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Characterization of Tau Antigens Isolated from Uninfected and Simian Virus 40-Infected Monkey Cells and Papovavirus-Transformed Cells

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Tau antigens (also known as cellular or nonviral tumor antigens) were detected in uninfected and simian virus 40-infected monkey cells after immunoprecipitation with serum from hamsters bearing simian virus 40-induced tumors (anti-T serum). These two proteins (56,000 daltons) were digested to similarly sized peptides with various amounts of *Staphylococcus aureus* V8 protease. The Tau antigen isolated from infected monkey cells was closely related but was not identical to the corresponding protein from human cells transformed by simian virus 40, as determined by two-dimensional mapping of their methionine-labeled tryptic peptides. Hamster cells transformed by various primate papovaviruses (simian virus 40, BK virus, and JC virus) synthesized indistinguishable Tau antigens, as determined by two-dimensional peptide mapping. When tested by the same procedure, these proteins and the ones made in monkey and human cells were found to be related to the Tau antigens isolated from simian virus 40-transformed mouse and rat cells. Based on these results, an "evolutionary tree" was constructed to show the relationship among the methionine-containing tryptic peptides of all of these proteins.

Tau antigens are cellular proteins (50,000 to 56,000 daltons) that are made in cells transformed by simian virus 40 (SV40) (2, 6–10, 13, 17) and that can be immunoprecipitated from extracts of these cells by using serum from hamsters bearing SV40-induced tumors (anti-T serum). Complexes consisting of the virus-coded tumor antigen (T-Ag, 94,000 daltons) and Tau antigens have been isolated from SV40-transformed cells (9, 12). Crawford et al. (4) were the first to show that antibodies made in response to gel-purified T-Ag could precipitate T and Tau antigens from lysates of transformed cells but could not precipitate gel-purified Tau antigen, indicating that the two proteins are not immunologically related and that Tau antigen was, in this case, precipitated from the cell lysates by virtue of its association with T-Ag.

Tau antigens are proteins that appear to be species specific. The proteins isolated from various lines of SV40-transformed mouse cells, for example, are very closely related to one another, as determined by two-dimensional mapping of their methionine-labeled tryptic peptides (16, 17). Tau antigens isolated from transformed cells of different species are not identical. However, they contain a number of methionine-tryptic peptides in common (16) or they can be cleaved with proteases to a few peptides that are similar in size (14).

Until recently, Tau antigens have been identified only in lines of rapidly proliferating cells (transformed by SV40 [2, 8–10, 17] or by other agents [5, 10]) and in SV40-infected nonpermissive cells (10). Linzer et al. (11) have now detected low levels of Tau antigens in uninfected, untransformed mouse 3T3 cells, and Melero et al. (13, 14) have recently found Tau antigens in SV40-infected monkey cells. I report that a species of Tau antigen could also be isolated from uninfected, untransformed monkey kidney cells and that this protein was very similar and possibly identical to the Tau antigen isolated from monkey cells infected with SV40. Furthermore, I describe the relationship of these proteins to Tau antigens isolated from various lines of SV40-transformed cells. Finally, I show that different primate papovaviruses induced the synthesis of the same or very similar Tau antigens in transformed hamster cells.

MATERIALS AND METHODS

Cells. SV40-transformed human cells (SV80) were originally isolated by Todaro et al. (20). SV40-transformed hamster cells were obtained from K. K. Takemoto. BK and JC virus-transformed hamster cells were isolated by Takemoto and Martin (18) and Walker et al. (21), respectively.

Infection of monkey cells. African green monkey kidney cells (BSC-1 or primary cells; Flow Laboratories, Inc.) were infected with SV40 at a multiplicity of 100 PFU per cell in Eagle medium supplemented with 2% fetal bovine serum.

Cell labeling, immunoprecipitation, and gel electrophoresis. Cultured cells were labeled for 3 h with L-[^{35}S]methionine as previously described (2). The cells were lysed and the immunoprecipitation reactions were performed as described by Simmons et al. (16), with the exception that the cell lysates (from 10^7 to 2×10^7 cells) were diluted to 10 ml with extraction buffer (2) prior to centrifugation to reduce background proteins in the immunoprecipitates. Acrylamide slab gel electrophoresis of sodium dodecyl sulfate-denatured proteins was performed as previously described (16).

Chromatography and electrophoresis of peptides. The procedure described by Cleveland et al. (3) was used in some experiments to compare two different proteins. After immunoprecipitation and gel electrophoresis, the acrylamide gels were dried and exposed to X-ray film to locate the labeled proteins. The appropriate gel slices were rehydrated in 0.125 M Tris (pH 6.8)–0.1% sodium dodecyl sulfate–0.001 M EDTA and placed on top of a slab gel consisting of 5% acrylamide in the stacking gel (3 cm long) and 20% acrylamide in the resolving gel (9 cm long) (3). Each gel slice was overlaid with 25 μl of the above buffer containing 10% glycerol, 0.01% bromophenol blue, and various amounts (0, 2, 10, or 100 μg) of *Staphylococcus aureus* V8 protease (Miles Laboratories, Inc.). Electrophoresis was carried out at 25 mA per gel until the dye reached the bottom of the stacking gel. The power was turned off for 30 min, and electrophoresis was resumed at the original amperage until the dye reached the bottom of the gel. The wet gels were soaked in 10 volumes of 1 M sodium salicylate (pH 6 to 7) for 75 min (1), dried, and exposed to Kodak XR2 film for 4 weeks at −70°C (fluorography).

Two-dimensional mapping of methionine-labeled tryptic peptides was performed as described previously (16).

RESULTS

Tau antigens isolated from uninfected and SV40-infected monkey cells. In a preliminary experiment, different batches of hamster anti-T serum were tested for their ability to immunoprecipitate T and Tau antigens from extracts of labeled SV40-transformed mouse cells. At the same concentration of serum, a few of these sera precipitated larger amounts of Tau antigens than most serum samples; however, the amounts of T-Ag they precipitated were about the same (see also 4). These superior anti-T sera were used in immunoprecipitation reactions with extracts of labeled monkey cells (BSC-1) that were either uninfected or infected with SV40. Figure 1a shows that two labeled proteins (94,000 and 56,000 daltons) were precipitated from infected cells with anti-T serum but not with normal serum. Uninfected monkey cells, on the other hand, contained only an immunoprecipitable 56,000-dalton protein (Fig. 1b). The larger protein (94,000 daltons) is most likely the virus-encoded T-Ag. The smaller proteins have

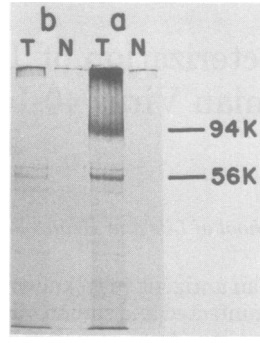

FIG. 1. *Acrylamide gel electrophoresis of proteins immunoprecipitated from uninfected (b) or SV40-infected (a) monkey cells with either normal (N) or anti-T (T) serum. Monkey kidney cells (BSC-1) were infected with SV40 at a multiplicity of 100 PFU per cell. At 22 h postinfection, these cells and other uninfected monkey cells were labeled with* L-[^{35}S]*methionine as described in the text. Extracts of these cells were incubated with either normal or anti-T hamster serum in immunoprecipitation reactions. Precipitated proteins were subjected to electrophoresis through 13% acrylamide gels (16) for 2.5 h at 25 mA, and the labeled proteins in the gel were detected by exposure to X-ray film. Molecular weights were estimated by the relative rates of migration of marker proteins with known molecular weights (2).*

the same approximate size (56,000 daltons) as Tau antigens isolated from SV40-transformed cells (16). For preparative purposes, most (70%) of the labeled extracts was incubated with anti-T serum in this experiment; the remainder was incubated with normal serum. Hence, the background bands (nonspecifically precipitated proteins) in Fig. 1 are more prominent in the tracks consisting of proteins precipitated with anti-T than with normal serum. Under the same conditions of labeling and immunoprecipitation, more radioactivity was detected in the 56,000-dalton protein from infected cells than in that from uninfected cells (Fig. 1). Small T-Ag was not resolved in these gels and migrated with the dye front.

Similar results were obtained when primary African green monkey kidney cells were substituted in this experiment for the BSC-1 cells (data not shown). This indicated that the 56,000-dalton immunoprecipitable protein was not artifactually produced in monkey cells that had been continuously passaged in culture. These experiments also show that the hamster anti-T sera that were used contained intrinsic anti-monkey 56,000-dalton protein activity.

The relationship between the immunoreactive 56,000-dalton proteins isolated from uninfected and SV40-infected monkey cells was determined by comparing the peptides of these proteins

produced by digestion with *Staphylococcus aureus* V8 protease (3). Figure 2 shows that the two proteins were cleaved to a number of similarly sized methionine-labeled peptides at various concentrations of protease. The small amounts of radioactivity in the protein from uninfected cells made it difficult to determine whether, at any one concentration of protease, the two digests contained identical peptide products. However, these peptide maps were quite similar and suggested that the 56,000-dalton proteins from uninfected and SV40-infected monkey cells were closely related.

Since the 56,000-dalton proteins from monkey cells could be immunoprecipitated with anti-T serum and since they had the same approximate molecular weight as the Tau antigens isolated from various lines of SV40-transformed cells, it was of interest to determine the relationship between these monkey proteins and Tau antigens. In a previous report from this laboratory (16), we showed that Tau antigens isolated from various lines of SV40-transformed cells were related to one another but were not identical and that a possible evolutionary relationship existed among Tau antigens of several cell species (mouse, rat, and human). It followed, then, that if the 56,000-dalton proteins from monkey cells were related to Tau antigens, the greatest homology between the two should be found between monkey and human cell proteins. I therefore compared the methionine-labeled tryptic peptides of the 56,000-dalton protein from SV40-infected monkey cells with those of Tau antigen isolated from SV40-transformed human cells (Fig. 3). These peptides were separated in two dimensions (electrophoresis and chromatography) on thin-layer cellulose plates. Figures 3a and b show the peptide fingerprints of the monkey and human proteins, respectively, and Fig. 3c shows the fingerprint of a 1:1 mixture of the peptides of these two proteins. These fingerprints indicate that these proteins contained approximately 16 similar methionine-labeled tryptic peptides. The monkey protein contained an additional five peptides, and the human protein contained an additional one (Fig. 3, arrows). Melero et al. (13) recently showed that comparable proteins from SV40-infected monkey cells and transformed human cells were indistinguishable, as determined by gel electrophoresis of peptides generated during limited digestions with *S. aureus* V8 protease. The data presented in this paper clearly show, however, that these proteins were distinguishable yet closely related. The results further show that uninfected and SV40-infected monkey cells synthesized Tau antigens and support a previous suggestion (16) that Tau antigens are evolutionarily conserved proteins.

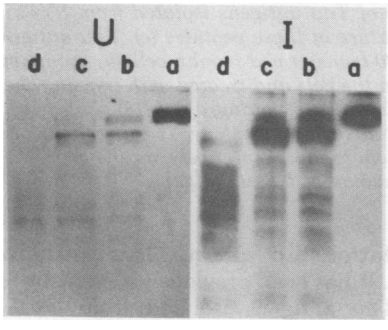

FIG. 2. *Electrophoresis of peptides generated by partial proteolysis of Tau antigens from uninfected or SV40-infected monkey cells: peptide maps of Tau antigen from infected (I) or uninfected (U) cells with 0 (a), 2(b), 10 (c), or 100 (d) μg of S. aureus V8 protease. The 56,000-dalton protein was isolated from ^{35}S-labeled uninfected or SV40-infected monkey cells by immunoprecipitation and gel electrophoresis. Protein samples were treated with various amounts of S. aureus V8 protease (0, 2, 10, or 100 μg) and subjected to electrophoresis through 20% acrylamide slab gels (3). The gels were fluorographed (1) and exposed to X-ray film at −70°C.*

Tau antigens of cells transformed by different papovaviruses. The evidence that is presently available suggests that Tau antigens are species-specific proteins (10, 14, 16, 17) and have the capacity to bind to T-Ag in transformed cells (9, 12). These studies were done primarily with cells infected with or transformed by SV40. Since the primate papovaviruses (SV40, BK virus, and JC virus) code for different (but related) T-Ag's (15), it might be suggested that these proteins interact with different cellular Tau antigens in transformed cells. Alternatively, the same form of Tau antigen could be synthesized in cells transformed by these various viruses, but only if the cells originated from the same animal species. To distinguish between these two possibilities, I compared the Tau antigens isolated from hamster cells transformed by SV40, BK virus, or JC virus. It should be pointed out that the lines of cells used were produced in different laboratories by transforming cells in culture with either virus (SV40) or DNA (BK virus) (18) or by culturing brain tumors that were induced with virus (JC virus) (21). Figure 4 shows that each of these transformants contained, in addition to T-Ag's (94,000 daltons), a species of Tau antigen (56,000 daltons) that could be immunoprecipitated with the homologous hamster anti-T serum. Since the T-Ag's of these papovaviruses are immunologically related (18, 19, 21), it was not surprising to find that the Tau antigens

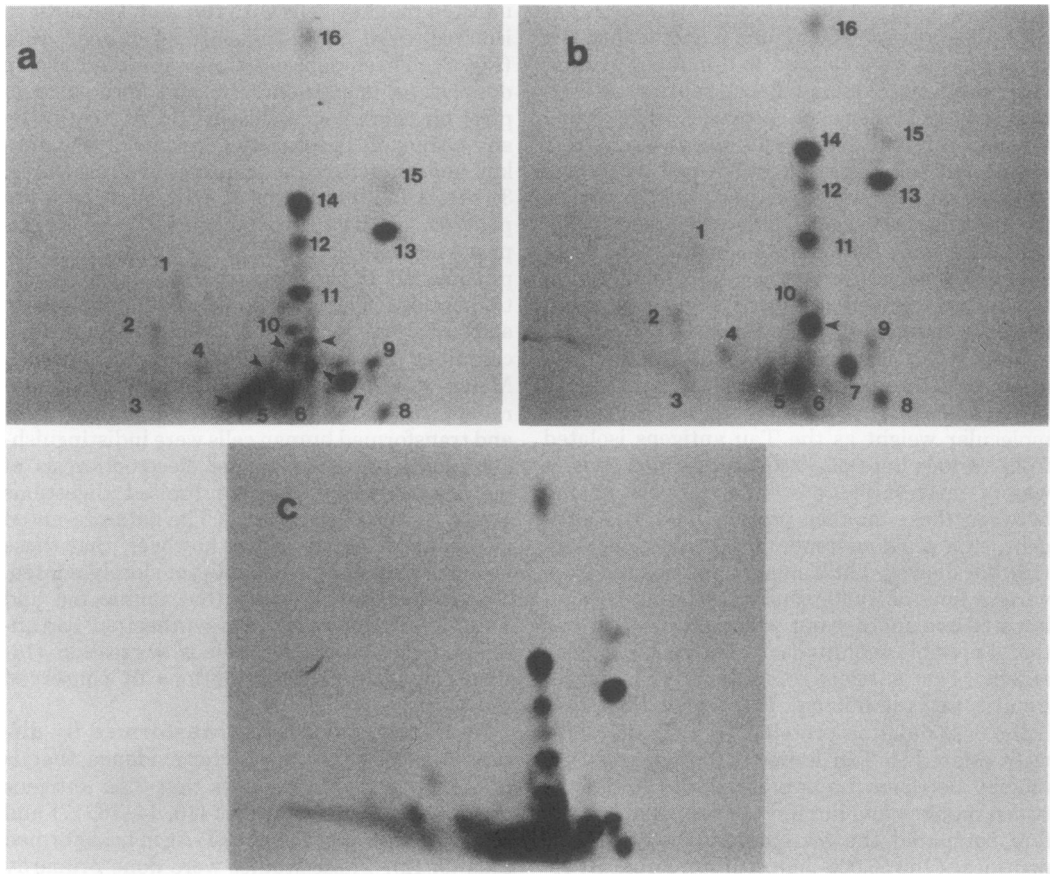

FIG. 3. *Fingerprints of methionine-labeled tryptic peptides of Tau antigens isolated from SV40-infected monkey (a), SV40-transformed human (b) cells, and a 1:1 mixture of these peptides (c). Tau antigens were isolated from ^{35}S-labeled SV40-infected monkey cells and SV40-transformed human cells by immunoprecipitation and gel electrophoresis. These proteins were eluted from the gels and treated with trypsin. The tryptic peptides were separated in two dimensions by electrophoresis and chromatography (16). Labeled peptides were detected on the thin-layer plates by fluorography using 2-methylnaphthalene. The directions of electrophoresis and chromatography were from left to right and bottom to top, respectively, as shown in the figure. The numbered peptides refer to those present in monkey and human cell Tau antigens.*

synthesized by each of these cell lines could be precipitated equally well with anti-SV40 T serum (data not shown).

To determine the relationship among these Tau antigen proteins, I compared their methionine-labeled tryptic peptides by two-dimensional peptide mapping (Fig. 5). In addition to the peptide maps shown in Fig. 5, a map of a mixture of peptides from Tau antigens of SV40- and JC virus-transformed cells was obtained (not shown). The methionine peptides of these three proteins were indistinguishable in two dimensions; hence, the same cellular protein is apparently made in hamster cells transformed by these various primate papovaviruses.

Relationship among Tau antigen peptides. It has been previously demonstrated that the Tau antigens isolated from SV40-transformed mouse, rat, and human cells contained a number of tryptic peptides in common (16). Since this study suggested that these proteins are evolutionarily related, it was appropriate to examine additional species of Tau antigen for possible similarities. The Tau antigen protein from monkey cells had already been compared to the human cell Tau antigen (Fig. 3), and its relationship to the mouse and rat proteins was determined by comparing the peptide fingerprints of these three proteins (16, and data not shown). Similarly, the peptide map of hamster

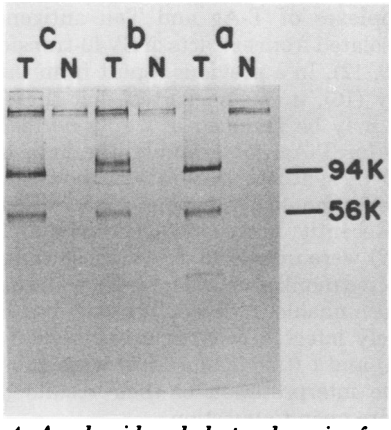

FIG. 4. *Acrylamide gel electrophoresis of proteins precipitated with normal (N) or anti-T (T) serum from hamster cells transformed by SV40 (a), BK virus (b), or JC virus (c). SV40-, BK virus-, or JC virus-transformed hamster cells were labeled with L-[^{35}S]methionine, and the labeled extracts of these cells were incubated with normal or anti-T serum. Precipitated proteins were subjected to acrylamide gel electrophoresis and detected by autoradiography.*

Tau antigen (Fig. 5) was compared with those of the other proteins. I was able to construct an "evolutionary tree" (Fig. 6) showing the relationship of the methionine-containing peptides of these various proteins. The number of peptides common to any two proteins is indicated by the sum of the numbers below the intersecting branches leading to these two proteins in Fig. 6. Thus, the human and rat Tau antigens share four + four = eight methionine-labeled peptides (16). The number of peptides unique to a particular protein is indicated on the single branch leading to that protein. All of these Tau antigen species appear to share four methionine peptides (peptides 4, 13, 14, and 16; Fig. 3). The rat, mouse, monkey, and human Tau antigens contain four additional shared peptides (peptides, 3, 5, 6, and 11; Fig. 3). The peptides of the hamster protein could be more closely related to the others than is indicated in Fig. 6 because I could not tell for certain that the protein lacked peptides 5 and 6. This protein is immunologically related to the monkey cell Tau antigen (and probably to all of the others) since hamster anti-

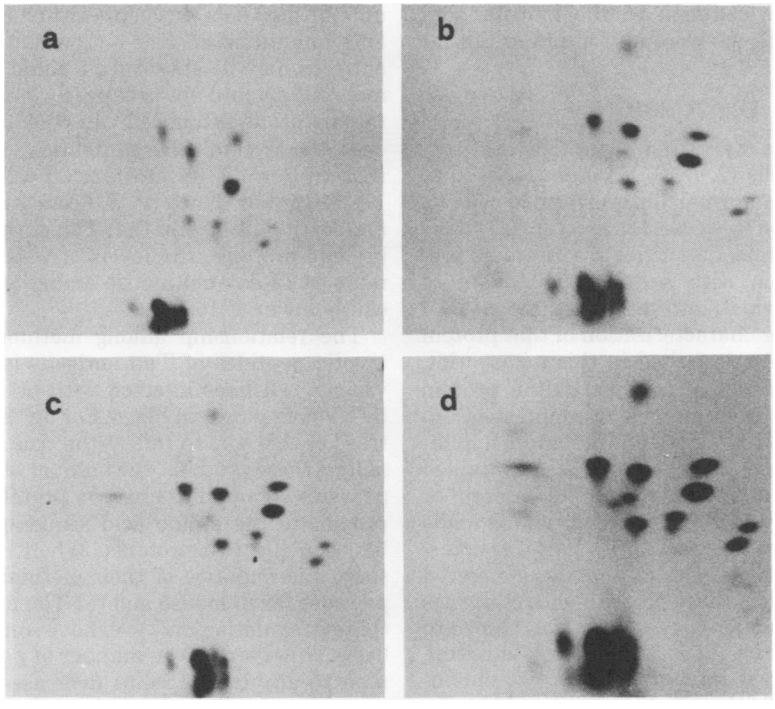

FIG. 5. *Fingerprints of methionine-labeled tryptic peptides of Tau antigens isolated from hamster cells transformed by SV40 (a), BK virus (b), JC virus (c), and a 1:1 mixture of BK and JC virus Tau antigen peptides (d). Tau antigens were isolated from ^{35}S-labeled hamster cells transformed by SV40, BK virus, or JC virus by immunoprecipitation and gel electrophoresis. The proteins were eluted from the gels, treated with trypsin, and subjected to two-dimensional fingerprinting. Labeled peptides were detected by fluorography.*

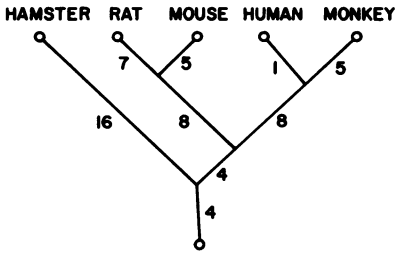

FIG. 6. *Relationship among the methionine-containing tryptic peptides of Tau antigens isolated from SV40-infected monkey cells and SV40-transformed human, mouse, rat, and hamster cells. For explanation, see text.*

T serum precipitated the monkey protein from uninfected cells (Fig. 1). The other eight numbered peptides in Fig. 3 (peptides 1, 2, 7, 8, 9, 10, 12, and 15) were found only in the human and monkey Tau antigens. It is interesting to note that the hamster Tau antigen appears to be the most dissimilar based on its methionine-containing tryptic peptides. In addition, all of the peptides shared by two distantly related proteins on the tree were also present in a third protein that is more closely related to one of the two (i.e., all of the peptides common to the hamster and mouse Tau antigens were also found in the rat protein).

DISCUSSION

Tau antigens were first identified in virus-transformed cells (2, 7–10, 17) and appeared to be missing from normal, untransformed cells (5, 10,17). Recently, however, Linzer et al. (11) have detected very small quantities of an immunoprecipitable protein with a molecular weight of 54,000 from normal, untransformed mouse 3T3 cells. No further characterization of that protein was reported. As described in this manuscript, detectable amounts of a 56,000-dalton protein were recovered from extracts of uninfected, untransformed monkey cells by immunoprecipitation with hamster anti-T serum that contained high levels of anti-Tau activity. This protein or one that is very closely related to it was made after infection of these cells with SV40, as determined by electrophoresis of peptides generated during digestion of these proteins with *S. aureus* V8 protease. The monkey protein was shown to be related to the Tau antigen isolated from SV40-transformed human cells by two-dimensional mapping of their methionine-labeled tryptic peptides. Compatible with the suggestion that these proteins are evolutionarily related, they were more closely related to each other than to the Tau antigens of other species (Fig. 6).

Complexes of T-Ag and Tau antigen have been isolated from extracts of SV40-transformed cells (9, 12). In a previous report from this laboratory (16), it was suggested that these complexes may be involved in a role normally reserved for T-Ag, for example, the induction of host DNA synthesis. If this were the case, these complexes should also be found in SV40-infected cells. Recently, however, McCormick and Harlow (12) were unable to detect these complexes in infected monkey cells. However, these authors were also unable to detect Tau antigens in productively infected cells, whereas Melero et al. (13, 14) and I (this manuscript) were able to do so. The interpretation of their results (12) is therefore open to question.

In spite of the fact that they can be detected in normal mouse (11) or monkey (this manuscript) cells, Tau antigens have not been detected in all cell lines that have been examined. For example, revertants of SV40-transformed mouse cells that do not express T-Ag have undetectable levels of Tau antigen (2; unpublished data). Furthermore, other lines of normal mouse cells (i.e., mouse fibroblasts; 7, 17) and one line of mouse embryonic carcinoma cells (402AX; unpublished data) also appear to have undetectable quantities of Tau antigens. Hence, these antigens may be absent from some lines of normal cells and are not necessarily associated with rapidly proliferating cells. In SV40-transformed cells, the level of these proteins is vastly greater than in normal, untransformed cells (5, 10, 11, 17). Recent evidence (P. T. Mora, personal communication) indicates that Tau antigens are embryonic proteins, the levels of which are maximum in 12-day-old mouse embryos and significantly lower at 16 days.

The relationship among methionine-labeled tryptic peptides of Tau antigens isolated from various cell lines infected with or transformed by SV40 is shown in Fig. 6. For an "evolutionary tree" of this sort to reflect the true relationship among these proteins, the number of methionine peptides common to any two proteins must approximate the amino acid sequence homology between the two proteins. When two proteins share the majority of their methionine-labeled peptides (as in mouse and rat Tau antigens; 16), this extrapolation may well have some statistical basis. However, as the number of peptides common to any two proteins decreases, the correspondence between peptide and sequence homologies becomes less significant. Therefore, the sequence relationship between the hamster Tau antigen and those of other species may be somewhat different than is indicated by the peptide data (Fig. 6).

Although the T-Ag's of SV40, BK virus, and JC virus are distinguishable (15), the Tau antigens produced in hamster cells transformed by these viruses are apparently the same. Furthermore, the Tau antigen from hamster cells transformed by polyoma virus is immunologically related since it could be precipitated with anti-SV40 T serum (unpublished data). Therefore, if complexes of T-Ag and Tau antigens exist in all of these cells, a similar Tau antigen must be able to recognize all of these different papovavirus T-Ag's. In transformed hamster cells, Tau antigens could recognize some common feature of papovavirus T-Ag's, perhaps similar sequences near the N-terminal ends (15) or a similar tertiary structure. Similarly, the SV40 T-Ag must also recognize a wide variety of Tau antigens in transformed cells of different species. Since these Tau antigens share common peptides, the conserved portions of these molecules may be the recognition sites for T-Ag.

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ADDENDUM IN PROOF

E. Harlow and L. Crawford (personal communications) have now recently detected Tau antigens and T-Ag-Tau antigen complexes in SV40-infected monkey cells by using monoclonal antibodies directed against Tau antigens.

LITERATURE CITED

1. **Chamberlain, J. P.** 1979. Fluorographic detection of radioactivity in polyacrylamide gels with the water-soluble fluor, sodium-salicylate. Anal. Biochem. **98**:132–135.
2. **Chang, C., D. T. Simmons, M. A. Martin, and P. T. Mora.** 1979. Identification and partial characterization of new antigens from simian virus 40-transformed mouse cells. J. Virol. **31**:463–471.
3. **Cleveland, D. W., S. G. Fischer, M. W. Kirschner, and V. K. Laemmli.** 1977. Peptide mapping by limited proteolysis in sodium dodecyl sulfate and analysis by gel electrophoresis. J. Biol. Chem. **252**:1102–1106.
4. **Crawford, L. V., D. C. Pim, and D. P. Lane.** 1980. An immunochemical investigation of SV40 T-antigens. 2. Quantitation of antigens and antibody activities. Virology **100**:314–325.
5. **DeLeo, A. B., G. Jay, E. Appella, G. C. Dubois, L. W. Law, and L. J. Old.** 1979. Detection of a transformation-related antigen in chemically induced sarcomas and other transformed cells of the mouse. Proc. Natl. Acad. Sci. U.S.A. **76**:2420–2424.
6. **Edwards, C. A. F., G. Khoury, and R. G. Martin.** 1979. Phosphorylation of T-antigen and control of T-antigen expression in cells transformed by wild type and *tsA* mutants of simian virus 40. J. Virol. **29**:753–762.
7. **Gaudray, P., M. Rassoulzadegan, and F. Cuzin.** 1978. Expression of simian virus 40 early genes in transformed rat cells is correlated with maintenance of the transformed phenotype. Proc. Natl. Acad. Sci. U.S.A. **75**: 4987–4991.
8. **Kress, M., E. May, R. Cassingena, and P. May.** 1979. Simian virus 40-transformed cells express new species of proteins precipitable by anti-simian virus 40 tumor serum. J. Virol. **31**:472–483.
9. **Lane, D. P., and L. V. Crawford.** 1979. T-antigen is bound to a host protein in SV40 transformed cells. Nature (London) **278**:261–263.
10. **Linzer, D. I. H., and A. J. Levine.** 1979. Characterization of a 54K dalton cellular SV40 tumor antigen present in SV40-transformed cells and uninfected embryonal carcinoma cells. Cell **17**:43–52.
11. **Linzer, D. I. H., W. Maltzman, and A. J. Levine.** 1979. The SV40 A gene product is required for the production of a 54,000 MW cellular tumor antigen. Virology **98**: 308–318.
12. **McCormick, F., and E. Harlow.** 1980. Association of a murine 53,000-dalton phosphoprotein with simian virus 40 larger-T antigen in transformed cells. J. Virol. **34**: 213–224.
13. **Melero, J. A., D. T. Stitt, W. F. Mangel, and R. B. Carroll.** 1979. Identification of new polypeptide species (48-55K) immunoprecipitable by antiserum to purified large T antigen and present in SV40-infected and -transformed cells. Virology **93**:466–480.
14. **Melero, J. A., S. Tur, and R. B. Carroll.** 1980. Host nuclear proteins expressed in simian virus 40-transformed and -infected cells. Proc. Natl. Acad. Sci. U.S.A. **77**:97–101.
15. **Simmons, D. T., and M. A. Martin.** 1978. Common methionine-tryptic peptides near the amino-terminal end of primate papovavirus tumor antigens. Proc. Natl. Acad. Sci. U.S.A. **75**:1131–1135.
16. **Simmons, D. T., M. A. Martin, P. T. Mora, and C. Chang.** 1980. Relationship among Tau antigens isolated from various lines of simian virus 40-transformed cells. J. Virol. **34**:650–657.
17. **Smith, A. E., R. Smith, and E. Paucha.** 1979. Characterization of different tumor antigens present in cells transformed by simian virus 40. Cell **18**:335–346.
18. **Takemoto, K. K., and M. A. Martin.** 1976. Transformation of hamster kidney cells by BK papovavirus DNA. J. Virol. **17**:247–253.
19. **Takemoto, K. K., and M. F. Mullarkey.** 1973. Human papovavirus, BK strain: biological studies including antigenic relationship to simian virus 40. J. Virol. **12**:625–631.
20. **Todaro, G. J., H. Green, and M. C. Swift.** 1966. Susceptibility of human diploid fibroblast strains to transformation by SV40 virus. Science **153**:1252–1254.
21. **Walker, D. L., B. L. Padgett, G. M. ZuRhein, A. E. Albert, and R. F. Marsh.** 1973. Human papovavirus (JC): induction of brain tumors in hamsters. Science **181**:674–676.