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Homologous Cellular Proteins Associated with Simian Virus 40 Small T Antigen and Polyomavirus Medium T Antigen

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The simian virus 40 small T-associated 56,000-M_r (56K) and 32K cellular proteins were shown to be closely related to the polyomavirus medium T-associated 61K and 37K cellular proteins as demonstrated by two-dimensional polyacrylamide gel electrophoresis and V8 protease peptide mapping.

Two cellular proteins with apparent molecular weights of 56,000 (56K protein) and 32,000 (32K protein) have been reported to be immunoprecipitated in association with simian virus 40 (SV40) small T, but not large T, by antisera from animals bearing SV40-induced tumors (SV40 anti-T) (15). Furthermore, the small T antigen of BK virus was shown to be associated with the 56K and 32K proteins (13).

Several reports have shown that polyomavirus medium T associates with the product of the *src* proto-oncogene $pp60^{c\text{-}src}$ (3, 5–7), the c-*yes* protein (11), and three other cellular proteins of apparent molecular weights of 88,000 (88K protein), 61,000 (61K protein), and 37,000 (37K protein) (9, 10). Medium T encoded by the nontransforming host range (*hrt*) mutants (1) did not associate with the 88K, 61K, and 37K cellular proteins and $pp60^{c\text{-}src}$ or formed less stable complexes, whereas medium T of other nontransforming mutants did interact with these proteins (2, 8, 14). Complex formation may be a necessary but insufficient requirement for transformation.

In the present study, we demonstrate that the SV40 small T-associated 56K and 32K proteins are identical to the medium T-bound 61K and 37K proteins. This was indicated originally by the finding that these proteins comigrated when analyzed together on the same polyacrylamide gel (Fig. 1). Medium T and the associated proteins were purified from [^{35}S]methionine-labeled polyomavirus-infected mouse 3T6 cells by affinity chromatography with one antipeptide serum. As described previously (9), the antipeptide serum used is specific for the medium T antigen, as the peptide used as the antigen is not found in the small T or large T antigens. Additional purification was achieved by immunoprecipitation with serum from hamsters bearing polyomavirus-induced tumors (polyomavirus antitumor serum). SV40 small T and the associated proteins were isolated by immunoprecipitation with SV40 anti-T serum from a mixture of [^{35}S]methionine-labeled monkey cell (CV1) extract and unlabeled SV40-infected CV1 extract (lane b). In this mixture of extracts, complex formation occurs between the unlabeled small T from infected cells and the labeled proteins from uninfected cells (12). This approach was used rather than direct immunoprecipitation of infected monkey cells to ensure that only complex formation with cellular proteins was studied. As a control, the immunoprecipitation was carried out with a mixture of labeled CV1 cell extract and unlabeled mutant DL-888-infected CV1 cell extract, which lacked small T (lane c). Similarly, mixing of the SV40-infected CV1 cell extract with [^{35}S]methionine-labeled mouse cell (10T1/2) extract resulted in complex formation between SV40 small T and two mouse proteins with apparent molecular weights identical to those of the medium T-associated 61K and 37K proteins (not shown). The different molecular weights reported previously for the SV40 small T- and polyomavirus medium T-associated proteins reflect differences in the gel systems used. These proteins will be referred to as 61K and 37K proteins in the rest of this report.

Further evidence that the 61K and 37K proteins were related was obtained by using an SV40 anti-T serum which recognizes the cellular proteins directly, in addition to the viral antigens (12). This serum was able to precipitate the proteins associated with polyomavirus medium T (data not shown). In addition, the proteins were shown to comigrate following two-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Fig. 2). The 61K protein migrated as a single spot on these gels, and the protein isolated in association with either polyomavirus medium T (panel a) or SV40 small T (panel b) comigrated when analyzed together (panel c). In each sample, the 37K protein was separated into three species, and these three comigrated in the mixture.

The 61K proteins were compared further by partial peptide mapping with V8 protease (4) (Fig. 3). In this analysis, the mouse 61K protein isolated in association with polyomavirus medium T (lanes a and b) was compared with the SV40 small T-associated 61K protein isolated both from monkey cells (lanes c to e) and from mouse cells (10T1/2) (lanes f and g). The 61K protein from all three sources showed the same pattern of proteolytic fragments. In addition to suggesting the identity of the polyomavirus medium T- and SV40 small T-associated 61K proteins, this analysis indicates that the 61K protein from different species is highly conserved.

Similar analyses with the 37K proteins were not successful, as these proteins did not yield distinct proteolytic fragments with a number of proteases. Additional evidence that the 37K protein is the same as that associated with the SV40 small T antigen was obtained by labeling extracts with *S*-[^{3}H-methyl]adenosyl-methionine (Fig. 4). It has been shown previously (12) that the 37K protein is a major labeled product under these conditions and that the 37K protein is the only labeled protein found in immunoprecipitates of SV40-infected cells. Similarly, the 37K protein was the only labeled protein observed in immunoprecipitates from cell

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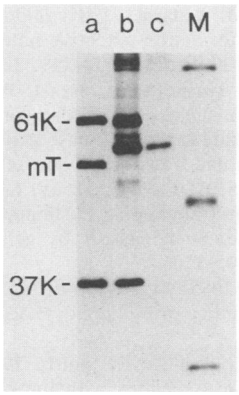

FIG. 1. Comparison of polyomavirus medium T- and SV40 small T-associated proteins by SDS-PAGE. Mouse 3T6 cells were infected with *dl*8, a transforming polyomavirus mutant with a deletion in the medium T gene. The cells were labeled with [^{35}S]methionine from 24 to 27 h after infection, and extracts were prepared in Nonidet P-40 lysis buffer described previously (9). Medium T and associated proteins were purified by affinity chromatography on antipeptide antibody columns (Glu-Glu–Sepharose) as described previously (9), followed by immunoprecipitation with serum raised in rats bearing polyomavirus-induced tumors (polyomavirus antitumor serum). Medium T and associated cellular proteins are shown in lane a. To prepare proteins associated with SV40 small T antigen, uninfected monkey cells (CV1) were labeled overnight with [^{35}S]methionine in medium that contained 1/18 the normal concentration of methionine. Extracts were prepared from these labeled uninfected cells in 0.5% Nonidet P-40 in Tris-buffered saline and then mixed with unlabeled extracts of monkey cells which had been infected with wild-type SV40 or a small T deletion mutant, *dl*-888, for 48 h. After incubation for 15 min at 37°C, extracts were immunoprecipitated with SV40 antitumor serum as described before (12). Cellular proteins which coprecipitated with small T antigen are shown in lane b; those which coprecipitated in the absence of small T are in lane c. The prominent protein present in lane c has not been further characterized. Lane M shows molecular size markers; from top to bottom: 69 kilodaltons (bovine serum albumin), 46 kilodaltons (ovalbumin), and 30 kilodaltons (carbonic anhydrase).

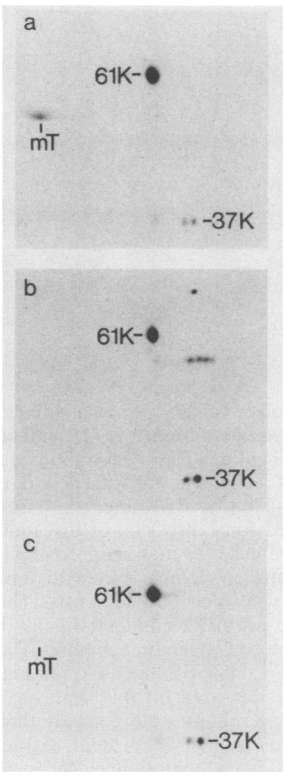

FIG. 2. Analysis of polyomavirus medium T- and SV40 small T-associated proteins by two-dimensional PAGE. Proteins were isolated as described in the legend to Fig. 1 and analyzed by two-dimensional PAGE as described before (14). The patterns shown are medium T-associated proteins (panel a) and proteins coimmunoprecipitated with extracts of wild-type SV40-infected cells (panel b). Lane c contains a mixture of the proteins shown in lanes a and b.

lines which express either the polyomavirus small T (lane e) or medium T (lane f) antigen.

Because the small T antigen of polyomavirus is almost entirely represented in the sequences of the medium T antigen, and because 61K and 37K cellular proteins were found with the SV40 small T antigen, it became of interest to determine whether the polyomavirus small T antigen could also interact with the same two cellular proteins. To examine this, mouse 10T1/2 cell lines which individually expressed the polyomavirus antigens were used for immunoprecipitation. As shown in Fig. 4, the 61K and 37K proteins were found associated with either the medium T (lane c) or the small T (lane d) antigen of polyomavirus.

In summary, two of the cellular proteins found in association with the polyomavirus medium T antigen are the same as those found in association with the SV40 small T antigen. In addition, the same two proteins associate with the polyomavirus small T antigen. Since complexes are not formed with the SV40 or polyomavirus large T antigens, it is likely that the unique sequence in SV40 small T antigen and sequences common to polyomavirus medium and small T antigens are involved in this interaction. This view is supported by the finding that the *hrt* mutations, located in the part of the polyomavirus genome encoding the region shared by these antigens, weaken the binding of the cellular pro-

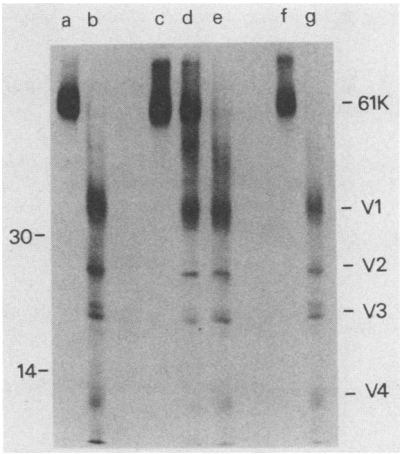

FIG. 3. Comparison of partial proteolytic maps of polyomavirus medium T- and SV40 small T-associated proteins. The 61K proteins isolated in association with either polyomavirus medium T or SV40 small T were excised from gels and digested with 10 ng (lane d) or 50 ng (lanes b, e, g) of V8 protease. Lanes a, c, and f were not digested with protease. The sources of the 61K proteins were polyomavirus-infected mouse cells, isolated by affinity chromatography (lanes a and b), and immunoprecipitates of wild-type SV40-infected monkey cells (lanes c, d, e) or uninfected mouse cells (lanes f and g).

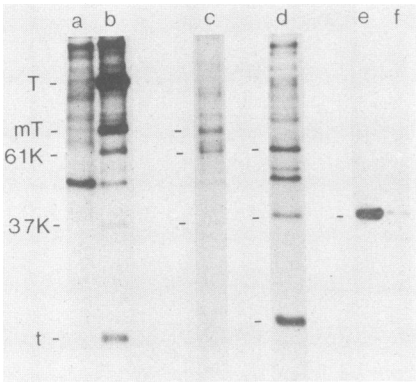

FIG. 4. Association of cellular proteins with either polyomavirus small T or medium T antigen. Mouse 10T1/2 cell lines which express either the small T or medium T antigen of polyomavirus were made by cotransfection of cells with pSV2neo and either pPyST1 or pPyMT1, kindly provided by R. Kamen. Individual G418-resistant clones were examined for expression of the appropriate viral protein. These lines were used for immunoprecipitation with polyomavirus antitumor serum, in comparison with uninfected cells and those infected with wild-type polyomavirus. The immunoprecipitated proteins shown are from uninfected cells (lane a), wild-type polyomavirus-infected cells (lane b), a medium T antigen-expressing cell line (lane c), and a small T antigen-expressing cell line (lane d). In the SDS-PAGE system used for this analysis (12), the polyomavirus medium T antigen migrates more slowly than the cellular 61K protein. To better visualize the 37K protein, extracts were incubated with *S*-[^{3}H-*methyl*]adenosyl-methionine before immunoprecipitation. As described previously (12), the 37K protein is the only labeled protein found in immunoprecipitates. Protein immunoprecipitated from the small T antigen-expressing cell line is shown in lane e and that from the medium T antigen-expressing cell line is in lane f. The reduced amounts of cellular proteins observed in the medium T-expressing cell line reflects the reduced amount of viral antigen present compared with the small T-expressing cell line.

teins, as do deletions in sequences unique to the SV40 small T antigen.

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