Presence in Growth-Arrested Cells of Cellular Proteins That Interact with Simian Virus 40 Small-t Antigen

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Two cellular proteins, 56K and 32K, found in association with simian virus 40 small-t antigen were not induced by viral infection. In addition, the proteins were expressed by cells in the growth arrest period, a time in which small-t function is of importance in infection and transformation.

The simian virus small-t antigen appears to be required for the efficient transformation of growth-arrested cells. A series of viable mutants with deletions in the 0.54 to 0.59 region of the genome affects only the viral small-t antigen, and these mutants are less efficient than wild-type (WT) virus in the transformation of non-growing cells (1, 2, 11). The use of actively growing cultures overcomes, in large part, the requirement for small-t antigen (6).

The simian virus 40 small-t antigen and those of other papovaviruses (9) can interact with two cellular proteins, 56K and 32K, such that the cellular proteins are immunoprecipitated indirectly using antitumor sera that recognize the small-t antigen (12). The two cellular proteins are non-phosphorylated cytoplasmic proteins that are found in cells of a variety of species. Although of a similar size, the 56K protein is distinct from those phosphoproteins of the 53K family that are induced by simian virus 40 infection and are found in a complex with the large-T antigen (3-5, 7).

For the interaction of small-t antigen and the cellular proteins to be of significance in transformation, the cellular proteins should be present in growth-arrested cells. We report here that the 56K and 32K proteins are synthesized by growth-arrested cells and that the synthesis of these proteins is not induced significantly by viral infection.

The 56K and 32K proteins were detected previously in uninfected cells by mixed-extract experiments (12) in which labeled extracts of uninfected cells were mixed with unlabeled extracts of infected cells before immunoprecipitation. Because of the inefficiency of small-t immunoprecipitation by most antisera and the relatively unstable nature of the small-t antigen interaction with cellular proteins, these experiments were difficult to quantitate. Recently, antisera from tumor-bearing animals have been identified that directly immunoprecipitate the 56K and 32K proteins in addition to the viral

small-t and large-T antigens (10). These antisera were used to compare the levels of the cellular proteins in infected cells and in growing and growth-arrested uninfected cells.

Infected or uninfected monkey kidney (CV-1) cells were labeled with [³⁵S]methionine in methionine-free medium for 3 h at 40 h postinfection. The cells were then extracted with buffers that contained Nonidet P-40 and immunoprecipitated under standard conditions (12). In the experiments shown in Fig. 1, extracts were adjusted for trichloroacetic acid-precipitable radioactivity, and portions of each extract were immunoprecipitated with antiserum 125, an antitumor serum that recognizes small-t antigen but not the cellular proteins, or with antiserum 133, one of the sera that recognizes the cellular proteins. When antiserum 125 was used, the 56K and 32K proteins were detected only in extracts of WT-infected cells (Fig. 1E). Immunoprecipitates of cells infected with dl-888, a mutant that lacks small-t antigen, did not contain the 56K and 32K proteins. In contrast, after immunoprecipitation with antiserum 133, the 56K and 32K proteins were observed in immunoprecipitates of uninfected (Fig. 1B), dl-888-infected (Fig. 1D), and WT-infected (Fig. 1F) cells. In these experiments, the efficiency of immunoprecipitation of the cellular proteins was monitored by reimmunoprecipitation of supernatant proteins with antiserum 133, and the efficiencies were found to be similar for all of the extracts.

Approximately the same quantities of the 56K and 32K proteins were observed in infected and uninfected cells. Excision of the proteins from the gels for direct counting showed that there was less than a twofold difference in radioactivity between the proteins from uninfected and infected cells. Thus, there is little apparent induction of the synthesis of these proteins by virus infection, in contrast to the large-T-associated 53K protein (5).

The 56K and 32K proteins were also synthesized by growth-arrested cells (Fig. 1G-L).

FIG. 1. Synthesis of 56K and 32K proteins by growth-arrested cells. Confluent 10-cm dishes of CV-1 cells were infected with mock, dl-888, or WT 830 lysates and placed in medium containing 5% serum (A to F) or serum-free medium containing 2 mM theophylline (G to L) for 40 h. The cells were then pulsed for 3 h with [35 S]methionine in methionine-free medium. Theophylline was added to the pulse medium of theophylline-treated cultures. The cells were then extracted with buffers that contained Nonidet P-40, and the extracts were adjusted to the same levels of trichloroacetic acid-precipitable radioactivity for immunoprecipitation. Antitumor sera used were 125, a serum that recognizes viral large-T and small-t antigens, and 133, a serum that directly immunoprecipitates the 56K and 32K proteins. Patterns shown are immunoprecipitated proteins from: (A,G) uninfected cells, serum 125; (B,H) uninfected cells, serum 133; (C,I) dl-888-infected cells, serum 125; (D,J) dl-888-infected cells, serum 133; (E,K) WT-infected cells, serum 125; (F,L) WT-infected cells, serum 133. VP1, Viral late protein.

CV-1 cells were incubated with 2 mM theophylline, an inhibitor of cyclic AMP phosphodiesterase, immediately after infection and labeled at 40 h postinfection with [35 S]methionine in the presence of theophylline. Under these conditions, the incorporation of [3 H]thymidine into uninfected cells decreased by 95%. In addition, dl-888-infected cells were growth arrested, whereas WT-infected cells were unaffected, as previously reported (8). In the present experiments, the incorporation of [3 H]thymidine into viral and cellular DNA in dl-888-infected cells was decreased 80% by theophylline. The decrease in viral as well as cellular DNA synthesis is reflected by the reduced synthesis of the viral late protein VP1.

Cellular 56K and 32K proteins were synthesized by CV-1 cells that had been growth arrested by theophylline addition. Extracts from theophylline-treated uninfected and infected cells were adjusted for levels of trichloroacetic acid-

precipitable radioactivity comparable to those in the untreated extracts and immunoprecipitated with antiserum 125 or antiserum 133. The quantities of the 56K and 32K proteins immunoprecipitated from theophylline-arrested cells with antiserum 133 were comparable to those found in untreated cultures. Similar results were obtained by using mouse 3T3 cells that were growth arrested by serum deprivation (data not shown).

Finally, the availability of the 56K and 32K proteins in nongrowing uninfected cells is supported by the stability of the two proteins (Fig. 2). Confluent CV-1 cells were labeled with [35 S]methionine for 3 h, and one plate was incubated in complete medium for 24 h in the presence of 2 mM theophylline. A significant amount of the 56K and 32K proteins persisted during the chase period. By excision of the bands from the gel, it was estimated that approximately 30% of the radioactivity found in the 56K and 32K proteins remained after the 24-h chase.

Taken together, the demonstration of the synthesis of the 56K and 32K proteins by strongly growth-arrested cells and the stability of the two proteins in nongrowing cells suggest that the cellular proteins are available to interact with

FIG. 2. Stability of the 56K and 32K proteins. Confluent dishes of CV-1 cells were labeled with [35 S]methionine in methionine-free medium for 3 h, and one plate was extracted. The other plate was washed with complete medium and incubated in complete medium containing 2 mM theophylline for 24 h before extraction. Patterns shown are immunoprecipitated proteins from: (A) pulse-labeled cells, serum 125; (B) pulse-labeled cells, serum 133; (C) pulse-chase-labeled cells, serum 133; (D) pulse-chase-labeled cells, serum 125.

small-t antigen once synthesized after infection. Although the functions of small-t antigen and the cellular proteins remain unknown, the complex between these proteins could exist in cells in the growth arrest period, a time in which small-t function is of primary importance.

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