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# Surface T-Antigen Expression in Simian Virus 40-Transformed Mouse Cells: Correlation with Cell Growth Rate

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**Cell growth control appears to be drastically altered as a consequence of transformation. Because the cell surface appears to have a role in modulating cell growth and simian virus 40 (SV40)-transformed cells express large T antigen (T-Ag) in the plasma membrane, we investigated whether surface T-Ag expression varies according to cell growth rate. Different growth states were obtained by various combinations of seeding density, serum concentration, and temperature, and cell cycle distributions were determined by flow microcytofluorometry. Actively dividing SV40-transformed mouse cell cultures were consistently found to express higher levels of surface T-Ag and T-Ag/p53 complex than cultures in which cells were mostly resting. In addition, the T-Ag/p53 complex disappeared from the surface of *tsA*7-transformed cells cultured under restrictive conditions known to induce complete growth arrest (39.5°C), although the surface complex did not disappear from other *tsA* transformants able to keep cycling at 39.5°C. These results suggest that surface SV40 T-Ag or surface T-Ag/p53 complex, or both, are involved in determining the growth characteristics of SV40-transformed cells.**

Simian virus 40 large tumor antigen (SV40 T-Ag) is a remarkably multifunctional polypeptide (for a review, see reference 43) that has among its functions the ability to induce cellular DNA synthesis (8, 20, 40, 48, 55). This ability has been mapped between 0.42 and 0.49 map units on the early region of SV40 DNA (48).

T-Ag is found in association with a host-coded cellular protein of about 53,000 molecular weight (p53), both in the nucleus (for a review, see reference 27) and on the surface (44–46) of SV40-transformed cells. The specific role of either nuclear- or surface-associated T-Ag/p53 complex in maintenance of transformation is unknown. Increased levels of p53 have been detected in proliferating cells (15, 38, 39), and serum stimulation of DNA synthesis in Swiss 3T3 cells is inhibited by microinjection of anti-p53 monoclonal antibodies (36, 37). Therefore, a role for p53 in control of normal cell proliferation is implicated. It is possible that T-Ag alters the normal mechanism(s) which control(s) cell division either directly or indirectly through an interaction with p53.

A first approach toward determining whether surface T-Ag, either by itself or when complexed with p53, plays a role in regulating proliferation of SV40-transformed cells is described in this study. A correlation was established between cell growth rate and the expression of T-Ag and T-Ag/p53 complex on the cell surface. The expression of T-Ag and T-Ag/p53 complex on the surface of actively dividing SV40-transformed mouse cells was elevated when compared with the levels found on resting cells. In addition, complete growth arrest of *tsA*7-transformed cells cultured under nonpermissive conditions was preceded by the disappearance of the T-Ag/p53 complex from the surface of those cells. These observations suggest that surface-associated T-Ag or T-Ag/p53 complex is involved in the alteration of normal growth control that accompanies SV40 transformation.

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## MATERIALS AND METHODS

**Cells and antisera.** The transplantable mKSA-Asc line of SV40-transformed BALB/c kidney cells (26, 63) and BALB/c 3T3 cells transformed by wild-type (WT) SV40 (WTB1a; 5) were included in this study. Also analyzed were three BALB/c 3T3 transformants induced by the SV40 temperature-sensitive mutants *tsA*7 (A7B4b), *tsA*30 (A30B3a), and *tsA*255 (A255B1b) originally established by Brockman (5). The A30B3a cell line is nearly as temperature resistant as WT transformants for growth properties but carries a temperature-sensitive viral genome and synthesizes a temperature-labile T-Ag (5). Cells were routinely cultured in Dulbecco modified Eagle minimal essential medium (DMEM; GIBCO Laboratories, Grand Island, N.Y.) supplemented with 10% fetal bovine serum (FBS) (Flow Laboratories, Inc., McLean, Va.) as described previously (44).

Ascitic fluid from hamsters with SV40-induced ascites tumors (HAF) (28) was used as a source of T antibodies. Monoclonal antibodies against p53 were obtained from PAb421 hybridoma supernatant fluids (22) which were concentrated 30-fold by $Na_2SO_4$ precipitation. Normal hamster serum (NHS) and monoclonal antibodies directed against human immunoglobulin (9N) served as controls.

**Radioactive labeling, immunoprecipitation, and electrophoresis.** Cells grown as monolayers were surface labeled in situ with $^{125}I$ by a lactoperoxidase-catalyzed reaction (44, 51). Labeled cells were either subjected to external immunoprecipitation (44) or disrupted with 1% Nonidet P-40 in 0.01 M Tris hydrochloride (pH 7.4), clarified by centrifugation, and then incubated with antiserum. Immune complexes were adsorbed with *Staphylococcus aureus* Cowan I strain, eluted, and analyzed by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and autoradiography (28, 44, 49).

**Cellular DNA content analysis and [$^3$H]thymidine incorporation.** The content of cellular DNA and the cell cycle distribution were determined on cells removed from tissue culture plates by trypsinization and then stained with ethidium bromide. DNA was quantitated by flow microcytofluo-

TABLE 1. Growth states of SV40-transformed mKSA-Asc cell cultures at different cell densities[a]

| Culture designation[b] | Density of monolayer | Total no. of cells ($\times 10^6$) | Incorporation of [$^3$H]TdR (cpm $\times 10^4$/$10^6$ cells) | % Cell cycle distribution | | | Ratio of S/$G_0$-$G_1$ | Growth state |
|---|---|---|---|---|---|---|---|---|
| | | | | $G_0$-$G_1$ | S | $G_2$-M | | |
| 1 | Sparse | 4.3 | 15.0 | 37.5 | 37.7 | 25.1 | 1.00 | Growing |
| 2 | Semiconfluent | 10.0 | 7.0 | 44.5 | 30.9 | 24.6 | 0.69 | Intermediate |
| 3 | Confluent | 22.0 | 4.0 | 52.2 | 26.6 | 21.2 | 0.51 | Mostly resting |

[a] Different numbers of mKSA-Asc cells were seeded into 100-mm tissue culture plates and cultured for 48 h at 37°C. Replicate cultures were then analyzed as described in the text to determine [$^3$H]TdR incorporation and cell cycle distribution and detection of T-Ag on the cell surface by radioiodination and immunoprecipitation (Fig. 1).

[b] Original seeding densities: culture 1, $1 \times 10^6$ cells; culture 2, $5 \times 10^6$ cells; culture 3, $10 \times 10^6$ cells.

rometry (57) with an Ortho 50H cell sorter. A total of $2 \times 10^4$ cells was counted per sample. The incorporation of [$^3$H]thymidine ([$^3$H]TdR) into trichloroacetic acid-precipitable material was performed as described previously (29). Cells were pulse-labeled for 30 min in medium containing 2.5 μCi of [$^3$H]TdR (ICN Pharmaceuticals, Inc., Irvine, Calif.; 64 Ci/mmol) per ml.

## RESULTS

**Surface T-Ag expression and cell growth rate.** It is recognized that the growth of transformed cells is not inhibited when contact is made with other cells at confluency. However, [$^3$H]TdR incorporation into trichloroacetic acid-precipitable material has revealed that transformed cells in confluent monolayers are less actively growing than those in sparse cultures (unpublished observations). Presumably, the proportion of transformed cells actually dividing in a population decreases as the number of cells in a culture increases, although cell division does not cease. Therefore, for this study it was reasoned that if different numbers of cells were seeded initially, cell cultures displaying different growth rates and cell cycle distributions would be available after a defined incubation period.

A confluent monolayer of mKSA-Asc cells in a 100-mm plate contains about $2 \times 10^7$ cells. Therefore, $1 \times 10^6$, $5 \times 10^6$, and $10 \times 10^6$ cells were seeded in regular growth medium into tissue culture plates and incubated for 48 h at 37°C. The cultures were then processed for [$^3$H]TdR incorporation, cell cycle distribution analysis, and detection of surface T-Ag as described above and in Table 1 and the legend to Fig. 1. At the time of analysis, the densities of the monolayers ranged from sparse to confluent (Table 1). The cells in all three samples were distributed throughout the phases of the cell cycle, showing that all three types of culture were still growing asynchronously. However, the S/$G_0$-$G_1$ ratios and the values for [$^3$H]TdR incorporation established that cultures 1, 2, and 3 displayed different growth rates that were inversely related to the total number of cells in each vessel (1, mostly growing; 2, intermediate growth rate; 3, mostly resting; Table 1).

Parallel cultures were surface iodinated by the lactoperoxidase-catalyzed reaction and subjected to external immunoprecipitation by NHS (Fig. 1, lane 1) or by HAF (lanes 2 to 4) to monitor surface T-Ag expression. It should be noted that the sample loaded in lane 2 ($\simeq 4 \times 10^6$ cells; cell population 1 in Table 1) had only about half the number of cells that were in the samples loaded into lanes 3 and 4 ($\simeq 10 \times 10^6$ cells; cell populations 2 and 3, respectively). Nevertheless, it is obvious that cultures in which cells were more actively growing (lane 2) expressed more surface T-Ag than those in which cells were mostly resting (lane 4).

A similar correlation between cell growth rate and expression of surface T-Ag was found when transformed cells were manipulated in a different way. Cultures maintained at 37°C in DMEM supplemented with 10% FBS contained mostly growing cells, whereas companion cultures maintained at 33°C in DMEM supplemented with only 1% FBS contained cells that were mostly resting. The growing cells were found to express more surface T-Ag that could be iodinated and immunoprecipitated than the resting cells (data not shown). This approach permitted a comparison of cells in sparse cultures that were in different growth states. Therefore, the apparent decrease in surface T-Ag expression by resting cells is not a consequence of cell-to-cell contact or an artifact due to an inability of antibodies to bind T-Ag because of some hindrance imposed by confluent monolayers.

**Shedding of surface T-Ag and cell growth rate.** We recently showed that the association of T-Ag with the cell surface is highly dynamic; T-Ag constantly appears and disappears from the surface of SV40-transformed cells (46). Therefore, the amount of surface T-Ag at any given time must represent a steady state reached as a sum of those two processes. Changes in the expression of surface T-Ag might reflect alterations in that equilibrium. An elevated expression of surface T-Ag in actively growing cell populations might be

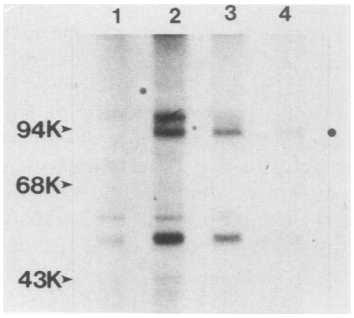


FIG. 1. Detection of SV40 surface T-Ag in mKSA-Asc cell cultures with different growth rates. These same cultures are characterized in Table 1: type 1, sparse and growing (lanes 1 and 2); type 2, semiconfluent (lane 3); and type 3, confluent, mostly resting (lane 4). Cell monolayers were surface iodinated by the lactoperoxidase technique, incubated with 1 ml of growth medium containing 50 μl of heat-inactivated NHS (lane 1) or HAF (lanes 2 to 4) for 30 min at 4°C, and disrupted by incubation for 30 min at 4°C with 1 ml of a 1% Nonidet P-40 solution (pH 7.4) containing 10% Trasylol. Immune complexes in the clarified cell lysates were adsorbed with Formalin-fixed, heat-inactivated *S. aureus* Cowan I strain and eluted and analyzed by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and autoradiography. Extracts from about $4 \times 10^6$ cells were loaded onto lanes 1 and 2, and extracts from about $10 \times 10^6$ cells were added to lanes 3 and 4. The positions of molecular weight markers appear on the left; K, $\times 10^3$. ●, T-Ag.

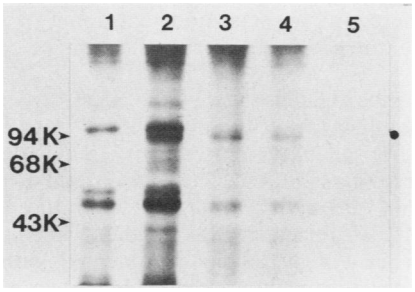


FIG. 2. Shedding of SV40 surface T-Ag from actively growing cell cultures. About $10^6$ mKSA-Asc cells were seeded into 100-mm plates and cultured for 48 h at 37°C. Cell monolayers were surface iodinated by the lactoperoxidase technique and subjected to external immunoprecipitation (as described in the legend to Fig. 1) by NHS (lane 1) or HAF (lanes 2 to 5) either immediately after labeling (lanes 1 and 2) or after 15 min (lane 3), 30 min (lane 4), or 60 min (lane 5) of incubation at 37°C. Molecular weight markers appear on the left; K, $\times 10^3$. These cells were equivalent to type 1, sparse cultures (Table 1; Fig. 1). ●, T-Ag.

the result either of an increased rate of entry of T-Ag molecules into the membrane or of a decreased velocity of shedding.

The rate of exposure of T-Ag on the cell surface might be related to the rate of synthesis of T-Ag in the cell. [$^{35}$S]methionine incorporation into total cellular T-Ag was the same in actively growing and resting cell cultures (data not shown). The total amount of intracellular T-Ag did not appear to change under different growth conditions. Therefore, elevated expression of surface T-Ag in actively growing cells does not appear to be the result of increased rates of synthesis of T-Ag molecules. However, it must be noted that we are currently unable to measure those molecules of intracellular T-Ag destined for association with the cell surface.

The kinetics of disappearance of surface T-Ag were then compared in cultures in which cells were mostly growing or mostly resting. This allowed us to analyze the other component affecting steady-state levels of T-Ag in the membrane.

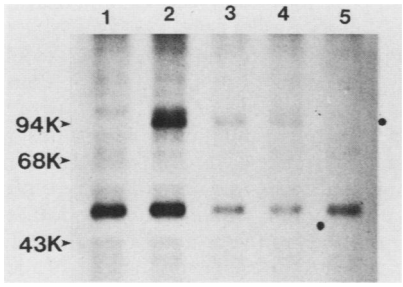


FIG. 3. Shedding of SV40 surface T-Ag from cells in cultures with low growth rates. About $10 \times 10^6$ mKSA-Asc cells were seeded into 100-mm plates and cultured for 48 h at 37°C. Cell monolayers were surface iodinated by the lactoperoxidase technique and subjected to external immunoprecipitation (as described in the legend to Fig. 1) by NHS (lane 1) or HAF (lanes 2 to 5) either immediately after labeling (lanes 1 and 2) or after 15 min (lane 3), 30 min (lane 4), or 60 min (lane 5) of incubation at 37°C. Molecular weight markers appear on the left; K, $\times 10^3$. These cells were equivalent to type 3, confluent cultures (Table 1; Fig. 1). This autoradiograph was overexposed relative to the one shown in Fig. 2. ●, T-Ag.

Cells in tissue culture plates equivalent to the sparse and confluent cultures characterized in Table 1 were surface iodinated by the lactoperoxidase-catalyzed reaction and subjected to external immunoprecipitation, either immediately after labeling or after different periods of incubation (15 to 60 min) at 37°C. The results showed no appreciable difference in the velocity of disappearance of surface T-Ag from the cultures exhibiting different cell growth rates. Iodinated T-Ag was lost with a half-life of <30 min from both sparse (Fig. 2) and confluent (Fig. 3) cell cultures. The autoradiograph obtained with confluent cell cultures (Fig. 3) had to be overexposed to be compared with that obtained with sparse cell cultures (Fig. 2).

**Expression of surface T-Ag and T-Ag/p53 complex and cell growth rate in temperature-sensitive transformants.** A series of WT SV40- and SV40 *tsA*-transformed cells was established by Brockman (5). All the *tsA* transformants except A7B4b continue to proliferate when cultured under restrictive conditions in DMEM supplemented with 1% FBS (9). Therefore, those cells were analyzed to test the hypothesis that the expression of the surface T-Ag or T-Ag/p53 complex, or both, might be related to cell growth rate.

We first determined whether the T-Ag/p53 complex was present on the surface of the SV40 *tsA* transformants when they displayed the transformed phenotype. WTB1a, A7B4b, A30B3a, and A255B1b transformed cells were cultured at 33°C. Subconfluent monolayers were surface iodinated by the lactoperoxidase-catalyzed technique and subjected to external immunoprecipitation with antibodies directed against T-Ag (HAF) or p53 (PAb421). Both T-Ag and p53 were coprecipitated by HAF from A7B4b, A255B1b, and A30B3a cells, as well as from WTB1a cells (data not shown). PAb421 monoclonal antibodies also were able to coprecipitate T-Ag and p53. Therefore, as expected from previous studies (44, 46, 47), T-Ag/p53 complexes were present on the surface of *tsA* transformants cultured at the permissive temperature.

We next examined the expression of T-Ag and T-Ag/p53 complex on the surface of WTB1a cells in relation to cell growth rate. WTB1a cells were cultured for 48 h in DMEM

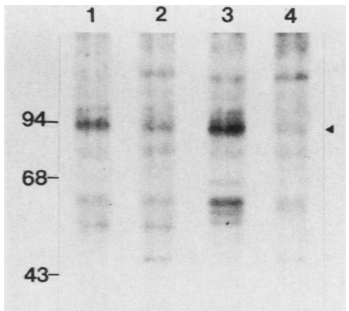


FIG. 4. Detection of SV40 T-Ag and T-Ag/p53 complex on the surface of WTB1a cells with different growth rates. Cell monolayers were surface iodinated by the lactoperoxidase technique and extracted with a 1% Nonidet P-40 solution (pH 7.4). The clarified cell lysates were incubated with 50 μl of HAF (lanes 1 and 2) or PAb421 concentrate (lanes 3 and 4) for 1 h at 4°C. The latter two samples were then incubated for 30 min at 4°C with 2 μl of rabbit immunoglobulin G anti-mouse immunoglobulins. Immune complexes were adsorbed with *S. aureus* Cowan I strain, eluted, and analyzed as described in the legend to Fig. 1. Molecular weight markers ($\times 10^3$) appear on the left. These same cultures are characterized in Table 2: semiconfluent (lanes 1 and 3); confluent (lanes 2 and 4). ◀ T-Ag.

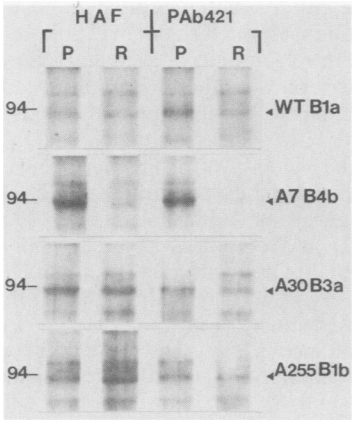


FIG. 5. Surface T-Ag and surface T-Ag/p53 complex on WT SV40- and *tsA*-transformed mouse cells at permissive and restrictive temperatures. WTB1a, A7B4b, A30B3a, and A255B1b cells were seeded (at different densities) into DMEM containing 10% FBS and incubated at 37°C for approximately 2 h. Medium was then replaced by DMEM supplemented with 1% FBS, and cells were shifted to either 33°C (P) or 39.5°C (R) and incubated for 48 h. Cells were then surface iodinated by the lactoperoxidase technique and disrupted with 1% Nonidet P-40 solution, and the clarified cell lysates were pre-precipitated by incubation for 1 h at 4°C with 15 μl of normal goat serum followed by adsorption with *S. aureus* Cowan I strain. After removal of the bacteria, supernatants were incubated for 1 h at 4°C with 15 μl of HAF or 50 μl of PAb421 monoclonal antibodies. The latter samples were then incubated for 30 min at 4°C with 2 μl of rabbit anti-mouse immunoglobulin G. Immune complexes were adsorbed with *S. aureus* Cowan I strain, and eluted antigens were analyzed by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and autoradiography. Samples per well were equivalent to $1.5 \times 10^7$ cells (WTB1a and A7B4b) and $0.6 \times 10^7$ cells (A30B3a and A255B1b). Position of phosphorylase *a* (molecular weight, 94,000) is indicated on the left. ◀, T-Ag.

supplemented with 10% FBS at 33°C or in DMEM supplemented with 1% FBS at 39.5°C. These combinations of serum concentration and temperature were used to obtain cells in different growth states. Replicate cultures were used for analysis of cell numbers and [$^3$H]TdR incorporation into cellular DNA and for detection of surface T-Ag and T-Ag/p53 complex (Fig. 4). The cultures from 33°C were semiconfluent ($10 \times 10^6$ cells each) at the time of analysis, and the cultures from 39.5°C were confluent ($17 \times 10^6$ cells each). The former incorporated $17.7 \times 10^4$ cpm of [$^3$H]TdR per $10^6$ cells during a 30-min pulse and the latter incorporated $5.3 \times 10^4$ cpm/$10^6$ cells.

T-Ag and p53 do not share antigenic cross-reactivity (27, 44). Hence, total iodinated surface T-Ag should be precipitated by HAF, but only those T-Ag molecules that are associated with p53 should be precipitated by PAb421 monoclonal antibodies. Therefore, the T-Ag bands in the autoradiograph (Fig. 4) precipitated by anti-p53 monoclonal antibodies represent the amount of surface T-Ag bound to the cellular protein. The results show that the expression of surface T-Ag in WTB1a cells is also related to cell growth rate, extending the results described above with the mKSA-Asc cells. Proliferating WTB1a cells expressed more T-Ag accessible to iodination (Fig. 4, lane 1) than did more stationary cells (lane 2). The amount of surface T-Ag/p53 complex paralleled those results (lanes 3 and 4). It appeared that most of the T-Ag at the cell surface of the WTB1a cells was complexed with p53 in growing cells (lanes 1 and 3), although there was a suggestion that some of the surface T-Ag is free (uncomplexed) in more stationary cells (lanes 2 and 4).

We next investigated whether the SV40 *tsA*-transformed cells displayed surface T-Ag or surface T-Ag/p53 complex, or both, after phenotypic reversion (after incubation at 39.5°C). Brockman (5) and Christensen and Brockman (9) reported that sensitivity to high temperature of the *tsA* mouse cell transformants was evident when cells were cultured in growth medium supplemented with 1% FBS. Therefore, previously determined cell numbers were seeded in DMEM containing 10% FBS and incubated at 37°C for approximately 2 h to maximize plating efficiencies. The medium was then replaced by DMEM supplemented with 1% FBS, and the cells were shifted to either 33 or 39.5°C and maintained under those conditions for 48 h. Nearly identical cell numbers were present in the various cultures after the 48-h incubation. Cells were then surface iodinated and processed as described in the legend to Fig. 5. Because lactoperoxidase-catalyzed surface iodination labels only externally exposed T-Ag (44, 45, 51), all labeled T-Ag detected in the autoradiographs corresponds to surface T-Ag.

The total amount of surface T-Ag (precipitated by HAF) expressed at 33°C (Fig. 5, HAF, P) appeared to be higher in *tsA* transformants than in the WT SV40 transformant. No dramatic change was observed in the level of surface T-Ag on cells cultured at the restrictive temperature (Fig. 5, HAF, R), with the notable exception of the A7B4b cells. Those cells showed a significant decrease in the amount of both surface T-Ag (Fig. 5, HAF, R) and surface T-Ag/p53 complex (Fig. 5, PAb421, R) at 39.5°C. By comparing the intensities of the labeled T-Ag bands precipitated by HAF (total surface T-Ag) and by PAb421 (T-Ag bound to p53), it is evident that most if not all surface T-Ag is associated with p53 in the different transformed cell lines grown at 33°C. Interestingly, nearly the same relative intensity bands of surface T-Ag were recovered by PAb421 from the cells cultured at 39.5 as at 33°C, except that there was a pronounced reduction in the T-Ag/p53 complex on the surface of A7B4b cells at the restrictive temperature. It should be noted that when the identical gels stained by Coomassie blue were analyzed, it was evident that the total amount of cellular T-Ag was not significantly altered in the A7B4b cells cultured under restrictive conditions. However, little or no T-Ag/p53 complex formation was detected at 39.5°C (data not shown).

Because markedly different growth rates would affect the detection of surface T-Ag at the two temperatures (Fig. 1 and 4), we analyzed the cell cycle distribution of the different cell lines under the experimental conditions described above. No significant differences were observed among the samples; all displayed cell cycle distributions typical of asynchronized cultures and similar $S/G_0$-$G_1$ ratios (Table 2). Therefore, variations in the amount of surface T-Ag (Fig. 5) cannot be ascribed to altered growth rates of the cells under the different experimental conditions.

## DISCUSSION

It has been postulated that the plasma membrane plays a role in modulating cell division (1, 7, 19). Several plasma membrane components have been shown to change periodically throughout the phases of the cell cycle (10, 11, 42, 53) or during the prereplicative phase after cell induction to enter the proliferative cycle (32, 33). Those components might be involved, either directly or indirectly, in the control of cell proliferation. Several cellular mechanisms which are

TABLE 2. Cell cycle distribution of WT and *tsA* transformants at the permissive and restrictive temperatures[a]

| Cell line | Temp (°C) | % Cell cycle distribution | | | Ratio of $S/G_0$-$G_1$ |
|---|---|---|---|---|---|
| | | $G_0$-$G_1$ | S | $G_2$-M | |
| WTB1a | 33 | 55.4 | 24.0 | 20.6 | 0.43 |
| | 39.5 | 63.3 | 21.4 | 15.3 | 0.34 |
| A7B4b | 33 | 55.6 | 23.3 | 21.1 | 0.42 |
| | 39.5 | 56.8 | 24.7 | 18.2 | 0.43 |
| A30B3a | 33 | 56.6 | 21.8 | 21.5 | 0.39 |
| | 39.5 | 53.5 | 24.1 | 22.4 | 0.45 |
| A255B1b | 33 | 51.9 | 26.5 | 21.6 | 0.51 |
| | 39.5 | 42.3 | 30.1 | 27.6 | 0.71 |

[a] Cells were cultured as described in the legend to Fig. 5, trypsinized, and resuspended in growth medium. A sample ($10^7$ cells) was washed with Tris-buffered saline solution, resuspended in hypotonic Tris-buffered saline solution, and stained with ethidium bromide as described previously (57). About $2 \times 10^4$ cells were counted in a flow microcytofluorometer, and the cell cycle distribution was determined by cell DNA content.

altered after transformation, such as contact inhibition of cell growth, are thought to be modulated by the plasma membrane. The insertion of a new protein such as T-Ag into the plasma membrane as a result of transformation by SV40 might induce changes in membrane characteristics that could result in altered control mechanisms.

SV40 T-Ag is associated with the host-coded p53 cellular protein in the plasma membrane of SV40-transformed cells (44–47). The biological role of that surface molecular complex remains to be determined. The data presented here show that expression of surface T-Ag and the T-Ag/p53 complex correlates with cell growth rate. More surface T-Ag and surface T-Ag/p53 complex is expressed in cultures in which most of the cells are dividing. The elevation in expression of surface T-Ag does not appear to be the result of increased rate of synthesis of intracellular T-Ag or increased stability of association of T-Ag with the membrane (decreased velocity of shedding). We do not know whether there are more molecules of T-Ag on the surface of growing cells or whether the molecules are arranged differently because of changes at the cell surface during the cell cycle that render T-Ag more accessible to iodination. The increase in cell size before mitosis and the resultant increase in surface area of such cells (2, 4, 21) might be related to the increased expression of surface T-Ag.

Although there are exceptions, most SV40 *tsA* transformants revert to the normal phenotype when incubated at the restrictive temperature (5, 6, 8, 25, 35, 41, 54). Three SV40 *tsA* transformants, as well as a WT transformant, were analyzed for the presence of surface T-Ag and T-Ag/p53 complex at both permissive and restrictive temperatures. The T-Ag and T-Ag/p53 complex were present on the surface of all *tsA* transformants cultured at the permissive temperature. One of those cell lines, the only one reported to exhibit complete growth arrest under nonpermissive conditions (9), failed to express surface T-Ag and surface T-Ag/p53 complex at the restrictive temperature. Perhaps surface T-Ag or surface T-Ag/p53 complex is functional in some growth-related process. In that event, its presence on the surface of A30B3a and A255B1b cells at 39.5°C would be expected, and its apparent absence on the A7B4b cells after 48 h at the nonpermissive temperature might presage their total growth arrest evident by 96 h (9).

Whether the presence of surface T-Ag, p53, or the complex itself is most relevant of the three to cell growth control remains to be determined. As discussed above, both T-Ag and the cellular p53 protein have been shown to be related to cell growth. In addition, it was reported recently that the levels of p53 are greatly decreased in Friend-erythroleukemia cells when they are induced to differentiate by dimethyl sulfoxide (3). Such a change in the expression of p53 precedes the decrease in cell growth rate that accompanies cell differentiation, suggesting once again that p53 has a role in cell proliferation control.

It is interesting to note that in several tumor virus systems the transforming proteins appear to be associated with the plasma membrane, including the Rous sarcoma virus *src* gene product (12, 60), Abelson virus p120 (62), the p21 *ras* protein (61), and the avian erythroblastosis virus *erbB* gene product (23). In addition, Willingham et al. (59) recently reported that an analog of the p21 transforming protein of the Harvey strain of murine sarcoma virus might be related to the regulation of normal cell growth control. That conclusion was based on the observation that the p21-related protein can be detected in normal epithelial cells only when they are actively growing. Recently, it has been observed that microinjection of purified H-*ras* protein stimulates quiescent cells to enter the S phase (18, 52). Equally important are the identification of the *sis* gene as a derivative of platelet-derived growth factor (16, 58) and the discovery of homology between the *erbB* oncogene and the DNA sequence that codes for the cytoplasmic and intramembranous domains of epidermal growth factor cell surface receptor (17). It is tempting to speculate that the SV40 T-Ag localized in the plasma membrane of transformed cells (13, 14, 24, 28, 34, 44, 46, 47, 49–51) may be exerting biological effects similar to those mediated by the membrane-associated retrovirus transforming proteins.

A functional role for surface T-Ag in transformation has been suggested recently by other studies in this laboratory. A mutant of SV40 [SV40(cT)-3] unable to transport T-Ag to the nucleus has been described (31). This mutated T-Ag is able to associate with the plasma membrane (30). In transfection studies with primary and established rodent cells, it has been demonstrated that the cytoplasmic mutant of SV40 is nearly comparable to WT virus in its ability to transform established cells, but sharply reduced in transforming frequency when assayed on primary cells (31a).

The lack of correlation between the presence of surface T-Ag and T-Ag/p53 complex and the expression of the transformed phenotype suggests that mere association of T-Ag with the cell surface is not sufficient to maintain the transformed phenotype. A similar observation has been made for polyoma-virus-transformed temperature-sensitive cells (56). Revertants to the normal phenotype continued to synthesize and insert into the plasma membrane the middle T antigen known to be crucial for polyomavirus transforming ability.

A less important, albeit practical, conclusion to be drawn from this study is that growing cells should be used in experiments that involve the detection of surface T-Ag. Cell populations with a low growth rate, such as confluent monolayers, may yield negative results because of the diminished expression of surface T-Ag.

In summary, a correlation between the expression of surface T-Ag and T-Ag/p53 complex and cell growth rate has been established in SV40-transformed mouse cells. This correlation suggests that the possibility should be considered that surface T-Ag or the T-Ag/p53 complex, or both, play a role, directly or indirectly, in control of cell proliferation.

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