Dynamic Nature of the Association of Large Tumor Antigen and p53 Cellular Protein with the Surfaces of Simian Virus 40-Transformed Cells

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A molecular complex of simian virus 40 large tumor antigen (T-Ag) and p53 cellular protein is present on the surface of simian virus 40-transformed mouse cells. The stability of the association of the two proteins with the cell surface was characterized. Cells were either surface iodinated by the lactoperoxidase technique or metabolically labeled with [³⁵S]methionine, and surface antigens were detected by differential immunoprecipitation with specific antibodies immediately after labeling or after incubation at 37°C. A rapid, concomitant disappearance of T-Ag and p53 from the cell surface was observed. The half-life of iodinated surface T-Ag was less than 30 min, whereas that of [³⁵S]methionine-labeled surface T-Ag was 1 to 2 h. Although T-Ag and p53 were rapidly lost, both were also rapidly replaced on the cell surface, since newly exposed molecules could be detected when cells were reiodinated after a 2-h chase period. Control experiments established that the loss of the surface molecules was not induced by the iodination reaction. The appearance of surface T-Ag was prevented when cellular protein synthesis was inhibited with cycloheximide. The disappearance and replacement of T-Ag and p53 appeared to be energy-independent processes, as neither was inhibited by sodium azide or 2,4-dinitrophenol. Incubation of iodinated cells at 4°C did block the loss of T-Ag and p53. These observations suggest that T-Ag and p53 are coordinately turned over in the plasma membrane. The nature of the association of the T-Ag-p53 complex with the cell surface can best be described as highly dynamic.

The plasma membrane is a highly dynamic cellular organelle, with its general structure changing throughout the cell cycle (3, 7, 19, 39, 54). Each stage during the life cycle of the cell is characterized by a specific array of molecules at the cell surface. The cycle-dependent expression of some, but not all, surface-associated molecules appears to reflect different individual turnover rates for each plasma membrane component (for a review, see reference 38).

Several mechanisms have been proposed to explain the way in which specific molecules are incorporated into and eliminated from the plasma membrane (for reviews, see references 37 and 38). The general hypothesis is that many plasma membrane proteins are probably synthesized and transferred across the rough endoplasmic reticulum membranes, following the pathways postulated for secretory proteins (5, 6). Specific sequences in the primary structure of the surface molecules would determine whether they remain attached to the plasma membrane (17, 45).

Internalization or secretion of a plasma membrane component in either a soluble form or in association with membrane vesicles could account for the disappearance of such a molecule from the cell surface. The release or secretion of a surface molecule toward the extracellular compartment is termed "shedding" (for a review, see reference 4). This shedding process is selective and influenced by the rate of cellular metabolism (4).

The shedding of many different plasma membrane components from the surface of both normal and tumor cells has been described. Proteins such as membrane-associated immunoglobulins (15), mouse alloantigens encoded by the major histocompatibility complex (16), human histocompati-

bility antigens (40), and blood group substances (40) have been shown to be shed from the surface of normal cells. Cell surface tumor-associated antigens are shed by murine (8) and human (9) melanoma cells and by methylcholanthrene-induced rat sarcoma cells (1, 12). In addition, tumor and transformed cells generally shed proteases and fibronectin (4).

Simian virus 40 (SV40)-infected and -transformed cells contain a virus-specific tumor antigen (T-Ag) that has a molecular weight of 94,000. Although the majority of the T-Ag is contained within the nucleus (41, 42), a small amount of this antigen is also present on the surface of such cells (13, 14, 23, 29, 33, 46, 47, 49, 51-53). Previous observations from this laboratory (30, 53) suggested that the surface-associated T-Ag is unstable and disappears from the surface as a consequence of being shed into the culture medium. Investigations described here have characterized in detail the shedding phenomenon in transformed cells. The results suggest that T-Ag that is lost from the cell surface is replaced rapidly by new T-Ag molecules, with neither the loss nor the replacement appearing to be an energy-dependent process. In addition, we have shown that the 53,000-dalton (53K or p53) cellular protein that has been found associated with large T-Ag, both in the nucleus (21, 28, 32, 33, 36, 51) and on the surface (33, 46, 47) of SV40-transformed cells, disappears concomitantly with T-Ag from the cell surface. The similarities in appearance and disappearance suggest that the two proteins may be coordinately turned over in the plasma membrane. The nature of the association of the T-Ag-p53 complex with the cell surface can best be described as highly dynamic.

MATERIALS AND METHODS

Cells. The transplantable mKSA-Asc line of SV40-transformed BALB/c kidney cells (25, 58) was routinely cultured

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as described previously (46). Subconfluent monolayers of cells grown in 100-mm tissue culture plates were used in every experiment.

Antisera. Ascitic fluid from hamsters bearing SV40-induced ascites tumors (hamster ascites fluid [HAF]; 29), mouse serum containing antibodies against mouse histocompatibility antigens (Mo α H-2.4; 46), and monoclonal antibodies with reactivity against mouse p53 cellular protein (PAb122; 22) or human immunoglobulins (9N) were used to immunoprecipitate the corresponding antigens from the surface of SV40-transformed cells.

Radioactive labeling and immunoprecipitation of surface T-Ag. Monolayers of mKSA-Asc cells were surface iodinated by the lactoperoxidase-catalyzed reaction (53). Cells to be metabolically labeled with either [35 S]methionine or [3 H]leucine were depleted of the specific amino acid by incubation for 2 h at 37°C in methionine- or leucine-free Eagle minimal essential medium (GIBCO Laboratories, Grand Island, N.Y.) supplemented with 2% dialyzed fetal bovine serum (GIBCO) and then labeled at 37°C for 15 min in 1 ml of the same medium containing 100 μ Ci of [35 S]methionine (>600 Ci/mmol; Amersham Corp., Arlington Heights, Ill.) or for 2 h in 1 ml of medium containing 100 μ Ci of [3 H]leucine (60 Ci/mmol; New England Nuclear Corp., Boston, Mass.).

Surface T-Ag was detected on surface-iodinated and [35 S]methionine-labeled cells by differential immunoprecipitation (46). Briefly, intact radiolabeled cells were incubated with growth medium containing a specific, heat-inactivated antiserum and extracted with detergent solutions. The immune complexes in the clarified cell lysates were precipitated with Formalin-fixed *Staphylococcus aureus* Cowan strain I, and the adsorbed antigens were eluted from the bacterial pellet. Nuclear T-Ag remaining in the supernatant fluids was precipitated by a second incubation with specific antibodies, the immune complexes were again adsorbed with *S. aureus*, and the antigens were eluted as described previously (46).

Gel electrophoresis and autoradiography. Immunoprecipitated antigens were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (51). The molecular weight markers used included phosphorylase *a* (94,000), bovine serum albumin (68,000), ovalbumin (43,000), carbonic anhydrase (30,000), and cytochrome *c* (11,700) (New England Nuclear). Autoradiography was performed with Kodak NSST X-ray films.

Measurement of proteins released into the medium. The amount of acid-insoluble radioactive material released into the medium by SV40-transformed cells was determined by precipitating the tissue culture supernatant fluids with trichloroacetic acid (TCA). Cells that had been metabolically labeled with [3 H]leucine, surface iodinated by the lactoperoxidase technique, or both metabolically and surface labeled were incubated at 37°C in 1 ml of growth medium. At different intervals the medium was collected, centrifuged at 3,000 rpm for 10 min at 4°C (International Equipment Co., Needham Heights, Mass.; model PR-6, rotor 269), and 125- μ l samples of the supernatant fluid were spotted in quadruplicate onto Whatman glass microfiber filters (934-AH; Fisher Scientific Co., Pittsburgh, Pa.) and dried (80°C for 20 min). Samples were precipitated by a single exposure to cold 10% TCA for 5 min and then washed for 5 min each in cold 5% TCA and cold 95% ethanol. Filters were dried as above and counted both in a gamma counter (Nuclear-Chicago Corp., Des Plaines, Ill.) and in a liquid scintillation counter (model LS-250; Beckman Instruments Inc., Fullerton, Calif.). To determine the total 3 H counts, all experimental 3 H values from double-labeled samples were corrected for detection of 125 I counts in the 3 H window.

Drugs, enzyme, and metabolic inhibitors. Cycloheximide (10 μ g/ml; Sigma Chemical Co., St. Louis, Mo.), Trasylol (10%; Mobay Chemical Co., New York, N.Y.), sodium azide (0.010 to 0.100 M; Sigma), and 2,4-dinitrophenol (0.001 M; Eastman Kodak Co., Rochester, N.Y.) were dissolved in growth medium. Trypsin (10 μ g/ml; ICN Nutritional Biochemicals, Cleveland, Ohio) was dissolved in a salt solution (140 mM NaCl, 5 mM KCl, 0.4 mM Na $_2$ HPO $_4$, 0.4 mM KH $_2$ PO $_4$, 5 mM dextrose, 5 mM NaHCO $_3$). Treatments with the different drugs and metabolic inhibitors were as described below and in the figure legends.

RESULTS

Disappearance of T-Ag and p53 from the surface of SV40-transformed cells. Lactoperoxidase-catalyzed radioiodination of molecules that are exposed on the cell surface was first described by Marchalonis et al. in 1971 (35). Since then, it has been the technique of choice to investigate the turnover of cell surface-associated molecules. We have used that technique to determine the kinetics of disappearance of large T-Ag and p53 cellular protein from the surface of SV40-transformed cells. Surface-iodinated mKSA-Asc cells were subjected to external immunoprecipitation by HAF (Fig. 1) or by anti-p53 monoclonal antibodies, PAb122 (Fig. 2), either immediately after labeling (Fig. 1, lane 2; Fig. 2, lane 2) or after different times of incubation at 37°C (chase period) in fresh growth medium. A significant decrease in the amount of 125 I-labeled T-Ag and p53 was observed after a 15-min chase (Fig. 1, lane 5; Fig. 2, lane 4). Both proteins had nearly (Fig. 1, lane 7) or completely (Fig. 2, lane 6) disappeared from the cell surface after a 1-h chase period. Note the similar kinetics of disappearance, regardless of whether anti-T-Ag or anti-p53 serum was used to immunoprecipitate the iodinated molecules.

Cells that had been metabolically labeled with [35 S]methionine for 15 min as described above were also subjected to external immunoprecipitation by HAF (Fig. 3) at the end of the 15-min pulse or after different periods of incubation at

FIG. 1. Disappearance of surface T-Ag (●) and p53 (○) detected by the use of T-antibodies. mKSA-Asc cells were surface iodinated and subjected to external immunoprecipitation by normal hamster serum (lane 1) or HAF (lanes 2 through 8) immediately after labeling (lanes 1 and 2) or after 5 min (lane 3), 10 min (lane 4), 15 min (lane 5), 30 min (lane 6), 60 min (lane 7), or 120 min (lane 8) of incubation in fresh growth medium at 37°C. Cells were disrupted with a Nonidet P-40 solution, and the immune complexes in the clarified cell lysate were adsorbed with *S. aureus* Cowan strain I. Antigens were eluted from the bacterial pellets and analyzed by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and autoradiography. Molecular weight markers are indicated at the left.

FIG. 2. Shedding of surface T-Ag (●) and p53 (○) detected by the use of anti-p53 monoclonal antibodies. mKSA-Asc cells were surface iodinated by the lactoperoxidase technique and subjected to external immunoprecipitation by 9N (lane 1) or PAb122 hybridoma supernatant fluid (lanes 2 through 7) immediately after labeling (lanes 1 and 2) or after 5 min (lane 3), 15 min (lane 4), 30 min (lane 5), 60 min (lane 6), or 120 min (lane 7) of incubation in fresh growth medium at 37°C. A 500- μ l sample of antibody-containing medium was used in each case. After a second incubation with goat anti-mouse immunoglobulins, cells were disrupted with a Nonidet P-40 solution, and the immune complexes in the clarified cell lysates were adsorbed with *S. aureus* Cowan strain I. Antigens were eluted from the bacterial pellets and analyzed by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and autoradiography. Molecular weight markers are indicated at the left.

37°C in fresh growth medium. [35 S]methionine-labeled large T-Ag and p53 could be detected on the surface of mKSA-Asc cells immediately after a 15-min pulse (Fig. 3, lane 1). An increase in the amount of both proteins was observed after a 15-min chase (Fig. 3, lane 2); that was followed by a gradual decrease during the ensuing 30- to 180-min chase (Fig. 3, lanes 3 through 6). The protein precipitated by normal serum (Fig. 3, lane 7) migrated slightly behind p53.

Selectivity of the disappearance of surface T-Ag and p53. Plasma membrane components appear to have different

FIG. 3. Appearance and disappearance of metabolically labeled surface T-Ag (●) and p53 (○). mKSA-Asc cells were metabolically labeled with [35 S]methionine for 15 min as described in the text. Labeled cells were subjected to external immunoprecipitation by normal hamster serum (lane 7) or HAF (lanes 1 through 6) immediately after labeling (lanes 1 and 7) or after 15 min (lane 2), 30 min (lane 3), 60 min (lane 4), 120 min (lane 5), or 180 min (lane 6) of incubation in fresh growth medium at 37°C. Cells were disrupted with a Nonidet P-40 solution, and the immune complexes in the clarified cell lysates were adsorbed with *S. aureus* Cowan strain I. Antigens were eluted from the bacterial pellets and analyzed by 7 to 15% gradient sodium dodecyl sulfate-polyacrylamide gel electrophoresis and autoradiography. Molecular weight markers are at the left.

turnover rates, which implies that the plasma membrane is neither synthesized nor degraded as a unit (for a review, see reference 38). The results described above suggest that large T-Ag and p53 protein might be coordinately incorporated and eliminated from the cell surface. It can be seen in Fig. 1 through 3 that some proteins that were non-specifically precipitated along with T-Ag exhibited variable patterns of change during the chase periods. Such observations suggest that the appearance and disappearance of large T-Ag and p53 from the surface of SV40-transformed cells might be a selective event.

To further substantiate this point the stability of histocompatibility antigens (mouse H-2 complex) on the surface of mKSA-Asc cells was also determined. H-2 antigens are composed of a heavy chain (45,000 daltons), which is an integral plasma membrane glycoprotein, and a noncovalently associated light chain (12,000 daltons), also known as β 2 microglobulin (24). Surface-iodinated mKSA-Asc cells were subjected to external immunoprecipitation by Mo α H-2.4 (Fig. 4) under the conditions described above. Both the 45K glycoprotein and β 2 microglobulin were detected on the cell surface immediately after the labeling (Fig. 4, lane 2). A gradual decrease of both molecules was evident during the chase period, although they were still detectable at the end of 2 h of incubation at 37°C (Fig. 4, lane 7). Therefore, H-2 antigens are probably more stable than T-Ag and p53 on the surface of SV40-transformed cells. It should be noted that in the gel system used the H-2 antigens migrated slightly ahead of the 43K and 11.7K markers.

Shedding of surface macromolecules is not induced by the lactoperoxidase-catalyzed iodination reaction. A critical point in studies of the release of iodinated molecules from the cell surface is whether the iodination reaction per se induces changes in the stability of such surface-associated molecules. It was shown above that T-Ag and p53 that had been metabolically labeled disappeared from the cell surface, as did the iodinated molecules. To demonstrate that the transformed cells are not markedly altered by the iodination

FIG. 4. Disappearance 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 normal mouse serum (lanes 1 and 8) or Mo α H-2.4 (lanes 2 through 7) immediately after labeling (lanes 1 and 2) or after 5 min (lane 3), 15 min (lane 4), 30 min (lane 5), 60 min (lane 6), or 120 min (lanes 7 and 8) of incubation in fresh growth medium at 37°C. Cells were disrupted with a Nonidet P-40 solution, and the immune complexes in the clarified cell lysates were adsorbed with *S. aureus* Cowan strain I. Antigens were eluted from the bacterial pellets and analyzed by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and autoradiography. Molecular weight markers are indicated at the left. Symbols: □, heavy chain; ■, β 2 microglobulin.

procedure, the total amount of protein secreted into the medium by iodinated and noniodinated cells was determined.

mKSA-Asc cells were metabolically labeled with [^3H]leucine as described above. The cells were then subjected to surface iodination, either by the standard method or by performing mock iodinations in which the lactoperoxidase enzyme was omitted or nonradioactive ^{127}I was used in place of ^{125}I . Cells on additional plates were labeled with [^3H]leucine or ^{125}I alone. Duplicate plates of radiolabeled cells were incubated with 1 ml of fresh growth medium for 30 min at 37°C. At the end of this incubation period, the amount of acid-insoluble [^3H]leucine-labeled molecules that had been released into the medium was determined as described above. The corrected values for TCA-precipitable ^3H -labeled proteins are presented in Table 1. It was evident that the total amount of cellular proteins released into the medium during a 30-min incubation was somewhat variable, but not markedly influenced by the surface iodination procedure.

That shedding of surface T-Ag and p53 is not induced by the lactoperoxidase-catalyzed reaction was also shown by the use of an inhibitor of protein synthesis. Preliminary studies established that treatment of mKSA-Asc cells with 10 μg of cycloheximide per ml for periods of time ranging from 1 to 6 h decreased the amount of [^{35}S]methionine incorporated into nuclear T-Ag to about 10% of that in untreated cells (data not shown). Therefore, cells that had been incubated in the presence of cycloheximide for different periods of time (1 to 6 h) were surface iodinated and subjected to external immunoprecipitation by HAF (Fig. 5). The amount of surface T-Ag accessible to iodination decreased with longer times of exposure of the cells to cycloheximide. After 6 h of treatment with the drug (Fig. 5, lane 5), a small amount of T-Ag was present, whereas no p53 could be detected. No difference was observed when cycloheximide was added once at the beginning of the 6-h incubation period and at 2-h intervals (Fig. 5, lanes 5 and 6, respectively). It is apparent that both T-Ag and p53 disappear from the cell surface in the absence of prior iodination manipulations. The rate of disappearance of T-Ag and p53 in the presence of cycloheximide appears somewhat slower than that observed in Fig. 1 and 2. This is probably due to a

FIG. 5. Effect of cycloheximide on the disappearance of surface T-Ag and p53. Subconfluent monolayers of mKSA-Asc cells were incubated at 37°C in fresh growth medium containing 10 μg of cycloheximide per ml for 1 h (lane 2), 2 h (lane 3), 4 h (lane 4), or 6 h (lane 5). Cycloheximide was added every 2 h to cells analyzed in lane 6. Control cells not exposed to cycloheximide are shown in lane 1. Cells were surface iodinated by the lactoperoxidase technique and subjected to external immunoprecipitation by HAF (lanes 1 through 6). Cells were disrupted with a Nonidet P-40 solution, and the immune complexes in the clarified cell lysates were adsorbed with *S. aureus* Cowan strain I. Antigens were eluted from the bacterial pellets and analyzed by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and autoradiography. Molecular weight markers are indicated at the left. Symbols: ●, T-Ag; ○, p53; ▽, 84K polypeptide.

replacement of released surface molecules by cytoplasmic proteins which had been synthesized before drug addition and inhibition.

It might be noted that an 84K T-Ag-reactive polypeptide was detected in the samples incubated for 6 h with cycloheximide (Fig. 5, lanes 5 and 6). An 84K polypeptide has been detected previously on the surface of mKSA-Asc cells that had been subjected to mild trypsinization before surface iodination (M. Santos and J. S. Butel, unpublished observations). Perhaps if protein synthesis is blocked, the last preformed molecules of T-Ag that become associated with the cell surface are not shed, but remain at that subcellular level. Because of the proteases abundant on the surface of transformed cells, the majority of those persisting T-Ag molecules might be cleaved at a sensitive site.

Mechanism of disappearance of surface-associated T-Ag and p53. Disappearance of surface molecules might result from either internalization or secretion toward the extracellular compartment. Shed molecules presumably might be subject to proteolytic degradation in the medium. Iodinated large T-Ag and p53 have not been identified within the cell at any time after labeling (data not shown). The data in Table 2 indicate that surface-iodinated proteins are released from mKSA-Asc cells into the culture medium; the addition of a protease inhibitor (Trasyolol) to the medium during the chase period had a minimal effect on the recoveries of iodinated proteins. Detectable amounts of large T-Ag can be immunoprecipitated from the medium after a 30-min chase, although quantitative recovery has never been achieved (data not shown).

Inhibitors were employed to determine the metabolic nature of the shedding of T-Ag and p53. mKSA-Asc cells were surface iodinated and subjected to external immunoprecipitation by normal hamster serum (Fig. 6, lane 1) or by HAF (Fig. 6, lanes 2 through 11) immediately after labeling (Fig. 6, lanes 1 and 2) or after a 2-h chase (Fig. 6, lanes 3

TABLE 1. Lack of influence of the lactoperoxidase-catalyzed reaction on shedding events from the surface of SV40-transformed cells^a

Cell labeling conditions				TCA-precipitable proteins in medium (³ H cpm × 10 ⁴)
Metabolically labeled	Surface iodination			
[³ H]leucine	¹²⁵ I	¹²⁷ I	Enzyme	
+ ^b	—	—	—	4.22
+	—	+	+	5.51
+	+	—	—	7.57
+	+	—	+	5.18

^a mKSA-Asc cells were metabolically labeled with [^3H]leucine as described in the text and subjected to the lactoperoxidase-catalyzed reaction under the conditions indicated in the table. Radiolabeled cells were then incubated in fresh growth medium at 37°C for 30 min. At the end of this incubation period, medium was collected from duplicate plates and centrifuged at 3,000 rpm for 10 min at 4°C, and radiolabeled proteins in the supernatant fluids were TCA precipitated and counted. ^3H counts per minute were corrected as described in the text. Each value is the average of duplicate samples.

^b +, Presence of reactant; —, absence of reactant.

TABLE 2. Release of iodinated surface-associated proteins from mKSA-Asc cells into the culture medium

Time after labeling (min)	Trasyolol in medium ^b	TCA-precipitable proteins in medium (¹²⁵ I cpm × 10 ⁶) ^c
15	—	3.86
30	—	4.77
30	+	4.92
60	—	5.38
120	—	5.48
120	+	6.34

^a mKSA-Asc cells grown as monolayers in 100-mm tissue culture plates were surface-iodinated by the lactoperoxidase technique and incubated in fresh growth medium at 37°C for different periods of time. At the end of each incubation period, medium was collected and centrifuged at 3,000 rpm for 10 min at 4°C, and iodinated proteins in the supernatant fluid were TCA precipitated and counted as described in the text.

^b Trasyolol (10%) was included in the culture medium during the incubation periods as a protease inhibitor.

^c Each value represents the average of two samples, and each TCA precipitation was performed in quadruplicate.

through 7). The chase was performed by incubating the iodinated cells for 2 h at 37°C in fresh growth medium (Fig. 6, lane 3) or in medium containing sodium azide (Fig. 6, lane 5), 2,4-dinitrophenol (Fig. 6, lane 6), or cycloheximide (Fig. 6, lane 7). A culture of cells was also incubated at 4°C during the 2-h chase (Fig. 6, lane 4). Shedding of surface-associated large T-Ag was blocked only by low temperature. Although not apparent in this gel due to the nonspecific precipitation of a similarly migrating protein, comparable results were obtained in other experiments for the shedding of p53 (data not shown).

Newly exposed T-Ag molecules could be detected on the surface of those cells that had been incubated for 2 h at 37°C when they were subjected again to surface iodination at the end of the incubation period (Fig. 6, lane 8). This replacement of surface T-Ag was not inhibited by sodium azide, 2,4-dinitrophenol, or cycloheximide (Fig. 6, lanes 9, 10, or 11, respectively). Similar results were observed with p53 (data not shown).

DISCUSSION

The stability of the association of SV40 T-Ag and p53 cellular protein with the surface of SV40-transformed mouse cells has been examined here in detail. Both T-Ag and p53 appear to associate transiently with the surface membrane; at least a portion of the population is then rapidly shed into the medium (30, 53). It has not been ruled out, however, that some of the loss may be a result of degradation of membrane-associated molecules. Control experiments established that the release of molecules was not induced by the lactoperoxidase-catalyzed surface iodination reaction. Shedding of surface T-Ag and p53 appears to be selective in that their loss does not mimic the pattern of release of other surface molecules, e.g., murine H-2 antigens.

Sodium azide, 2,4-dinitrophenol, and cycloheximide, compounds routinely used as metabolic inhibitors, do not block the shedding of T-Ag and p53. These results suggest that the shedding of T-Ag and p53 is not an energy-dependent cellular event and that concomitant protein synthesis is not required. In contrast, both proteins fail to be released into the medium when iodinated cells are incubated at 4°C. Since temperatures lower than physiological can induce a

drastic decrease in the fluidity of the plasma membrane (11), one conclusion is that the release of surface T-Ag and p53 occurs by a process affected by membrane fluidity (e.g., diffusion).

The concomitant disappearance of both surface T-Ag and p53, monitored by T-antibodies as well as p53 antibodies, suggests that the two proteins are being shed coordinately, possibly as a complex. This hypothesis is substantiated by the fact that a complex between T-Ag and p53 has been detected on the surface of SV40-transformed mouse cells (46, 47). Whether these proteins are shed in association with phospholipids from the plasma membrane is presently not known. Emerson and Cone (15, 16) and Liepins and Hillman (31) have shown that active cellular metabolism and physiological temperatures are required when shedding of surface molecules is mediated by plasma membrane vesicles. In contrast, Emerson and Cone (15, 16) have demonstrated that membrane-associated immunoglobulin M (IgM) is shed into the medium as a molecular unit in the absence of demonstrable associated cellular proteins or phospholipids and that shedding of the IgM is not energy dependent and is inhibited by low temperatures. These data might be extrapolated to speculate that the shedding of T-Ag and p53 may occur by a process similar to that of IgM. Selectivity of shedding of different surface macromolecules also appears to be controlled by the cytoskeleton (16). Whether shedding of T-Ag and p53 is influenced by the cellular filamentous network is presently under investigation.

T-Ag and p53 molecules that are lost from the cell surface are rapidly replaced, since new molecules can be iodinated on the cell surface after a 2-h chase. Therefore, the turnover of surface T-Ag and p53 is the net result of a combination of shedding (or degradation or both) and exposure of molecules. The results presented here suggest that T-Ag and p53 are rapidly lost from the exterior of the cell once they are exposed on the cell surface (estimated half-life of iodinated T-Ag, less than 30 min; Fig. 1 and 2). The disappearance of T-Ag and p53 from the surface of [³⁵S]methionine-labeled cells (Fig. 3) or cycloheximide-treated cells (Fig. 5) seems to

FIG. 6. Disappearance and reexposure of surface T-Ag (●). mKSA-Asc cells were surface iodinated by the lactoperoxidase technique and subjected to external immunoprecipitation by normal hamster serum (lane 1) or HAF (lanes 2 through 11) immediately after labeling (lanes 1 and 2) or after a 2-h chase (lanes 3 through 11). Cells were incubated in fresh growth medium at 4°C (lane 4) or at 37°C (lanes 3 and 5 through 11). During the chase period at 37°C, cultures were incubated in the presence of sodium azide (lanes 5 and 9), 2,4-dinitrophenol (lanes 6 and 10), or cycloheximide (lanes 7 and 11). Cells in lanes 8 through 11 were surface iodinated for a second time after the chase period, but before external immunoprecipitation by HAF. Cells were disrupted with a Nonidet P-40 solution, and the immune complexes in the clarified cell lysates were adsorbed with *S. aureus* Cowan strain I. Antigens were eluted from the bacterial pellets and analyzed by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Molecular weight markers are indicated at the left.

be a slower process (estimated half-life, 1 to 2 h). This apparent contradiction in kinetics might reflect the fact that different experimental designs labeled different populations of molecules. The total pool of surface-destined T-Ag and p53 molecules synthesized during the labeling period would be detectable in the metabolically labeled experiments. In contrast, only those molecules externally exposed at the time of surface labeling would be detected as iodinated molecules.

Very recently, the post-translational modification of plasma membrane-associated T-Ag by acylation has been reported (26, 27). Other membrane-associated viral transforming proteins have also been found recently to contain tightly bound lipid (20, 50). It has been postulated that plasma membrane proteins that do not contain hydrophobic sequences might be anchored in the lipid bilayer via linked fatty acids (48). Since the primary sequence of large T-Ag contains no pronounced hydrophobic regions (18, 43), its association with the plasma membrane might be mediated by acylation. However, considering the dynamic nature of the association of T-Ag with the cell surface, it might be postulated that although insertion of T-Ag into the plasma membrane could be mediated by acylation, the subsequent release of the molecules might be due to conformational changes.

Shedding has been shown to be a phenomenon occurring with both normal and tumor or transformed cells (4). Release of molecules from the surface of tumor cells appears to play a crucial role in determining the malignancy of a tumor (4). It has been suggested that circulating tumor antigens might act as blocking factors, thereby increasing the metastatic potential of a given tumor. Existing evidence suggests that large T-Ag, either alone or in association with other surface proteins such as p53, constitutes the SV40 tumor-specific transplantation antigen (2, 10, 34, 44, 56; for reviews, see references 55 and 57). Therefore, shedding of T-Ag and p53 from the surface of SV40-transformed mouse cells might have a role in either determining the extent of tumor growth or influencing the immune response of the host.

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