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# Complex of Simian Virus 40 Large-T Antigen and Host 53,000-Molecular-Weight Protein in Monkey Cells

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Mouse cells transformed by simian virus 40 (SV40) have been shown to contain a complex of the virus-coded large-T antigen with a host 53,000-molecular-weight (53K) protein. Initial attempts to detect a similar complex in lytically infected cells were unsuccessful, and it therefore seemed that the complex might be peculiar to transformed or abortively transformed nonpermissive cells. Immunoprecipitation of [$^{32}$P]phosphate-labeled extracts of SV40-infected CV-1 African green monkey kidney cells with antibodies specific for large-T or the 53K protein revealed that the large-T–53K protein complex was formed during lytic infection. Only a minor fraction of the large-T present was associated with 53K protein, and large-T and the 53K host protein cosedimented during centrifugation through sucrose gradients. We used monospecific sera and monoclonal antibodies to study the rate of synthesis and phosphorylation of the 53K protein during lytic infections. Infection of CV-1 cells with SV40 increased the rate of synthesis of the 53K protein fivefold over that in mock-infected cells. At the same time, the rate of phosphorylation of the 53K protein increased more than 30-fold compared with control cultures. Monkey cells transformed by UV-irradiated SV40 (Gluzman et al., J. Virol. **22**:256–266, 1977) also contained the large-T–53K protein complex. The formation of the complex is therefore not a peculiarity of SV40-transformed rodent cells but is a common feature of SV40 infections.

In many respects, cells transformed by simian virus 40 (SV40) are similar to cells infected by the virus at early times after infection. In both cases there is little or no expression of the late proteins but substantial expression of both large-T and small-t antigens. These two polypeptides, with molecular weights of about 85,000 and 20,000, respectively, come from overlapping coding regions and correspond to most of the coding capacity of the early region (4). In immunoprecipitates from lysates of transformed mouse cells, a third polypeptide with a molecular weight of about 53,000 (53K) is found (2, 3, 6, 10, 13, 14, 17, 19). This phosphoprotein is host coded (14) and is found in a complex with large-T (12). The complex can be immunoprecipitated from lysates of these cells with antisera raised against either gel-purified large-T (12) or 53K protein (16). Although the complex of the 53K protein with virus-coded large-T is prominent in transformed cells, several attempts to detect the complex in productively infected monkey cells had failed. The presence of the complex in transformed cells and its apparent absence in infected monkey cells led us to ask whether the large-T–53K protein complex was a peculiarity of SV40-transformed cells. We first examined infected monkey cells by using a high-titer antiserum against large-T and a specific monoclonal antibody against the 53K protein. This revealed the presence of the large-T–53K protein complex. We then compared uninfected, infected, and transformed monkey cells and found that the cells all contain 53K protein at various levels and that infection of CV-1 monkey cells with SV40 had a striking effect on the rate of synthesis and phosphorylation of the 53K protein.

## MATERIALS AND METHODS

**Cells and virus.** The CV-1 strain of African green monkey kidney cells was used as a permissive host for infection with SV40, strain SV-S. C2, C6, and C11 cells are SV40 transformants of CV-1 cells. These cells were transformed by infection with UV-irradiated SV40 and were kindly provided by E. Winocour. The SVA31E7 cell line is an SV40-transformed BALB/c 3T3 A31 mouse cell and was provided by Y. Ito. Cell lines were grown on 50- or 90-mm plastic tissue culture dishes (NUNC) in the Dulbecco modification of Eagle minimal essential medium (E4) supplemented with 10% calf serum.

**SV40 infection of CV-1 cells.** Media from confluent monolayers of CV-1 cells were aspirated, and the cell sheet was covered by a minimal quantity (1.0 ml/90-mm dish or equivalent) of E4 containing the appropriate dilution of SV40. Mock infections were performed identically except that dilutions of unin-

fected CV-1 lysate were substituted for the virus dilutions. Adsorption was allowed to continue for 2 h at 37°C, and then E4 with 10% calf serum was added.

**Preparation of radioactive extracts.** Proteins were labeled by growing cells in the presence of [$^{35}$S]-methionine (1 mCi/90-mm dish, 1,000 to 1,300 Ci/mmol; Radiochemical Centre) or [$^{32}$P]phosphate (1 mCi/90-mm dish, carrier-free; Radiochemical Centre) in 1 ml of E4 supplemented with 2% dialyzed calf serum but lacking either methionine or phosphate, respectively. After 3 h the labeling period was terminated by washing the cells once in cold Tris-buffered saline (150 mM NaCl, 50 mM Tris, pH 7.4) and then lysing the cells in 1.0 ml of lysis buffer (1% Nonidet P-40, 150 mM NaCl, 20 mM Tris, pH 8.0, 0°C). The cell debris was removed by centrifugation, and the lysates were used immediately for immunoprecipitations.

**Sera.** Antisera against large-T were prepared by hyperimmunizing a New Zealand white female rabbit with purified adenovirus-SV40 hybrid D2 protein (D. Lane, personal communication) and was kindly provided by D. Lane. Culture supernatant or ascitic fluid from the clone 122 hybridoma was used as a source of anti-53K activity and was the kind gift of E. Gurney (8).

**Immunoprecipitation and electrophoresis of polypeptides.** Lysates were either used without any further treatment or pre-precipitated by incubating them overnight at 4°C with 1 volume of normal rabbit serum (100 μl/90-mm dish) and then adding 10 volumes of a 10% suspension of Formalin-treated *Staphylococcus aureus* Cowan I (SAC) (9). After 15 min at 4°C, SAC was removed by centrifugation, and the SAC incubation was repeated. Samples of the supernatants were mixed with 0.5 ml of NET-GEL buffer (150 mM NaCl, 5 mM EDTA, 50 mM Tris, 0.25% gelatin, 0.02% $NaN_3$, pH 7.4) and immunoprecipitated for 1 h at 4°C with antiserum, hybridoma culture supernatant, or hybridoma ascitic fluid. The immunoprecipitates were collected on 20 to 40 volumes of 10% SAC per volume of antiserum or equivalent, washed three times in NET-GEL buffer, and then eluted from SAC by heating at 85°C for 10 min in sample buffer (2.0% sodium dodecyl sulfate [SDS], 100 mM dithiothreitol, 10% glycerol, 50 mM Tris, pH 6.8). SAC was removed by centrifugation, and the supernatants were loaded on 10% or 12% polyacrylamide gels (11). Polypeptides were separated by electrophoresis for 5 to 6 h at 100 to 150 V. Gels were stained in Coomassie blue (0.5% Coomassie brilliant blue, 50% trichloroacetic acid), destained in methanol-acetic acid, dried, and autoradiographed on Kodak SB-5 film. Radioactivity in individual bands was determined by the procedure of Ward et al. (20).

**Sucrose gradient centrifugation.** Proteins from radiolabeled cells were sedimented through sucrose gradients as described by McCormick and Harlow (16). Briefly, 400 μl of a Nonidet P-40 lysate was layered on a 4.6-ml, 5 to 20% sucrose gradient (1% Nonidet P-40, 150 mM NaCl, 10 mM dithiothreitol, 20 mM Tris, pH 7.4) with a 0.4-ml 60% sucrose cushion. After the gradients were centrifuged at 25,000 rpm in an SW50.1 rotor for 15 h at 4°C, they were each fractionated, and then individual fractions were immunoprecipitated.

## RESULTS

**Presence of large-T-53K complex during productive infection.** Large-T can be found in at least two distinct forms in SV40-transformed mouse cells (16). A large proportion of large-T is found in a high-molecular-weight complex with a host 53K protein. Using qualitative assays, several groups have failed to detect or have had difficulty detecting an analogous complex in productive infections of monkey cells (3, 10, 12, 16). Because of the potential significance of this distinction between SV40-transformed cells and productively infected cells, we decided to determine whether the large-T–53K complex was absent in productive infections.

Using a series of titrations with a potent antiserum specific for large-T, we were able to immunoprecipitate both large-T and a phosphorylated 53K polypeptide from productively infected CV-1 cells (Fig. 1). CV-1 cells were infected with SV40 and pulsed with [$^{32}$P]phosphate at 48 h after infection. Aliquots from lysates of these cultures were then immunoprecipitated with increasing concentrations of serum raised against purified adenovirus-SV40 hybrid D2 protein. Even with as little as 0.001 μl of anti-D2 serum, we were able to detect both large-T and the 53K species. As measured by film darkening on this and shorter exposures, radioactivity in the large-T and 53K bands increased

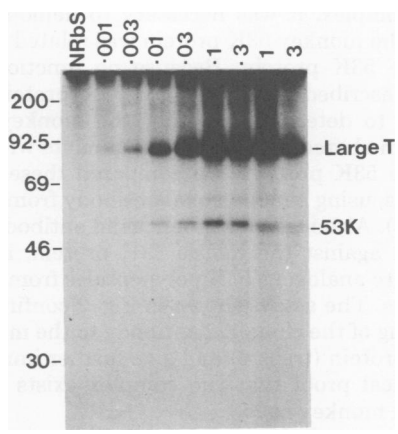


FIG. 1. *Titration of extracts from productively infected CV-1 cells with anti-D2 serum. A confluent monolayer of CV-1 cells was infected with SV40 (100 PFU per cell). At 48 h after infection, the cells were pulsed with [$^{32}$P]phosphate. Aliquots of the lysate (20 μl) were precipitated with normal rabbit serum or increasing volumes of anti-D2 serum. Immunoprecipitates were collected on 30 μl of SAC, and polypeptides were separated by SDS-polyacrylamide gel electrophoresis. Gels were dried and autoradiographed on Kodak SB-5 film.*

proportionally until 0.1 μl of anti-D2 serum was added. Because each aliquot used in these immunoprecipitations represented 1/50 of a confluent monolayer of infected CV-1 cells on a 90-mm petri dish, we could calculate that 5 μl of anti-D2 serum was sufficient to precipitate all of the radioactive large-T from $3 \times 10^6$ cells.

The two tracks on the far right of Fig. 1 also demonstrate two potential difficulties in detecting the monkey 53K protein. Note that the rabbit immunoglobulin G (IgG) heavy-chain monomer and the 53K species migrated with almost identical mobilities, causing the 53K band to be pushed slightly forward and to lose its sharpness. Therefore, increasing the antibody concentrations to ensure the immunoprecipitation of all of the large-T present is likely to mask the presence of the 53K species. This is in contrast to the mouse 53K protein which migrates faster than the rabbit IgG heavy-chain monomer (see below) and often is sharpened by increasing the antibody concentration. Also, note that 3 μl of the IgG-rich anti-D2 serum did not precipitate as much phosphorylated large-T or 53K protein as did 1 μl. This suggests that the antibody-binding capacity of SAC (30 μl of a 10% suspension) had been exceeded in this titration.

Although the coordinate precipitation of large-T and the 53K protein with an anti-large-T serum that has no detectable activity against the monkey 53K protein (see below) is a strong immunochemical argument for the presence of the complex, it was necessary to demonstrate that the monkey 53K protein was related to the mouse 53K protein. Because no function has been ascribed to the mouse 53K protein, we chose to determine whether the monkey 53K protein shared antigenic determinants with the mouse 53K protein. We compared these same lysates, using a monoclonal antibody from clone 122 (8). Although this monoclonal antibody was raised against the mouse 53K protein, it also binds to analogous 53K polypeptides from other species. The assays shown in Fig. 2 confirm the binding of the clone 122 antibody to the monkey 53K protein (track 6) and give further immunochemical proof that the complex exists in infected monkey cells.

The cascade immunoprecipitations shown in Fig. 2 also demonstrate that lysates from productive infections of CV-1 cells contain both large-T that is not complexed to the 53K protein (track 10) and 53K protein that is not bound to large-T (track 5). Free large-T is a common feature of all SV40-transformed cell lines that have been examined to date (16), but free 53K protein has only been reported in an SV40-transformed human cell, SV80 (8, 13).

Physical evidence for the presence of the complex is shown in Fig. 3. The complex from transformed mouse cells has previously been shown to sediment at 23S (16). The 53K protein and large-T from infected monkey cells also sedimented together at approximately 20S, suggesting an approximate molecular weight for the complex of $5 \times 10^5$. These data also confirm the specificity of the 122 monoclonal antibody. Note that the clone 122 antibody did not precipitate large-T from the top of the gradient, although large-T was present, as shown by precipitation with anti-D2 serum (data not shown).

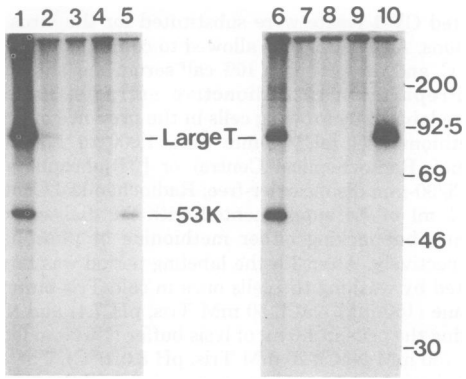


FIG. 2. *Cascade immunoprecipitation of extracts from productively infected CV-1 cells. A confluent monolayer of CV-1 cells was infected with SV40 (100 PFU per cell). At 48 h after infection, the cells were pulsed with [$^{32}$P]phosphate, and lysates were prepared from these cells. A small sample of the extract was given three sequential immunoprecipitations with anti-D2 serum (tracks 1, 2, and 3). Any remaining antibody-antigen complexes were cleared by an additional round of SAC incubation (track 4) before immunoprecipitation with anti-53K monoclonal antibody (track 5). The inverse sequence is shown in tracks 6 to 10, beginning with three rounds of anti-53K monoclonal antibody (tracks 6, 7, and 8) and ending with an immunoprecipitation with anti-D2 serum (track 10).*

**Induction of 53K protein synthesis after SV40 infection.** The presence of the large-T–53K protein complex in productive infection raises the question of whether virus infection changes the regulation of synthesis or phosphorylation of the host 53K protein. To ascertain the effect of virus infection, we labeled cell cultures with [$^{35}$S]methionine or [$^{32}$P]phosphate at various times after infection. Samples from lysates of these cultures were then immunoprecipitated with an excess of antibody specific for large-T or the 53K protein. The immunoprecipitates were separated by SDS-polyacrylamide gel electrophoresis (Fig. 4 and 5). Figure 4 shows the change in synthesis and phosphorylation of large-T during infection, as estimated by precipitation with an excess of anti-D2 serum. The

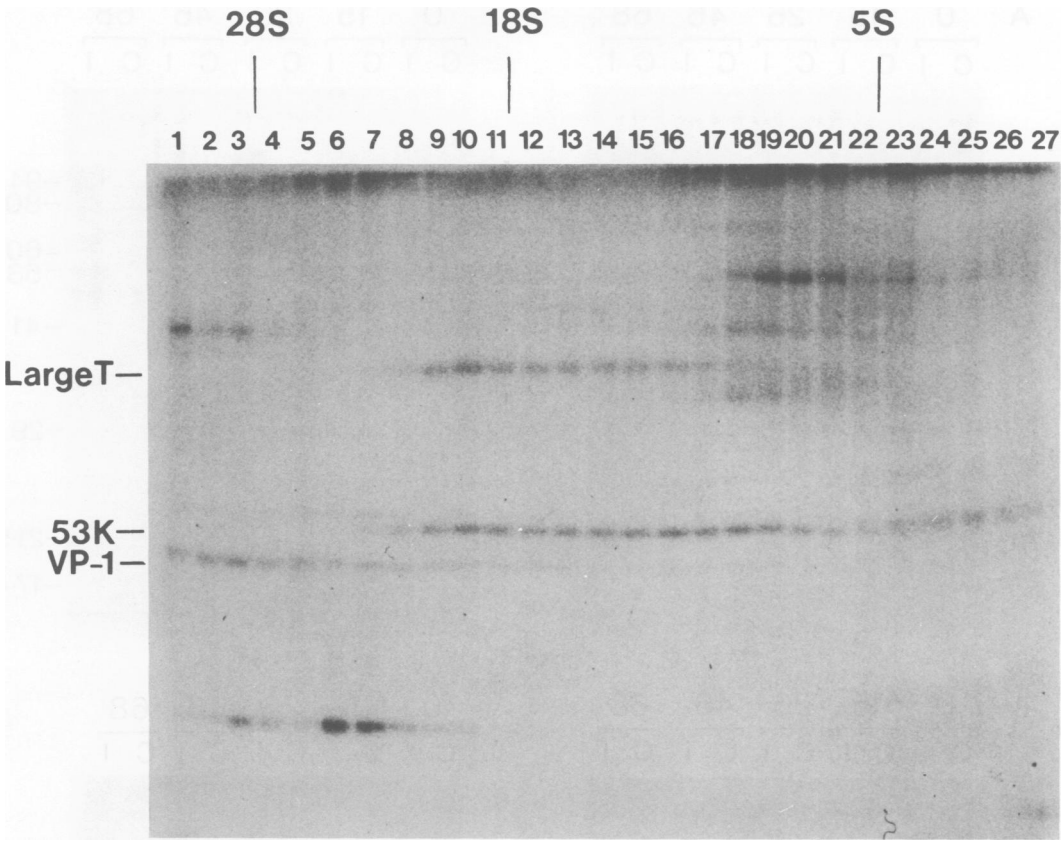


FIG. 3. *Sucrose gradient centrifugation of extracts from productively infected CV-1 cells. A confluent monolayer of CV-1 cells was infected with SV40 (100 PFU per cell). At 48 h after infection, cells were pulsed with [$^{32}$P]phosphate. The lysate was layered over a 5 to 20% sucrose gradient and centrifuged for 15 h at 25,000 rpm. The gradient was fractionated, and each fraction was immunoprecipitated with anti-53K monoclonal antibody. Immunoprecipitates were collected on SAC, and polypeptides were separated by SDS-polyacrylamide gel electrophoresis. Gels were dried and autoradiographed on Kodak SB-5 film. Sedimentation was from right to left.*

peak of large-T synthesis was at 26 h after infection, whereas the peak of phosphorylation was at 46 h after infection. Although the rates of both synthesis and phosphorylation of large-T had distinct peaks during infection, the synthesis and phosphorylation of that fraction of the 53K protein that was bound to large-T continued to increase throughout infection. This was seen most clearly with immunoprecipitation of total 53K protein, using an excess of the clone 122 antibody (Fig. 5). As shown in Fig. 5, precipitation of mock-infected CV-1 cells with the clone 122 antibody shows a 53K band which was not precipitated by the anti-D2 serum (Fig. 4). This confirmed that anti-D2 serum has no detectable activity against the monkey 53K protein and completes the immunochemical argument for the presence of the complex. Both large-T and the 53K protein can be precipitated from lysates of productively infected cells with antisera that recognize only one component of the complex.

Because the rates of synthesis and phosphorylation of the 53K protein in mock-infected CV-1 cells remained relatively constant throughout the time course, we were able to use these rates as base lines to calculate the changes induced by SV40 infection. The 53K protein bands from Fig. 5 were excised, and the radioactivity was measured. The rates of synthesis and phosphorylation of the 53K protein relative to mock-infected cultures are shown in Fig. 6. The rates of synthesis and phosphorylation were strikingly different. The synthesis of the 53K protein rose by 5-fold compared with the mock-infected cultures, whereas the rate of phosphorylation increased by more than 30-fold.

**Presence of large-T-53K complex in SV40-transformed monkey cells.** The pres-

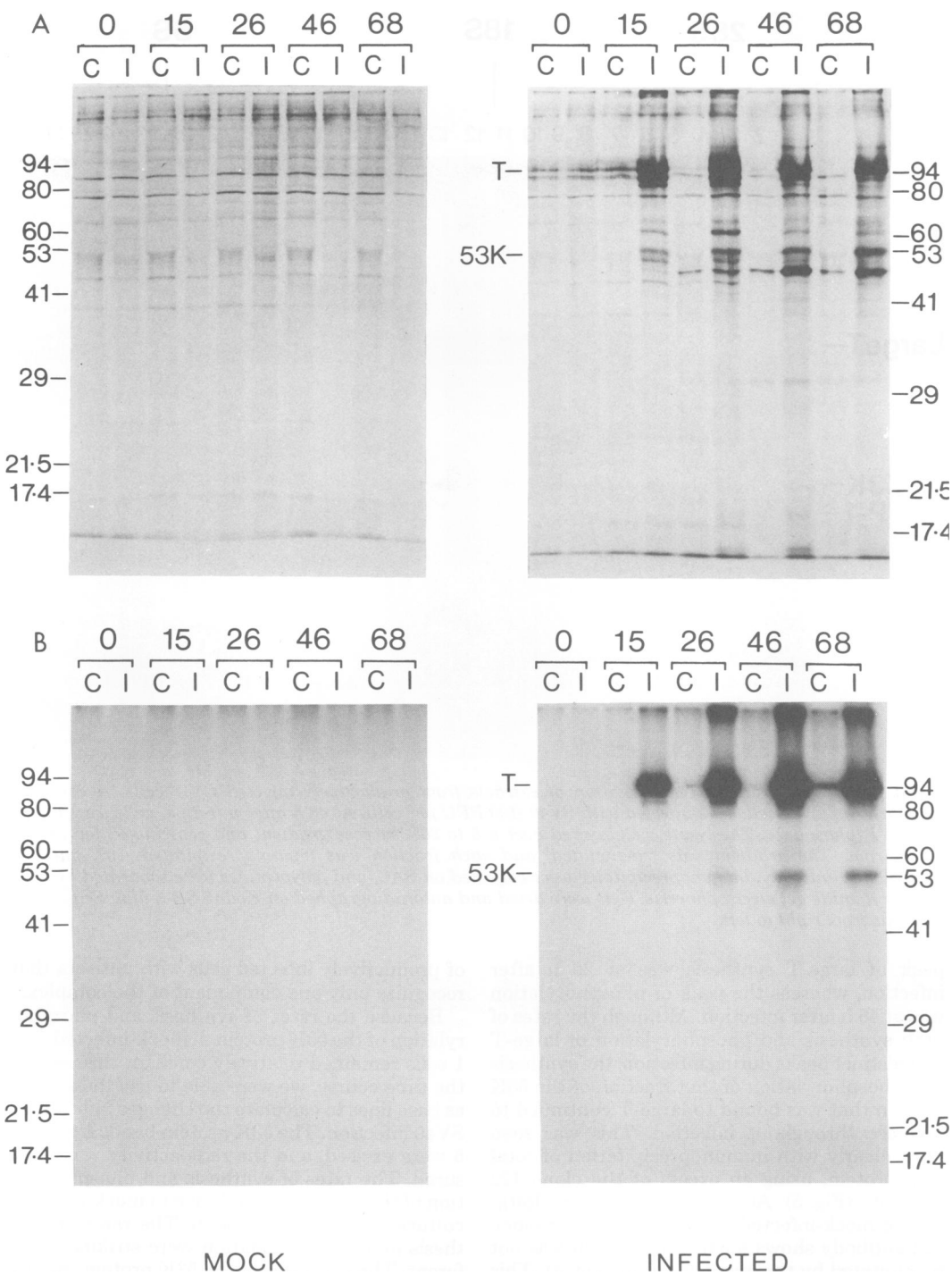


FIG. 4. *Synthesis and phosphorylation of large-T during productive infection of CV-1 cells. Confluent monolayers of CV-1 cells were infected with SV40 (20 PFU per cell) (right) or mock infected (left). At 15, 26, 46, and 68 h after infection, cells were pulsed with [$^{35}$S]methionine (A) or [$^{32}$P]phosphate (B). Lysates were precipitated with either normal rabbit serum (C) or anti-D2 serum (I). Immunoprecipitates were collected on SAC, and polypeptides were separated by SDS-polyacrylamide gel electrophoresis. Gels were dried and autoradiographed on Kodak SB-5 film.*

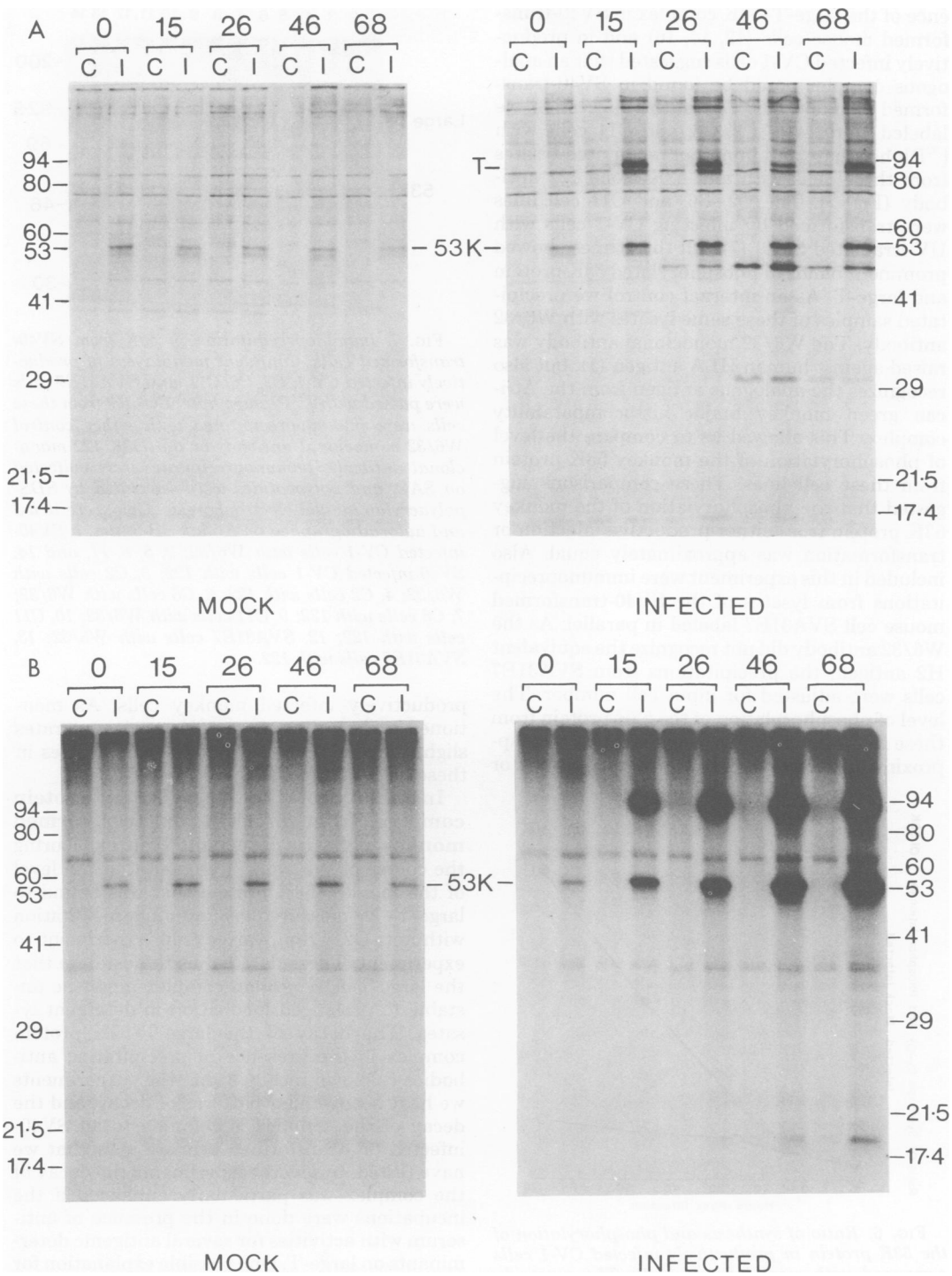


FIG. 5. *Synthesis and phosphorylation of the 53K protein during productive infection of CV-1 cells. Confluent monolayers of CV-1 cells were infected with SV40 (20 PFU per cell) (right) or mock infected (left). At 15, 26, 46, and 68 h after infection, cells were pulsed with [$^{35}$S]methionine (A) or [$^{32}$P]phosphate (B). Lysates were precipitated with either control hybridoma supernatant (C) or anti-53K hybridoma supernatant (I). Immunoprecipitates were collected on SAC, and polypeptides were separated by SDS-polyacrylamide gel electrophoresis. Gels were dried and autoradiographed on Kodak SB-5 film.*

ence of the large-T-53K complex in SV40-transformed mouse cells (12, 14, 16) and in productively infected CV-1 cells suggested that an analogous complex could be found in SV40-transformed monkey cells. To test this suggestion, we labeled cultures of C2, C6, and C11 cells with [$^{32}$P]phosphate and immunoprecipitated lysates from these cells with anti-53K clone 122 antibody (Fig. 7). The C2, C6, and C11 cell lines were transformed by infecting CV-1 cells with UV-irradiated SV40 (7). All three lines showed prominent bands of phosphorylated 53K protein and large-T. As an internal control we precipitated samples of these same lysates with W6/32 antibody. The W6/32 monoclonal antibody was raised against human HLA antigen (1), but also recognizes the analogous antigen from the African green monkey major histocompatability complex. This allowed us to compare the level of phosphorylation of the monkey 53K protein from these cell lines. These comparisons suggested that the phosphorylation of the monkey 53K protein from either productive infection or transformation was approximately equal. Also included in this experiment were immunoprecipitations from lysates of the SV40-transformed mouse cell SVA31E7 labeled in parallel. As the W6/32 antibody did not recognize the equivalent H2 antigen, the precipitations from SVA31E7 cells were adjusted for input cell number. The level of phosphorylation of the 53K protein from these SV40-transformed mouse cells was approximately equal to that of the transformed or

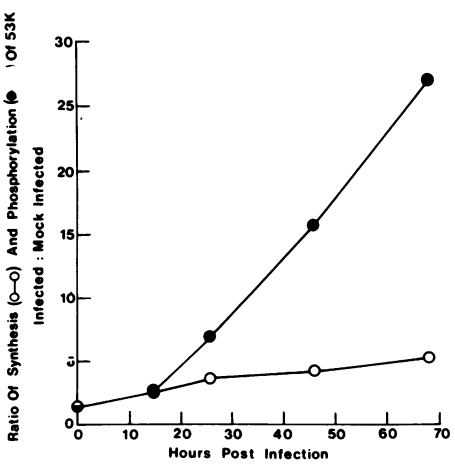


FIG. 6. *Ratio of synthesis and phosphorylation of the 53K protein in productively infected CV-1 cells compared with mock-infected cells. The autoradiographs shown in Fig. 5 were used as templates to excise the 53K bands from the dried polyacrylamide gel. The radioactivity in the excised gel pieces was determined as described in the text.*

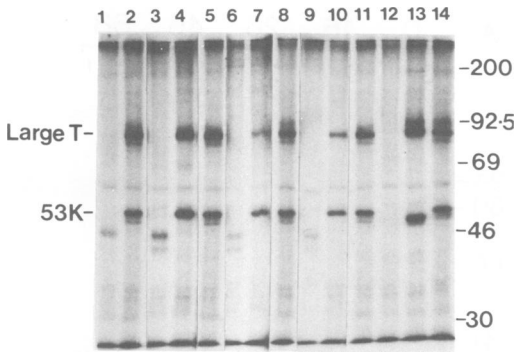


FIG. 7. *Immunoprecipitation of 53K from SV40-transformed cells. Confluent monolayers of productively infected CV-1, C2, C6, C11, and SVA31E7 cells were pulsed with [$^{32}$P]phosphate. Lysates from these cells were immunoprecipitated with either control W6/32 monoclonal antibody or anti-53K 122 monoclonal antibody. Immunoprecipitates were collected on SAC, and polypeptides were separated by SDS-polyacrylamide gel electrophoresis. Gels were dried and autoradiographed on Kodak SB-5 film. 1, SV40-infected CV-1 cells with W6/32; 2, 5, 8, 11, and 14, SV40-infected CV-1 cells with 122; 3, C2 cells with W6/32; 4, C2 cells with 122; 6, C6 cells with W6/32; 7, C6 cells with 122; 9, C11 cells with W6/32; 10, C11 cells with 122; 12, SVA31E7 cells with W6/32; 13, SVA31E7 cells with 122.*

productively infected monkey cells. As mentioned earlier, the mouse 53K species migrates slightly faster than the monkey 53K species in these gel systems.

**Instability of the large-T-53K protein complex from infected or transformed monkey cells in detergent lysates.** During the course of this work, we noted that the level of the monkey 53K protein that was bound to large-T, as measured by immunoprecipitation with anti-D2 serum, varied from experiment to experiment. These data led us to postulate that the large-T-53K protein complex might be unstable to prolonged incubation in detergent lysates. The decay of the large-T-53K protein complex in the presence of precipitating antibodies is shown in Fig. 8. In other experiments we have seen similar patterns of decay, and the decay of the complex is common to all SV40-infected or -transformed primate cells that we have tested. In several experiments the decay of the complex was particularly enhanced if the incubations were done in the presence of antiserum with activities for several antigenic determinants on large-T. One possible explanation for these data is that binding of an antibody to one particular determinant on large-T changes the equilibrium constant for the association or dissociation of the large-T-53K protein complex.

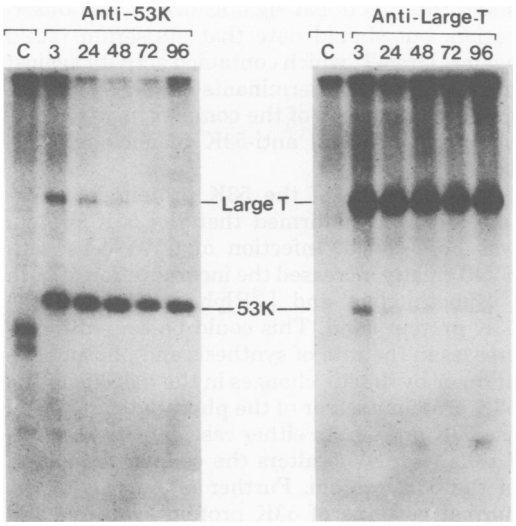


FIG. 8. *Decay of the large-T-53K protein complex in detergent lysates. A semiconfluent monolayer of C11 cells was pulsed with [$^{32}$P]phosphate for 3 h. The cells were lysed in 1% Nonidet P-40, and antibody was added to aliquots. Immunoprecipitation was allowed to continue for 3, 24, 48, 72, or 96 h. Incubation was terminated by collecting the immunoprecipitates on SAC, and the immunoprecipitates were washed and separated by SDS-polyacrylamide gel electrophoresis. Gels were dried and autoradiographed on Kodak SB-5 film. Anti-53K was clone 122 ascitic fluid; anti-large-T was anti-D2 serum. Controls were harvested at 3 h.*

This might be accomplished by either stabilizing the free form of large-T or by binding of an antibody to a site on large-T involved in its association with the 53K protein. These two possibilities are under investigation.

## DISCUSSION

Changes in host macromolecular synthesis are common consequences of many viral infections of eucaryotic cells. For example, infection of many mammalian cells with SV40 results in the stimulation of host DNA synthesis. Presumably, this serves to amplify the machinery for DNA synthesis needed for replication of the SV40 genome. Modification of host macromolecular synthesis during infection with relatively simple viruses suggests an intimate and specific relation between viral and host regulatory elements. Lane and Crawford (13) detected the presence of a complex between one of the SV40 nonstructural proteins, large-T, and a 53K protein in SV40-transformed mouse cells. Careful demonstration by Linzer and Levine (14) that this 53K protein was host coded raised the possibility that the large-T-53K complex was a specific example of virus interaction with host cell biochemistry. These speculations have been strengthened by two important observations. DeLeo et al. (5) showed elevated levels of a 53K protein (p53) in all transformed mouse cells that they examined, and Linzer et al. (15) reported the induction of 53K protein synthesis during SV40 infection of mouse cells. If the large-T-53K protein complex is an example of virus-host interaction, the structure of viral large-T must have evolved concomitantly with the evolution of the host 53K protein. Several workers failed to demonstrate an analogous complex during infections of monkey cells, the natural host for SV40 (12, 16). Although Melero et al. (17) reported the presence of a 55K protein in immunoprecipitates from productive infections of monkey cells, these results are difficult to interpret because the sera that were used to immunoprecipitate large-T and the 55K protein had activity against both polypeptides in their own right (17). The apparent absence of the large-T-53K protein complex during infections of monkey cells suggested that the presence of the complex in infections and transformation of nonpermissive mouse cells was fortuitous. Because the binding of the mouse 53K protein to large-T was stable to harsh conditions such as high salt (4 M NaCl), partial reduction (100 mM dithiothreitol), and incubations in chelating agents (5 mM EDTA) and nonionic detergents (1% Nonidet P-40) (16), it seemed unlikely that the formation of the complex in transformed mouse cells was fortuitous. The strong affinity of large-T for the mouse 53K protein suggested that there was a strong selective (and possibly essential) advantage for a large-T molecule that could bind to a monkey 53K protein equivalent. While this manuscript was in preparation, two indications that there was a 53K protein equivalent in monkey cells were published. Gurney et al. (8) isolated a hybridoma that produced anti-mouse 53K monoclonal antibodies which immunoprecipitated a monkey 53 K protein. Also, Simmons et al. (18) have described the tryptic peptide analyses of a 53K protein from SV40-transformed monkey cells. We have used the monoclonal antibody of Gurney et al. (8) together with a potent anti-T serum to examine productively infected African green monkey cells for the presence of the large-T-53K protein complex. Using the stringent criteria outlined previously by McCormick and Harlow (16), we have shown that a complex between SV40 large-T and a host 53K protein was formed during these infections. Both large-T and the 53K phosphoproteins were precipitated from lysates of infected CV-1 cells by antibodies specific for only

one component of the complex, and both proteins sedimented together during sucrose gradient centrifugation.

We have used similar criteria to demonstrate the presence of the large-T–53K protein complex in SV40-transformed monkey cells. Both the 53K protein and large-T can be precipitated from lysates of all three monkey transformed cell lines that we have tested with monoclonal antibodies active against either component of the complex. As with other examples of the complex, the two proteins cosediment on sucrose gradients (E. Harlow, unpublished data). These experiments also allowed us to determine that there was no appreciable difference between the levels of phosphorylation of the 53K proteins in transformed and productively infected CV-1 cells.

Although no function has been ascribed to the 53K protein or the large-T–53K protein complex, the monkey 53K protein described here is analogous to the mouse 53K protein. Both are host phosphoproteins that bind to large-T, and their synthesis is increased after infection with SV40 (15; this manuscript). The mouse and monkey 53K proteins share at least one antigenic determinant as demonstrated by immunoprecipitation with monoclonal antibodies raised against the mouse 53K protein (8; this manuscript). Also, the large-T–53K protein complex is located in the nucleus of transformed mouse cells (16) and infected monkey cells (E. Harlow, unpublished data).

Several possible explanations for the difficulty initially experienced in detecting the large-T–53K protein complex during productive infection emerged from these experiments. Because the amount of free large-T was always in great excess compared with the large-T bound to the 53K protein, it would be simple to show a prominent large-T band but little or no 53K protein. In these experiments the ratio of free to bound large-T was at least 10:1, and in one experiment it was as high as 1,000:1. We assume that these differences reflect the number of SV40 genomes present in the infected cells that can serve as coding sequences for large-T. Because the monkey 53K protein and rabbit IgG heavy-chain monomer migrate on polyacrylamide gels with almost identical mobilities, as little as 1 μl of an IgG-rich antiserum was sufficient to broaden the monkey 53K protein band and mask its presence. A third problem in identifying the large-T–53K protein complex during productive infections is the relative instability of the complex. The instability of the large-T–53K protein complex in detergent lysates appears to be a common feature of all SV40-infected or -transformed primate cells (unpublished data). We do not understand the functional significance of this observation, but should note that antiserum raised against large-T, which contained activity against many antigenic determinants on large-T, seems to speed the decay of the complex in vitro compared with several anti-53K monoclonal antibodies.

The presence of the 53K protein in mock-infected cells confirmed that the 53K protein was host coded. Infection of CV-1 cells with SV40 greatly increased the incorporation of both [$^{35}$S]methionine and [$^{32}$P]phosphate into the 53K protein band. This could be caused by increases in the rate of synthesis and phosphorylation or by drastic changes in the half-life of the 53K protein itself or of the phosphate groups on the 53K protein. In either case, infection of CV-1 cells with SV40 alters the cellular regulation of the 53K protein. Further alterations of the normal patterns of 53K protein synthesis and phosphorylation were revealed by comparing the relative changes in the incorporation of [$^{35}$S]methionine and [$^{32}$P]phosphate. The incorporation of [$^{35}$S]methionine rose by 5-fold compared with mock-infected cultures, whereas the level of phosphorylation rose by 30-fold. This disparity could be caused by several phenomena, but a discussion of the possible mechanisms will be more meaningful when careful measurements of the changes in half-life and amount of the 53K protein during infection are completed. Nevertheless, it is likely that the modification of the synthesis and phosphorylation of the 53K protein is essential for some stage of the SV40 life cycle. How the virus mediates the modification and whether this modification is associated with changes in cellular function must await further work.

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## LITERATURE CITED

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