Association of Cellular 56,000- and 32,000-Molecular-Weight Proteins with BK Virus and Polyoma Virus t-Antigens

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Two cellular proteins (molecular weights: 56,000 and 32,000) were specifically co-immunoprecipitated by simian virus 40 and BK virus t-antigens and by polyoma virus non-T early proteins.

In a previous study (20), we demonstrated that two cellular proteins with apparent molecular weights of 56,000 (56K) and 32K were specifically immunoprecipitated in association with the simian virus 40 (SV40) t-antigen. The 56K and 32K proteins were found in infected and in uninfected cells. To demonstrate the presence of the proteins in uninfected cells, extracts of radiolabeled uninfected cells were mixed with unlabeled extracts of SV40-infected cells before immunoprecipitation. The specificity of the interaction was shown by the use of SV40 deletion mutants that do not synthesize intact small t-antigen and by the use of specific antisera (10). The 56K and 32K proteins were not present in immunoprecipitates of cells infected with t-antigen deletion mutants or when antisera that do not recognize the viral t-antigen were used for immunoprecipitation.

Other transforming papovaviruses code for the synthesis of t-antigens that resemble the SV40 t-antigen both in size and in sequences of cysteine-rich regions of the proteins (2, 3, 17). Consequently, it was of interest to determine whether t-antigens of the human papovavirus BK virus (BKV) and the mouse virus polyoma could promote the co-immunoprecipitation of cellular proteins from infected cells.

Immunoprecipitation of proteins from BKV-infected cells was studied by using human embryonic kidney (HEK) cells that had been infected with wild-type BKV for 72 h at 37°C (8, 19). The infected cells were pulsed with [³⁵S]-methionine for 24 h, then extracted with 0.5% Nonidet P-40 in Tris-buffered saline, pH 8. Extraction buffers included 2 to 5 mM dithiothreitol. Immunoprecipitation was done according to standard procedures (20). Proteins were immunoprecipitated by using antisera from hamsters bearing tumors of the SV40-transformed cell line, THE-2 (10). The SV40 and BKV large T- and small t-antigens cross-react immunologically (11, 15), and the antitumor sera raised

against THE-2 cells interacted with these proteins at high efficiency. Antisera raised in animals bearing BKV-induced tumors were not used because all sera tested in these laboratories lacked detectable antibody to viral small t-antigens and recognized only large T-antigens of SV40 and BKV.

Immunoprecipitates of BKV-infected HEK cells contained the viral proteins, T and t (21K), but also contained two proteins with apparent molecular weights of 56K and 32K (Fig. 1B). Electrophoretically similar 56K and 32K proteins were also present in immunoprecipitates of SV40-infected monkey kidney (CV-1) cells (Fig. 1C), but not in immunoprecipitates of cells infected with SV40 deletion mutants that lacked t-antigen (Fig. 1D). To determine whether the 56K and 32K proteins observed in immunoprecipitates of BKV-infected cells were cellular in origin, mixed extract experiments were done. Uninfected CV-1 cells were labeled with methionine and then mixed with unlabeled extracts of cells infected with SV40 or BKV. After incubation at 4°C for 10 min, antisera were added. As shown in Fig. 1, two proteins with apparent molecular weights of 56K and 32K were observed in the immunoprecipitates of the mixtures. The dependence of the precipitation of the cellular proteins on BKV t-antigen is shown in Fig 1E. When antiserum that did not recognize t-antigen was used, the two cellular proteins were not observed. This antiserum did recognize the BKV and SV40 large T-antigens, additional evidence that the large T-antigens were not involved in the co-immunoprecipitation of the cellular 56K and 32K proteins.

Similar results have been obtained with labeled HEK cells and a variety of cells from other species as the source of cellular proteins. We do not know if the 56K and 32K proteins observed are identical in all species, but we have never observed species differences in electrophoretic migration. Of interest, however, is that the pro-

FIG. 1. Co-immunoprecipitation of cellular proteins with BKV *t*-antigen. Direct immunoprecipitation of proteins from infected cells was done with BKV-infected HEK cells or SV40-infected CV-1 cells. Both wild-type SV40 and the *t*-antigen deletion mutant, d1-888, were used in this experiment. BKV-infected HEK cells were labeled with [35 S]methionine in complete medium from 72 to 96 h postinfection. SV40-infected CV-1 cells were pulsed with methionine for 3 h in methionine-free medium at 40 h postinfection. The patterns shown are autoradiograms of immunoprecipitable proteins from (A) BKV-infected HEK cells, nonimmune serum; (B, H) BKV-infected HEK cells, antitumor serum; (C) wild-type SV40-infected CV-1 cells, antitumor serum; and (D) d1-888-infected CV-1 cells, antitumor serum. To examine co-immunoprecipitation of cellular proteins, uninfected CV-1 cells were labeled with methionine and extracted with Nonidet P-40. The extract was mixed before immunoprecipitation with extracts of BKV-infected HEK cells or extracts of SV40-infected CV-1 cells. Immunoprecipitation was done either with antitumor serum that recognized both *T*- and *t*-antigens (anti-*T,t*) or with antitumor serum that recognized only the large *T*-antigen (anti-*T*). The patterns shown are cellular proteins found in immunoprecipitates after mixing with extracts of (E) unlabeled BKV-infected HEK cells, anti-*T* serum; (F) unlabeled BKV-infected HEK cells, anti-*T,t* serum; and (G) SV40-infected CV-1 cells, anti-*T,t* serum.

teins are cellular in origin and that they are observed in immunoprecipitates only in the presence of viral *t*-antigen.

In contrast to BKV and SV40, polyoma virus codes for the synthesis of three early gene products, the 21K *t*-antigen, the 56K middle *T*-anti-

gen, and the 100K large *T*-antigen. These proteins are antigenically distinct from those synthesized by SV40 and BKV. Thus, the interaction of polyoma early proteins with the cellular 56K and 32K proteins was of particular interest. To look for such an interaction, the polyoma-transformed cell line F111(KV) was used for both direct immunoprecipitation and mixing experiments. This cell line was particularly useful because the polyoma large *T*-antigen was not expressed (Fig. 2) as is the case in other known polyoma-transformed cell lines (7). As compared to nonimmune serum, the polyoma antitumor serum used in these experiments specifically immunoprecipitated three proteins (56K, 32K, and 21K [*t*-antigen]) from the F111(KV) cells. It is most likely that the 56K protein observed is the polyoma middle *T*-antigen, because this protein is expressed by all polyoma-transformed cells and appears to be essential for polyoma virus transformation (18). Evaluation of these immunoprecipitates for a cellular 56K protein was not possible because of the presence of the viral 56K protein. A protein in the 32K region of the gel was observed, but it was not known whether the protein was present because of an association with the polyoma early gene products. Other

FIG. 2. Immunoprecipitable proteins of F111(KV)-transformed cells. The polyoma-transformed rat cell line was labeled with [35 S]methionine for 3 h and then extracts were prepared by using buffers containing 0.5% Nonidet P-40 and 5 mM dithiothreitol. Extracts were immunoprecipitated with either nonimmune hamster serum (A) or antipolyoma serum (B).

laboratories have described proteins of this size class in immunoprecipitates of polyoma virus-infected cells and have shown that peptide maps of these proteins are unlike the maps of the viral proteins (5, 12, 14).

Unlabeled extracts of the transformed cell line supported the co-immunoprecipitation of the cellular 56K and 32K proteins from uninfected cells when polyoma antitumor serum was used (Fig. 3). In the experiment shown here, uninfected cells were labeled and used as a source of the cellular proteins, but similar results were obtained with uninfected mouse 3T3 cells (data not shown). In all experiments with polyoma proteins, it was found that the presence of dithiothreitol was critical for co-immunoprecipitation of the cellular proteins. This observation is of particular interest because of the conservation of the cysteine-rich region of all the viral t-antigens.

Because the transformed cell line did not express polyoma large T-antigen, the co-immunoprecipitation of the cellular proteins was most likely due to the polyoma small t- or middle T-antigens. Polyoma middle T-antigen shares nearly all the small t-antigen amino acid sequence (3, 17). Middle T-antigen, however, is membrane associated, whereas small t-antigen is a cytoplasmic protein (6). Our studies have shown that the cellular 56K and 32K proteins are soluble cytoplasmic proteins in both infected and uninfected cells (20) in a variety of extraction conditions. In addition, direct attempts to find the cellular proteins in membrane fractions of the cell have been made, and the 56K and 32K proteins have never been detected in membrane-associated form (unpublished observations). Consequently, it seems most likely that the polyoma small t-antigen promoted the co-immunoprecipitation of the cellular 56K and 32K proteins, although involvement of the middle T-antigen, particularly in Nonidet P-40 extracts, cannot be ruled out.

The functions of the viral small t-antigens or of the cellular 56K and 32K proteins are not known. SV40 t-antigen is necessary for efficient transformation of growth-restricted cells (1, 9, 16); polyoma hr-t mutants that lack both small t- and middle T-antigens are almost totally transformation defective (18). A role for these gene products in disorganization of actin cable structure has been defined by microinjection experiments, for both polyoma virus (13) and SV40 (4). An intriguing possibility is that the cellular 56K and 32K proteins play some role in actin cable structure and that the interaction of the cellular proteins with t-antigens may explain the loss of organized cable structure in infected cells. However, we have no evidence to support

FIG. 3. Co-immunoprecipitation of cellular proteins with polyoma proteins. Methionine-labeled extracts were prepared from uninfected CV-1 cells, and then a portion of the extract was mixed with unlabeled extracts of polyoma-transformed or SV40-infected cells before immunoprecipitation. The patterns shown are cellular proteins found in immunoprecipitates after mixing with extracts of (A) polyoma-transformed cells, nonimmune serum; (B) polyoma-transformed cells, antipolyoma serum; (C) wild-type SV40-infected CV-1 cells, anti-T,t serum; and (D) dl-888-infected CV-1 cells, anti-T,t serum.

this possibility, and the cellular proteins have not been detected in cytoskeletal preparations (unpublished observations). Nonetheless, the interaction of the cellular 56K and 32K proteins with early proteins of papovaviruses from different species supports the likelihood that the interaction is of consequence to the expression of the function of the papovavirus t-antigens.

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