., Arlington Heights, Ill.)-labeled phosphorylase *a* (94K), bovine serum albumin (68K), ovalbumin (43K), carbonic anhydrase (30K), and cytochrome *c* (11.7K) obtained from Pharmacia Fine Chemicals, Piscataway, N.J., were used as molecular weight markers. Autoradiography was performed with NS5T X-ray film.

**Immunofluorescence reaction.** Hybridoma supernatant fluids and the corresponding concentrates were screened for nuclear activity by indirect immunofluorescence (IF) with acetone-fixed SV40-transformed cells as described previously (42).

## RESULTS

**Definition of antigenic structure of surface T-ag and nuclear T-ag of SV40-transformed cells with monoclonal antibodies.** The correlation between structure and function of a given protein is well established. Therefore, studies designed to investigate the function(s) of a macromolecule are greatly facilitated if its structure is known. Functions attributable to nuclear T-ag are better elucidated than are those for surface T-ag; several functions have already been mapped to specific domains of the polypeptide (see above). A comparison between the structures of surface T-ag and nuclear T-ag would be a useful prelude to ascertaining the function(s) of large T-ag on the surfaces of SV40-infected and -transformed cells. We initiated such a comparison through the use of a series of T-ag-reactive monoclonal antibodies.

All the monoclonal antibodies used in this study have been previously characterized (9, 14, 22, 23). The antigenic sites on the T-ag molecule recognized by these monoclonal antibodies are indicated in Table 1. Supernatant fluids (47) and fluid concentrates (prepared as described above) from the anti-T-ag hybridomas were tested for reactivity against nuclear T-ag by IF on acetone-fixed SV40-transformed cells (Table 1). Monoclonal antibodies directed against cellular protein p53 were treated similarly. All the preparations displayed a positive nuclear reaction.

The differential immunoprecipitation technique has been shown to be both sensitive and selective in distinguishing surface T-ag and nuclear T-ag in the same cells (46), and the specific polypeptides corresponding to labeled antigens can be identified in autoradiographs of polyacrylamide gels. Therefore, this technique was chosen to compare the antigenic structures of both surface T-ag and nuclear T-ag.

Surface-iodinated mKSA-Asc cells were subjected to external immunoprecipitation with hybridoma supernatant concentrates. Each concentrate was adjusted, based on data in Table 1, so that equivalent titers of nuclear reactivity were used. An important caveat about normalizing the antibody titers in this way is that acetone fixation of the nuclear T-ag might adversely affect the binding of a given monoclonal antibody to its antigenic site and cause underestimation of its actual titer. All monoclonal antibodies tested were able to precipitate surface T-ag (Fig. 1) and coprecipitate p53 cellular protein. The p53 band appears partially masked in this figure by another protein which is precipitated nonspecifically even by the control 9N (Fig. 1, lane 11).

TABLE 1. Reactivity of monoclonal antibodies with SV40 T-ag and cellular protein p53

| Monoclonal antibody | Binding site on T-ag[a] | Nuclear reactivity (IF titer)[b] |
|---|---|---|
| PAb416 | 0.43–0.65 (A) | 640 |
| PAb419 | 0.60–0.65 (A) | >640 |
| PAb430 | 0.60–0.65 (A) | 160 |
| PAb100 | 0.28–0.44 (C) | 100 |
| PAb204 | 0.29–0.37 (C) | 320 |
| PAb101 | 0.17–0.28 (C) | 640 |
| PAb405 | 0.17–0.28 (C) | >640 |
| PAb414 | 0.17–0.39 (C) | 512 |
| PAb423 | 0.17–0.39 (C) | 400 |
| PAb122 | None[c] | 512 |
| PAb421 | None[c] | 640 |

[a] The region of SV40 DNA (in map units) encoding the portion of T-ag containing the antigenic determinant recognized by the monoclonal antibody; data taken from Harlow et al. (23), Deppert et al. (14), and Clark et al. (9). The letters in parentheses indicate that the antigenic determinant is near the amino (A) or the carboxy (C) end of the T-ag polypeptide.

[b] Nuclear reactivity was determined by an IF test as indicated in the text. Titer corresponds to the reciprocal of the highest dilution at which nuclear staining was still positive.

[c] Monoclonal antibodies directed against mouse cellular protein p53.

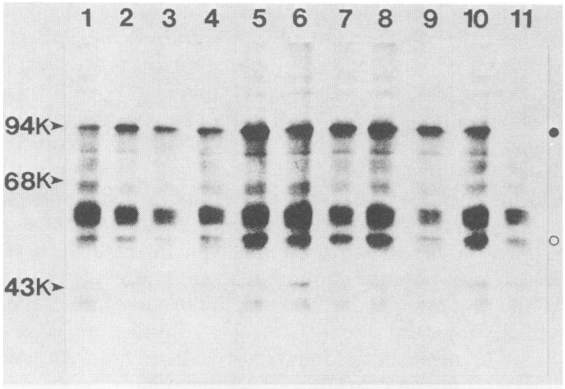


FIG. 1. Detection of surface T-ag and p53 on SV40-transformed cells by monoclonal antibodies. mKSA-Asc cells were surface iodinated by the lactoperoxidase technique and subjected to external immunoprecipitation with equivalent titers of nuclear T-ag reactivity for each hybridoma supernatant concentrate. Lanes: 1, PAb416; 2, PAb419; 3, PAb430; 4, PAb100; 5, PAb101; 6, PAb405; 7, PAb414; 8, PAb423; 9, PAb122 (unconcentrated supernatant fluid); 10, HAF; and 11, 9N. Incubations with monoclonal antibodies were followed by incubations with goat antimouse immunoglobulins. Cells were then disrupted with an NP-40 solution, and the immune complexes in the clarified cell lysates were adsorbed with SACI. Eluted antigens were analyzed by 12% SDS-PAGE and autoradiography. Molecular weight markers are indicated on the left. Symbols: ●, large T-ag; ○, p53.

Importantly, monoclonal antibodies directed against p53 (PAb122) also coprecipitated both T-ag and p53 (Fig. 1, lane 9), substantiating our previous observations that a T-ag–p53 complex is present on the surface of SV40-transformed cells (46).

It appears, therefore, that antigenic determinants located at both the carboxy and the amino termini of the T-ag molecule are exposed on the transformed cell surface. It should be noted, however, that relatively less iodinated T-ag was bound by monoclonal antibodies directed against determinants on the amino terminus (Fig. 1, lanes 1 through 3) than by antibodies directed against the carboxy terminus (Fig. 1, lanes 5 through 8). PAb100 (Fig. 1, lane 4), which is directed toward a determinant more internal on the carboxy end of the molecule than are other carboxy-specific monoclonal antibodies, behaved more like the antibodies directed toward the amino terminus of T-ag. These interpretations must be viewed with a certain amount of caution, as the binding sites on T-ag have not been mapped very precisely for some of the monoclonal antibodies; apparent reactivities may have been influenced in the mapping studies by the conformation of truncated polypeptides used to define provisional binding sites.

The surface reactivity of the monoclonal antibodies was compared with the nuclear reactivity by performing the internal immunoprecipitation reaction with all of them. [$^{35}$S]methionine-labeled cells were first subjected to external immunoprecipitation with HAF to remove membrane-associated complexes of T-ag and p53 protein. Samples (100 μl) from the clarified cell lysate were then reacted with 5 μl of each of the different hybridoma supernatant concentrates; a secondary antibody was added before the immune complexes were adsorbed with SACI and processed as indicated above. Both large T-ag and p53 protein were coprecipitated by all the monoclonal antibodies (Fig. 2). It is informative to note that preparations which had reacted poorly with surface T-ag, such as PAb416 and PAb430 (Fig. 1, lanes 1 and 3), precipitated large amounts of nuclear T-ag (Fig. 2, lanes 6 and 4, respectively). Therefore, a weak surface reactivity does not necessarily reflect a lower concentration or a lower affinity of antibody.

In addition to p53, another protein, presumably cellular in origin, was precipitated by PAb204 (Fig. 2, lane 8). This protein migrated at ca. 66K and probably corresponds to the one previously described by Crawford et al. (13) as being reactive with PAb204. We believe that the 75K polypeptide which appears to be specifically precipitated by the monoclonal antibodies is a degradation product of T-ag.

**Demonstration of antigenic determinants shared by native surface T-ag and denatured nuclear T-ag with polyclonal antisera.** Monoclonal antibodies established that there were several antigenic determinants in common between nuclear T-ag and surface T-ag. Well-characterized polyclonal sera were next employed to substantiate that conclusion. Those sera were also examined to clarify their disparate reactivities exhibited in previous IF tests. Soule et al. (55) observed that several antisera raised in different ways against surface-associated T-ag gave a positive surface IF reaction on unfixed SV40-transformed cells but failed to recognize nuclear T-ag in acetone-fixed cells. In contrast, antisera prepared against nuclear T-ag did recognize both surface T-ag and nuclear T-ag under comparable experimental conditions. One of the antisera (HaαS) directed against surface T-ag was shown to be able to precipitate detergent-solubilized nuclear T-ag. It was postulated that surface-associated T-ag and nuclear T-ag probably represent highly related molecules which exhibit different conformations due to variable modifications, processing, or other unrecognized effects of being incorporated in different microenvironments. Some of those previously described immunological reagents (55) were analyzed in the differential immunoprecipitation test.

Surface-iodinated mKSA-Asc cells were subjected to external immunoprecipitation (Fig. 3) by normal sera (normal

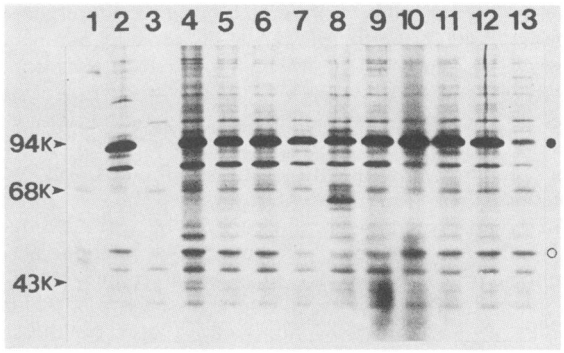


FIG. 2. Detection of nuclear T-ag and p53 from SV40-transformed cells by monoclonal antibodies. mKSA-Asc cells were metabolically labeled with [$^{35}$S]methionine for 2 h as indicated in the text. Labeled cells were subjected to external immunoprecipitation with HAF to remove surface T-ag and p53 and were then disrupted with an NP-40 solution. Samples (100 μl) of the clarified cell lysates were incubated for 1 h at 4°C with 5 μl of normal hamster serum (NHS) (lane 1), HAF (lane 2), or 9N (lane 3) or with 5 μl of supernatant concentrates of PAb430 (lane 4), PAb419 (lane 5), PAb416 (lane 6), PAb100 (lane 7), PAb204 (lane 8), PAb414 (lane 9), PAb101 (lane 10), PAb405 (lane 11), PAb423 (lane 12), or PAb421 (lane 13). After a second incubation with 2 μl of goat antimouse immunoglobulins, immune complexes were adsorbed with SACI, and the antigens were eluted and analyzed by 12% SDS-PAGE and autoradiography. Molecular weight markers are indicated on the left. Symbols: ●, large T-ag; ○ p53.

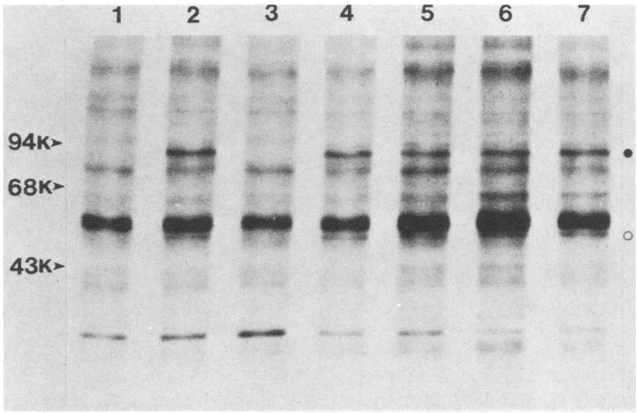


FIG. 3. Detection of surface T-ag and p53 on SV40-transformed cells with different antisera. mKSA-Asc cells were surface iodinated by the lactoperoxidase technique and subjected to external immunoprecipitation by NRS (lane 1), RαT (lane 2), NMS (lane 3), MoαS (lane 4), MoαT (lane 5), MoαNuc (lane 6), or MoαPM (lane 7). Cells were disrupted with an NP-40 solution, and the immune complexes in the clarified cell lysates were adsorbed with SACI. Antigens were eluted from the bacterial pellets and analyzed by 7 to 15% gradient SDS-PAGE and autoradiography. Molecular weight markers are indicated on the left. Symbols: ●, large T-ag; ○, p53.

rabbit serum [NRS], lane 1; normal mouse serum [NMS], lane 3), nuclear IF-positive sera (RαT, lane 2; MoαT, lane 5; MoαNuc, lane 6), and surface IF-positive–nuclear IF-negative sera (MoαS, lane 4; MoαPM, lane 7). All the antisera precipitated large T-ag from the surface of these SV40-transformed cells. In addition, p53 cellular protein was coprecipitated by all the antisera. The heavy band at 55K is believed to be precipitated nonspecifically since it also appeared with NRS and NMS.

To test the reactivity of these immunological reagents with nuclear T-ag, mKSA-Asc cells were metabolically labeled with [$^{35}$S]methionine for 2 h and then subjected to differential immunoprecipitation. Nuclear T-ag and p53 protein were again coprecipitated by all the antisera (Fig. 4, lanes 2, 3, and 5 through 8), including those unable to react with nuclear T-ag by IF. These results confirm those previously obtained by Soule et al. (55) and establish the generality of the phenomenon. Together with the monoclonal antibody data, these reactions suggest that surface-associated T-ag and nuclear T-ag share multiple antigenic determinants and probably have the same or very similar primary structure, although different tertiary structures or modifications are not ruled out.

**Definition of antigenic structure of surface T-ag and nuclear T-ag of SV40-infected cells with monoclonal antibodies.** Large T-ag is present on the surfaces of SV40-infected cells, as well as on transformed cells. Whereas surface T-ag might have a role in regulating the transformed phenotype, no biological function has been postulated for T-ag on the surfaces of SV40-infected permissive cells. If surface T-ag has some specific transformation-related function, then differences might exist between surface T-ag from SV40-transformed and -infected cells. Therefore, we examined the antigenic structure of surface T-ag on SV40-infected TC7 cells, using the monoclonal antibodies mentioned above. WT SV40 and *dl*1263, which lacks 33 base pairs (encoding 12 amino acids) at 0.20 map units (11, 64), were used. Cells infected with *dl*1263 synthesize a slightly truncated large T-ag which migrates at 89K and has a reduced adenovirus helper function (10). Both of these modified properties probably reflect an altered carboxy terminus.

It was of interest to determine whether the modified T-ag would also be exposed on the cell surface. As shown below, surface T-ag was detected on *dl*1263-infected cells. Since the reactivities of surface T-ag on WT SV40- and *dl*1263-infected cells were the same with the monoclonal antibodies tested, only the results obtained with the deletion mutant are presented.

Cells infected with *dl*1263 were surface iodinated by the lactoperoxidase-catalyzed reaction 24 h postinfection and then subjected to the external reaction of the differential immunoprecipitation technique with equivalent titers of the monoclonal antibodies. All antibodies were able to precipitate T-ag from the surface of infected cells (Fig. 5). It should be noted that similar amounts of surface T-ag were precipitated by monoclonal antibodies directed against either the carboxy or the amino terminus of the T-ag polypeptide. This was in contrast to the results obtained with transformed cells (Fig. 1). Hybridomas PAb100 and PAb204 exhibited variable surface reactivity, sometimes displaying weak reactivity and occasionally displaying negative surface reactivity. When surface T-ag was detected with hybridomas PAb100 (Fig. 6, lane 4) and PAb204 (Fig. 6, lane 5), the amount of surface T-ag precipitated by those two reagents was markedly smaller than that precipitated in parallel by other antibodies, such as HAF and PAb414 (Fig. 6, lanes 3 and 6, respectively). The 84K band apparent in some anti-T-ag immunoprecipitates (Fig. 6, lanes 3 and 6) probably represents a degradation product of T-ag. The poorer resolution of autoradiographs of iodinated proteins makes it difficult to distinguish the lower-molecular-weight band when surface-iodinated cells are analyzed.

None of the reagents, including HAF, appeared to coprecipitate p53 cellular protein from the surface of infected cells. Importantly, monoclonal antibodies against p53 failed to precipitate either p53 or T-ag from the surface of infected cells (Fig. 5, lane 9). Since p53 has been detected in WT SV40-infected cell lysates (24), where it also appears to be tightly associated with large T-ag, it was important to

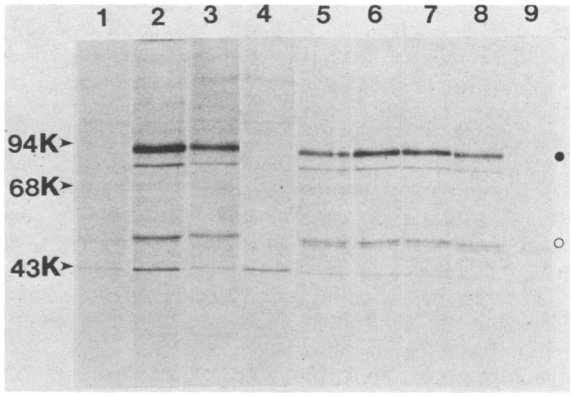


FIG. 4. Detection of nuclear T-ag and p53 from SV40-transformed cells with different antisera. mKSA-Asc cells were metabolically labeled with [$^{35}$S]methionine for 2 h as described in the text. Labeled cells were disrupted with an NP-40 solution, and the clarified cell lysates were incubated overnight at 4°C with NHS (lane 1), HAF (lane 2), RαT (lane 3), NRS (lane 4), MoαS (lane 5), MoαT (lane 6), MoαNuc (lane 7), MoαPM (lane 8), or NMS (lane 9). Immune complexes were adsorbed with SACI, and then antigens were eluted from the bacterial pellets and analyzed by 7 to 15% gradient SDS-PAGE and autoradiography. Only 10% of the total immunoprecipitate was loaded in each lane. Molecular weight markers are indicated on the left. Symbols: ●, large T-ag; ○, p53.

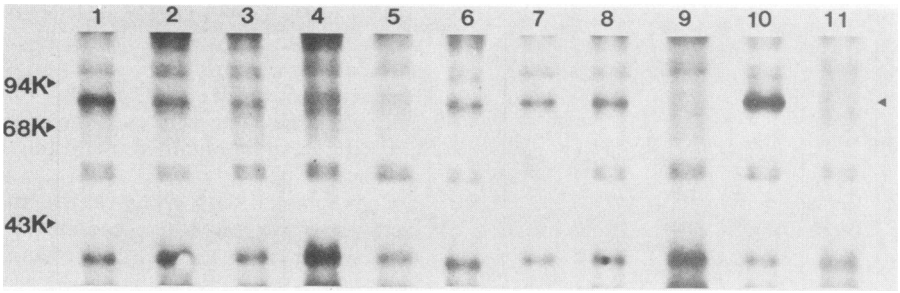


FIG. 5. Surface reactivity of SV40 *dl*1263-infected cells with monoclonal antibodies. TC7 cells grown as monolayers (ca. $6 \times 10^6$ cells per culture) were infected with *dl*1263 at a multiplicity of infection of 10 and surface iodinated by the lactoperoxidase technique 24 h later. Labeled cells were subjected to external immunoprecipitation by incubation for 30 min at 4°C with 1 ml of growth medium containing 50 μl of PAb416 (lane 1), 50 μl of PAb419 (lane 2), 200 μl of PAb430 (lane 3), 300 μl of PAb100 (lane 4), 100 μl of PAb204 (lane 5), 50 μl of PAb405 (lane 6), 50 μl of PAb414 (lane 7), 160 μl of PAb423 (lane 8), 50 μl of PAb421 (lane 9), 50 μl of HAF (lane 10), or 50 μl of 9N (lane 11). Cells were then incubated with 1 ml of growth medium containing 5 μl of rabbit antimouse immunoglobulins for 30 min at 4°C and disrupted with an NP-40 solution, and the immune complexes in the clarified cell lysates were adsorbed with SACI. Eluted antigens were analyzed by 12% SDS-PAGE and autoradiography. Molecular weight markers are indicated on the left. Arrowhead at right, Truncated large T-ag.

determine whether the intracellular complex could be detected in our system with the monoclonal antibodies.

Cells infected with *dl*1263 were labeled with [$^{35}$S]methionine for 2 h from 22 to 24 h postinfection, disrupted with an NP-40 solution, and immunoprecipitated as indicated in the legend to Fig. 6. As expected, both large T-ag and p53 were coprecipitated by HAF (Fig. 7, lane 8). All the monoclonal antibodies, with the possible exception of PAb204 (Fig. 7, lane 4) and PAb414 (Fig. 7, lane 7), were able to coprecipitate large T-ag and p53. Larger amounts of PAb204 and PAb414 also failed to precipitate the T-ag–p53 complex (data not shown). p53 was also precipitated from mock-infected cells by PAb421 (Fig. 7, lane 11). Under the labeling conditions employed, the amounts of p53 recovered from the mock-infected and SV40-infected cells appeared to be nearly comparable. Therefore, the deletion of 12 amino acids from the carboxy terminus of the T-ag polypeptide does not alter its ability to associate with p53, and the intracellular T-ag–p53 complex in SV40-infected cells is recognized by the majority of the monoclonal antibodies.

If the molar ratio between T-ag and p53 immunoprecipitated from the nucleus of infected cells was the same in immunoprecipitates from the cell surface, it is doubtful that a surface complex would have been detected in our assay. These results, therefore, do not demonstrate a complete absence of p53 at the cell surface. However, if any complex is present at the surface, the amounts are small and below the limits of detection. By comparing Fig. 2 and 7 (the latter is overexposed to show p53), it is evident that the amount of intracellular T-ag–p53 complex is greater in SV40-transformed cells than in SV40-infected cells. (Each lane in Fig. 7 represents the cell lysate from 10-fold more cells than each lane in Fig. 2.) This is presumably due to a lower level of p53 in the infected permissive cells.

It is interesting to note that PAb419, PAb204, and PAb414 coprecipitated cellular proteins of ca. 33K, 36K, and 40K, respectively, from SV40-infected cells (Fig. 7, lanes 1, 4, and 7, respectively). Such cellular proteins might possess antigenic sites in common with the T-ag polypeptide, as previously suggested by Crawford et al. (13).

## DISCUSSION

The presence of large T-ag on the surface of SV40-transformed cells is well documented (see above). The majority of the surface-associated molecules are complexed with host protein p53 (46, 47). The intriguing possibility exists that those surface-associated molecules might be related to the role of the viral protein in maintenance of the transformed phenotype or tumor-specific transplantation antigen expression or both (for reviews, see references 60 and 65).

As a step toward understanding the biological function(s) of surface T-ag, we have determined its antigenic structure in the plasma membrane of transformed cells and compared it with surface T-ag expressed on SV40-infected cells. Based on the reactivities of nuclear T-ag and surface T-ag with different immunological reagents, including a series of anti-T-ag monoclonal antibodies, it is apparent that the two forms of T-ag are highly related molecular entities. It should be stressed, however, that the results do not imply complete

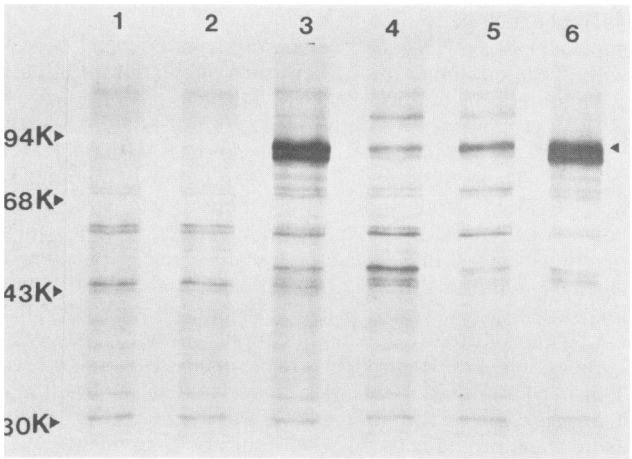


FIG. 6. Detection of T-ag on the surface of SV40 *dl*1263-infected cells. TC7 cells grown as monolayers (ca. $6 \times 10^6$ cells per culture) were infected with *dl*1263 at a multiplicity of infection of 10 (lanes 3 through 6) or were mock infected (lanes 1 and 2). Cells were labeled with [$^{35}$S]methionine (100 μCi/ml) from 22 to 24 h postinfection and then subjected to external immunoprecipitation by incubation for 30 min at 4°C with 1 ml of growth medium containing 50 μl of 9N (lane 1), 50 μl of HAF (lanes 2 and 3), 300 μl of PAb100 (lane 4), 300 μl of PAb204 (lane 5), or 50 μl of PAb414 (lane 6). After a second incubation for 30 min at 4°C with 1 ml of growth medium containing 5 μl of rabbit antimouse immunoglobulins, cells were disrupted with an NP-40 solution, and the immune complexes in the clarified cell lysates were adsorbed with SACI. Eluted antigens were analyzed by 12% SDS-PAGE and autoradiography. Molecular weight markers are indicated on the left. Arrowhead at right, Truncated large T-ag.

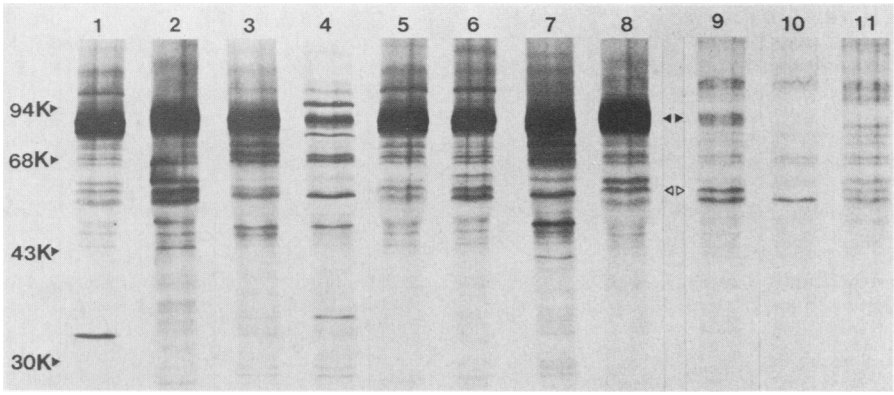


FIG. 7. Detection of nuclear T-ag and p53 from SV40 *dl*1263-infected cells. About $6 \times 10^6$ TC7 cells were infected with *dl*1263 at a multiplicity of infection of 10 (lanes 1 through 10) or were mock infected (lane 11) and labeled with [$^{35}$S]methionine (100 μCi/ml) from 22 to 24 h postinfection. Labeled cells were disrupted with an NP-40 solution, and the clarified cell lysates were incubated for 1 h on ice with 50 μl of PAb419 (lane 1), 50 μl of PAb430 (lane 2), 150 μl of PAb100 (lane 3), 150 μl of PAb204 (lane 4), 50 μl of PAb405 (lane 5), 50 μl of PAb412 (lane 6), 50 μl of PAb414 (lane 7), 50 μl of HAF (lane 8), 50 μl of PAb421 (lanes 9 and 11), or 50 μl of 9N (lane 10). After a second incubation for 30 min at 4°C with 2 μl of rabbit antimouse immunoglobulins, immune complexes were adsorbed with SACI, and the antigens were eluted and analyzed by 12% SDS-PAGE and autoradiography. Molecular weight markers are indicated on the left. Solid arrowheads, Truncated large T-ag; open arrowheads, p53.

identity between surface T-ag and nuclear T-ag. In fact, it has recently been reported (31) that surface T-ag is acylated, whereas nuclear T-ag is not. Surface T-ag seems to be folded and arranged in such a way that antigenic determinants located on both the amino and the carboxy thirds of the molecule are exposed toward the extracellular compartment. Deppert and Walter (18) have recently reached the same conclusion by using IF tests and antisera made against purified T-ag and a synthetic peptide corresponding to the carboxy terminus of T-ag. Monoclonal antibodies that recognize the carboxy end of T-ag appear to be relatively more efficient at precipitating surface T-ag from SV40-transformed cells than are antibodies directed toward determinants on the amino end. This observation suggests that the carboxy terminus of T-ag might be more exposed than the amino terminus to the exterior of the transformed cells. Whether this is a consequence of the tertiary conformation of T-ag or of its disposition in the plasma membrane remains to be investigated. Because the binding sites on the T-ag polypeptide have not been defined precisely for most of the monoclonal antibodies, more specific conclusions cannot be drawn regarding the exposure of T-ag on the cell surface.

Some large T-ag appears to be continuously lost or shed from the cell surface while being concomitantly replaced with new molecules (33, 48, 56). If the carboxy end of T-ag protrudes first on the cell surface, then at any given time most if not all of the externally exposed T-ag molecules should have their carboxy termini available to be recognized and bound by specific antibodies. As observed, monoclonal antibodies directed against the carboxy end of the T-ag molecule should be the most efficient at precipitating surface T-ag. However, the carboxy terminus of the T-ag polypeptide does not appear to be essential for mediating the insertion of T-ag into the plasma membrane, as has been shown for other membrane proteins (5, 57). We have demonstrated that a deletion of 12 amino acids at the carboxy end, which alters adenovirus 2 helper function (10), does not block exposure of T-ag on the cell surface. Truncated large T-ag molecules which lack the amino terminus can be incorporated into the surface of adenovirus 2-SV40 hybrid-infected cells (16–18). Truncated large T-ag lacking the carboxy terminus is able to be exposed on the cell surface and serve as a target for cytotoxic T lymphocytes (61). Therefore, it might be speculated that neither end of the T-ag polypeptide is mandatory for insertion into the plasma membrane. Acylation of surface T-ag molecules has been reported (31). This might be the mechanism by which T-ag is incorporated into the plasma membrane, rather than a specific sequence of the polypeptide being the determining factor.

Some subtle but intriguing differences were observed when infected cells were compared with transformed cells. First, large T-ag on the surface of WT SV40- and *dl*1263-infected cells was recognized by monoclonal antibodies directed against either the amino or the carboxy terminus. Interestingly, no differences were observed among those monoclonal antibodies directed against sites on either terminus of the T-ag polypeptide with respect to ability to interact with and precipitate surface T-ag, in contrast to the relatively more efficient detection of surface T-ag on transformed cells by carboxy-specific monoclonal antibodies. Second, monoclonal antibodies PAb100 and PAb204 showed minimal reactivity with T-ag on the surface of SV40-infected cells, whereas both reacted with T-ag on the surface of SV40-transformed cells. Finally, no T-ag–p53 complexes were detected on the surface of WT SV40- and *dl*1263-infected cells, an observation in agreement with previous cell fractionation data (54). Perhaps the association of T-ag with p53 alters the conformation of T-ag on the surface of transformed cells, facilitating a higher reactivity with those monoclonal antibodies directed against the carboxy end of the polypeptide. It is possible that the T-ag–p53 complex may be present on the surface of SV40-infected cells below the limits of detection of our assay system. However, the presence of readily detectable levels of the complex on the surface of SV40-transformed cells may indicate that the complex serves some transformation-related biological role at that location.

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