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Identification and Partial Characterization of New Antigens from Simian Virus 40-Transformed Mouse Cells

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Two new species of antigens were detected in simian virus 40-transformed mouse cells, in addition to the large (94,000 daltons) and small (20,000 daltons) tumor antigens. These antigens were immunoprecipitated from cell extracts by using anti-T serum and not normal, nonimmune serum. One of these was a protein with a molecular weight of approximately 130,000 and was present in some but not all SV40-transformed mouse cells. The other, which we have named Tau antigen, has a molecular weight of 56,000 as estimated by electrophoresis through acrylamide gels and was found in all virus-transformed cells examined. The 130,000-dalton antigen contained about 15 methionine-tryptic peptides which were also present in the large SV40 tumor antigen as determined by ion-exchange chromatography. This strongly suggested that the protein was virus coded. The 56,000-dalton Tau antigen appeared to share only two methionine-tryptic peptides with the large species of SV40 tumor antigen, as determined by ion-exchange and paper chromatographies. Our results are compatible with a cellular origin for Tau antigen. However, our data do not exclude the possibility that this protein contains sequences specified by the virus DNA.

Simian virus 40 (SV40) and polyoma virus are small double-stranded DNA-containing viruses which can reproduce lytically in permissive cells or induce transformation of nonpermissive cells. The SV40 and polyoma virus genomes are divided into early and late regions. Only the early region is expressed in lytically infected cells before the onset of virus DNA replication and in virus-transformed cells.

SV40-infected and transformed cells synthesize virus-specific antigens which can be immunoprecipitated using serum obtained from animals bearing virus-induced tumors (anti-T serum) (1, 5, 34). These tumor antigens (T Ag's) are proteins with molecular weights of 80,000 (80K) (large T Ag) (9) and 20K (small T Ag) (23), although the molecular weight of the large T Ag species as determined by acrylamide gel electrophoresis is somewhat higher (86K to 100K) (1, 5, 34). In addition to the large and small T Ag forms, polyoma virus specifies a third antigen (middle T Ag) with a molecular weight of 55K to 63K (10, 26, 29). This protein may be associated with the plasma membrane of polyoma virus-infected mouse cells (11).

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The large T Ag has been implicated in the replication of viral DNA in productively infected cells (31) and in the establishment and maintenance of the transformed state (4, 17, 20, 32). In addition, T Ag induces transplantation immunity in mice that are challenged with SV40-induced tumors (2, 6).

In this communication, we report that at least two new species of SV40 transformation-specific antigens can be identified in transformed mouse cells by immunoprecipitation with anti-T serum. One of these, called Tau antigen, was found in all SV40-transformed cells examined and had a molecular weight of 56K. A second antigen (130K daltons) was detected in only a portion of the transformed cell lines. Analysis of their methionine-labeled tryptic peptides indicated that the 130K-dalton antigen contained approximately 15 peptides which were also present in the large T Ag species. The Tau antigen, on the other hand, contained only two peptides that appeared to be constituents of large T Ag as well.

MATERIALS AND METHODS

Cells. A cloned AL/N cell line (designated 210) was transformed by SV40 (strain 776). Two independent transformants (lines 214 and 215) were selected by cloning single cells as described by Winterbourne and Mora (35), and were T Ag positive as determined by

indirect fluorescent antibody staining (22). In addition, one cloned T Ag-negative sister cell line (213) was maintained in culture as a control. Approximately 10^6 to 10^7 SV40-transformed cells (line 215) were injected into syngeneic AL/N mice to obtain tumor lines which could be T Ag negative. Four tumors were selected without bias from separate mice, and cell lines were established as previously described (19). Tumor lines 221, 222, and 224 were T Ag positive as determined by fluorescent antibody staining, whereas line 223 was T Ag negative.

A similar procedure was used to obtain SV40-transformed BALB/c mouse cells from a spontaneously transformed cell line (3T12-4-T, designated 301). After transformation with SV40, we obtained a single T Ag-positive cloned cell line (303) as described by Winterbourne and Mora (35). These cells were injected into syngeneic BALB/c mice, and two tumor cell lines (314, 315) were derived from this passage. Both were T Ag positive.

A separate culture of cloned 301 cells was transformed by SV40, and a T Ag-positive transformant (312) was injected into syngeneic mice. One tumor line (323) was established in culture.

The T AL/N (clone 3) and the derived SV40-transformed mouse cell line (subclone 1) have been described elsewhere (19). BALB/c (11A8) transformants were obtained from G. Todaro.

Cell labeling procedures. Transformed cells were grown in 150-cm^2 CoStar tissue culture flasks and were labeled with 75 μCi of L-[^{35}S]methionine per ml or 100 μCi of $H_3{}^{32}PO_4$ per ml for 3 h at 37°C in 10 ml of methionine-free or phosphate-free Eagle medium, respectively, containing 2% dialyzed fetal calf serum. Primary cultures of African green monkey kidney cells were infected with SV40 at a multiplicity of 500 PFU/cell and labeled between 23 and 24 h postinfection with 100 μCi of [^{3}H]methionine per ml as described above.

Radioimmunoprecipitation. Labeled cells were washed three times in ice-cold 0.01 M Tris–0.001 M Na_2HPO_4–0.137 M NaCl (pH 7.4) and scraped off the surface of the flask in the same buffer. The cells were pelleted at 1,000 × *g* for 10 min at 4°C and resuspended in 0.01 M Tris, 0.5% Triton X-100, 0.5% sodium deoxycholate, 10% glycerol, 10^{-4} M each of phenylmethylsulfonyl fluoride and L-1-tosylamide-2-phenylethylchloromethyl ketone, pH 9.0 (extraction buffer, modified from Schwyzer [25]). The material was incubated at 4°C for 3 h with occasional shaking and centrifuged at 100,000 × *g* for 1 h. The supernatants (approximately 1 ml) were carefully removed, and 15 μl of heat-inactivated (56°C for 30 min) normal hamster serum or anti-T serum was added. The anti-T sera were obtained from hamsters carrying SV40-induced tumors and were provided by J. Gruber (National Cancer Institute).

Swollen protein A-Sepharose (Pharmacia Co.) was washed three times and resuspended in extraction buffer to a final concentration of 50% (vol/vol). The washed protein A-Sepharose was added to each reaction (120 μl per tube), and the samples were incubated at 4°C for 3 h with vigorous shaking. The Sepharose was centrifuged at 2,000 rpm for 5 min at 4°C, washed five times in 0.1 M Tris–0.5 M LiCl–1% 2-mercaptoethanol (pH 9.0) (25), and resuspended in 75 μl of gel electrophoresis sample buffer (0.0625 M Tris, 3% sodium dodecyl sulfate, 5% 2-mercaptoethanol, 10% glycerol, and 0.005% bromophenol blue, pH 7.0). The samples were incubated at 100°C for 3 min, and the Sepharose was pelleted by centrifugation. Supernatants were carefully removed and subjected to electrophoresis through vertical 10% polyacrylamide slab gels as described by Laemmli (15). The following proteins were used as molecular weight markers: phosphorylase *a* (94K), bovine serum albumin (66K), ovalbumin (45K), DNase I (31K), trypsinogen (24K) and lactoglobulin (18.4K). Electrophoresis was carried out at 10 mA per gel. To detect unlabeled proteins in the gel, the slab gel was stained overnight in 0.1% Coomassie blue–50% methanol–10% acetic acid–40% water at room temperature and destained in the same solution in the absence of the dye. To detect labeled proteins, the gels were imbedded with 2,5-diphenyloxazole and exposed to X-ray film at −70°C as described elsewhere (3).

Chromatography of tryptic peptides. The methods used for eluting proteins from acrylamide gels, treating them with trypsin, and subjecting the resulting peptides to Chromobeads ion-exchange chromatography have been described before (28).

Column fractions containing peptides of interest were pooled together, and the peptides were lyophilized to dryness and dissolved in a small volume of 0.01 M acetic acid. Samples (10 to 20 μl) were applied side by side to Whatman no. 1 paper and subjected to descending chromatography. The solvent system consisted of butanol–water–pyridine–acetic acid (15:12:10:3). When the solvent front reached the end of the paper, the paper was dried in a chemical hood and sliced in 1-cm strips. A 0.6-ml volume of 30% acetic acid was added to each strip. The samples were incubated at 50°C for 1 h and counted for radioactivity in 10 ml of scintillation counting fluid.

RESULTS

Detection of new antigens in SV40-transformed cells. SV40-transformed AL/N mouse cells were examined for antigens that could be immunoprecipitated with anti-T serum. Cells (lines 214 and 215) were labeled with [^{35}S]methionine, and the labeled proteins in the immunoprecipitates were subjected to electrophoresis through polyacrylamide gels. In addition to the large T Ag (94K daltons) and small T Ag (20K daltons), a new species of antigen (56K daltons) was easily identified in the precipitates (Fig. 1, A [T] and B [T]). This protein, called Tau antigen because of its lack of extensive peptide homology with T Ag's (see below), was absent from the precipitates of these labeled cell extracts using normal, nonimmune serum (Fig. 1, A [N] and B [N]). Similarly, Tau antigens could not be detected in the spontaneously transformed parent (210) or sister (213) cell line of lines 214 and 215 (Fig. 1, C and D). A protein that migrated just behind Tau antigens was

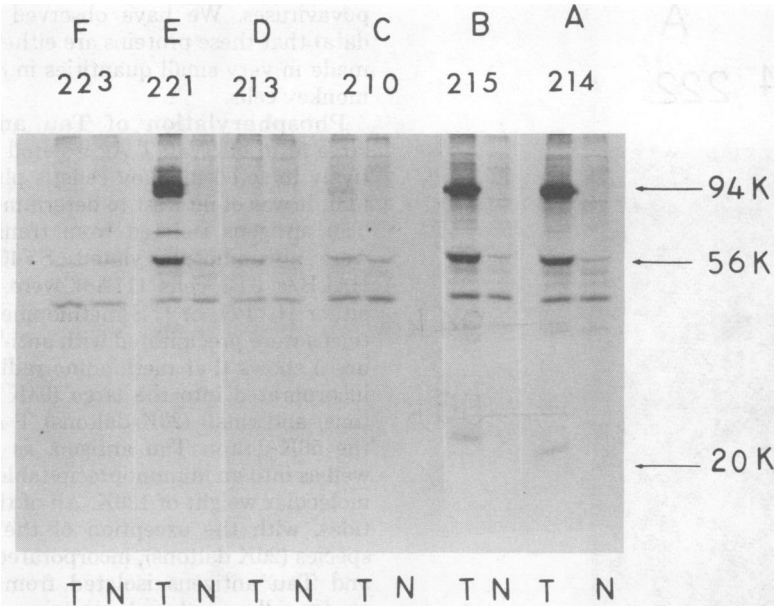

FIG. 1. *Polyacrylamide gel electrophoresis of [^{35}S]methionine-labeled proteins precipitated from AL/N mouse cells. SV40-transformed AL/N mouse cells (lines 214, 215, and 221), spontaneously transformed cells (lines 210 and 213), and T Ag-negative revertant cells (line 223) were labeled with [^{35}S]methionine. Extracts of these cells were incubated with either normal or anti-T serum and with protein A-Sepharose. The precipitated labeled proteins were subjected to acrylamide gel electrophoresis and detected in the gel by fluorography (3). The tracks indicated by T or N contained proteins precipitated with anti-T or normal hamster serum, respectively. Molecular weight values indicated for some of the labeled polypeptides were estimated by the relative migration rates of marker proteins with known molecular weights.*

sometimes observed in our immunoprecipitates (Fig. 1). This protein was judged to be a contaminating cell protein since it was brought down with the normal serum (N) as well as the anti-T serum (T).

To determine the stability of Tau antigen after passage of transformed cells in animals, we induced tumors in immunocompetent syngeneic mice using a cloned T Ag-positive transformant (215). Four tumor lines (221–224) were isolated, and all but one (223) were T Ag positive as determined by indirect immunufluorescence (22). The T Ag-positive cell lines contained all three previously detected immunoprecipitable antigens (94K, 56K, and 20K) (Fig. 1, E and Fig. 2, A and B). On the other hand, the T Ag-negative revertant tumor line (223) lacked all of these antigens, including the 56K-dalton protein (Fig. 1, F).

A second series of independently transformed mouse cells were studied to confirm these observations. SV40-transformed BALB/c 3T12-4-T cells and tumor lines derived from these cells were examined for the presence or absence of Tau antigens. All T Ag-positive transformants (303, 314, 315, and 323) contained the 56K-dalton antigen, whereas the parent, spontaneous non-SV40 transformant (301) lacked this protein (Fig. 3). In addition, one of the cell lines derived from a tumor (315) contained a much larger (130K daltons) immunoprecipitable protein. An immunoprecipitable protein of approximately the same size (130K daltons) has been detected in a line of SV40-transformed cells by Lichaa and Niesor (16).

The presence of multiple species of large T Ag (90K to 105K daltons) in SV40-transformed cell lines has been described elsewhere (12, 16). Several of our cell lines contained these various T Ag forms (see Fig. 1–5). It is unlikely that they represent degradation products of a single species of large T Ag, since there is a great deal of variability in the large T Ag polypeptides isolated from different cell lines by the same experimental technique (12, 16). One of the SV40-transformed tumor cell lines that we examined (221) contained an additional immunoprecipitable protein (68K daltons) which has not been characterized further. It should be pointed out that some cell lines (notably 315) lacked detectable amounts of small T Ag (20K daltons). This observation is probably not too important in light of the fact that rather small amounts of this protein were immunoprecipitated from most

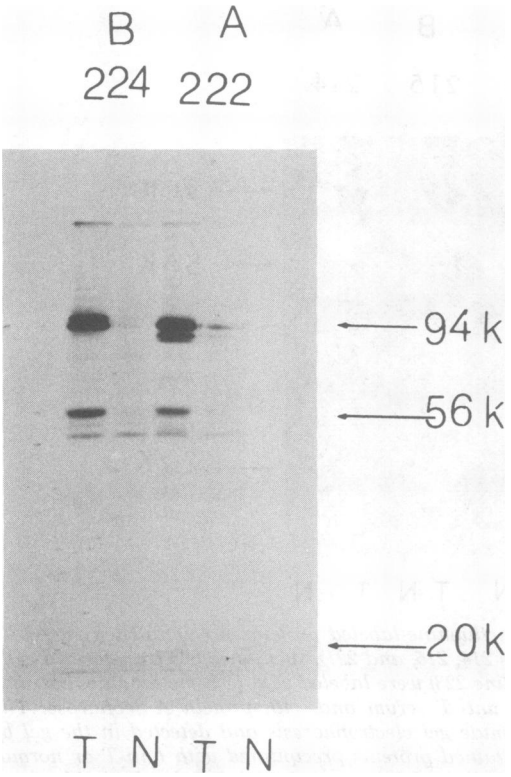

FIG. 2. *Polyacrylamide gel electrophoresis of labeled proteins precipitated from SV40-transformed mouse AL/N cells. The cell lines that were labeled (222 and 224) were T Ag positive as determined by indirect immunofluorescence. For details see the legend to Fig. 1.*

of the T Ag-positive transformants that we examined. This, in turn, might be due to the fact that rather long (3 h) labeling periods were used in our experiments.

Since the virus DNA in the transformed mouse cell lines described above had not been characterized, we decided to examine an SV40-transformed AL/N line (subclone 1) which contained a single copy of integrated viral DNA per cell (14). Figure 4 (A) shows that this transformant contained the expected T Ag species as well as the 56K-dalton antigen, whereas its spontaneously transformed parent (T AL/N clone 3) lacked all of these proteins (Fig. 4, B). In fact, all SV40-transformed cells tested thus far, including transformed rat, rabbit, and human cells (data not shown), contained an immunoprecipitable protein with a molecular weight of 56K. Since Tau antigens could not be detected in spontaneously transformed cells and in tumor cell lines which reverted to a T Ag-negative state, these results suggested to us that Tau antigens were specific to transformation by papovaviruses. We have observed (unpublished data) that these proteins are either not made or made in very small quantities in SV40-infected monkey cells.

Phosphorylation of Tau antigens. The large form of SV40 T Ag isolated from productively infected monkey cells is phosphorylated (33). It was of interest to determine if the T and Tau antigens isolated from transformed cells were also phosphorylated. SV40-transformed BALB/c 3T3 cells (11A8) were labeled with either $H_3{}^{32}PO_4$ or [^{35}S]methionine, and the antigens were precipitated with anti-T serum. Figure 5 shows that methionine radioactivity was incorporated into the large (94K to 100K daltons) and small (20K daltons) T Ag forms and the 56K-dalton Tau antigen, as predicted, as well as into an immunoprecipitable protein with molecular weight of 130K. All of these polypeptides, with the exception of the small T Ag species (20K daltons), incorporated ^{32}P. Thus, T and Tau antigens isolated from transformed mouse cells are phosphoproteins.

Methionine-labeled tryptic peptides of the 56K antigen. Since all of the SV40-transformed cells that we examined possessed a 56K-dalton Tau antigen in addition to T Ag's, it was important to determine the relationship of this protein to the large and small T Ag's of SV40. We therefore compared the methionine-labeled tryptic peptides of the 56K protein isolated from SV40-transformed mouse cells (line 315) to those of large T Ag isolated from infected monkey cells. Figure 6 shows that the large T Ag contained 18 to 19 methionine-tryptic peptides as determined by ion-exchange chromatography, in agreement with a previous report (28). The same tryptic peptide profile was obtained when the large T Ag species was isolated from several different SV40-transformed cell lines (unpublished results). The Tau antigen isolated from line 315 contained 16 resolvable methionine-tryptic peptides, of which 4 (numbered 2, 3, 13, and 18) coeluted with peptides present in the 94K antigen. These 16 peptides and the methionine-labeled tryptic peptides of Tau antigen (56K daltons) isolated from a different line of SV40-transformed BALB/c mouse cells (11A8) were indistinguishable by ion-exchange chromatography (data not shown).

To determine if each of these four peptides in Tau antigen was identical to the coeluting peptide in T Ag, the appropriate peptides of each protein were first selected by ion-exchange chromatography and then analyzed further by descending paper chromatography. Paper chromatography was chosen to separate peptides in the second dimension because this method re-

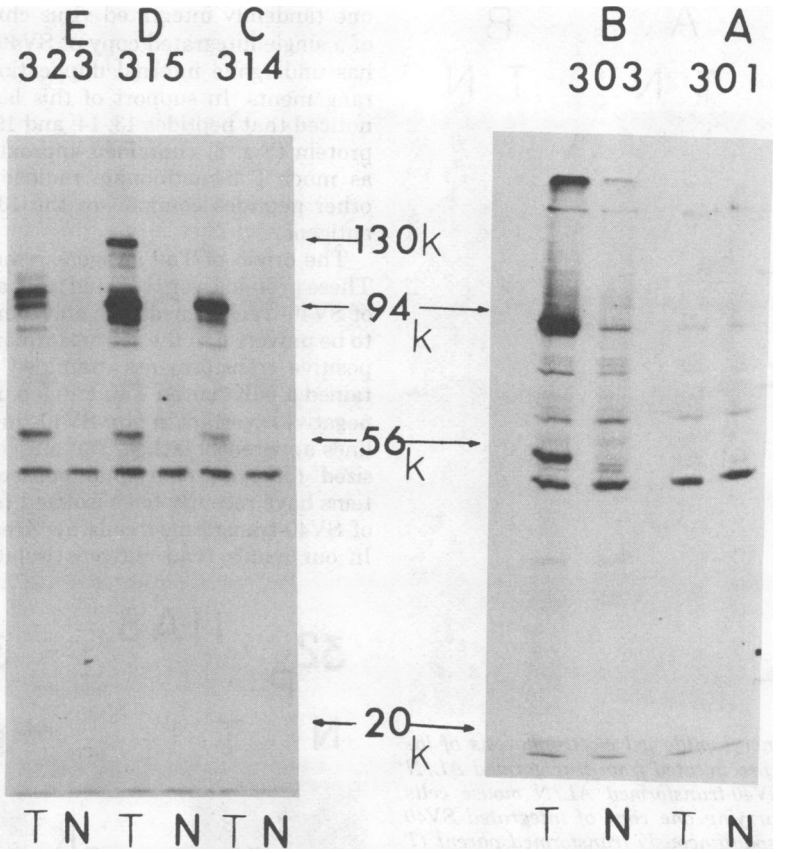

FIG. 3. *Polyacrylamide gel electrophoresis of labeled proteins precipitated from transformed BALB/c mouse cells. Spontaneously transformed (301) and SV40-transformed (303, 314, 315, and 323) BALB/c mouse cell lines were labeled with [^{35}S]methionine and analyzed for immunoprecipitable antigens as described in the legend to Fig. 1.*

solves peptides on the basis of hydrophobicity, unlike Chromobeads chromatography which resolves peptides on the basis of ionic charge. Peptides numbered 2 and 3 (see Fig. 6) isolated from either protein behaved as single peptides by paper chromatography, but the coeluting peptides migrated to different positions (data not shown). This showed that the coeluting peptides in "2" or "3" were not identical. Peptide 13 in Tau antigen actually consisted of three or possibly four peptides, of which at least one comigrated with a peptide in the "peptide 13" fraction of large T Ag on the chromatography paper (Fig. 7A, fraction 20). Similarly, the "peptide 18" fraction from Tau antigen consisted of several methionine-labeled peptides of which one (Fig. 7B, fraction 17) chromatographed with a peptide from large T Ag. These results indicate that two methionine-labeled tryptic peptides are probably shared by T and Tau antigens. One of these, the major peptide in the "peptide 13" fraction of large T Ag (Fig. 7A, fraction 20), is a constituent of the small T Ag of SV40 as well (27). Therefore, it can be localized to the region in the 94K antigen which is also shared by small T Ag (i.e., between 0.59 and 0.66 on the SV40 chromosomal map) (7, 21). The map position in T Ag of the second cochromotographing peptide (Fig. 7B, fraction 17) is not known.

Methionine-labeled tryptic peptides of the 130K antigen. The relationship between the 130K-dalton immunoprecipitable protein that we detected in SV40-transformed mouse cells and the large T Ag species was determined by comparing their methionine-labeled tryptic peptides by ion-exchange chromatography. Fifteen peptides of the 130K antigen coeluted with peptides in the 94K large T Ag and four did not (Fig. 8). Three peptides that were present in the 94K T Ag were absent from the 130K protein (Fig. 8). These are the large T Ag peptides 6, 12, and 15. Peptides 6 and 15 are constituents of the

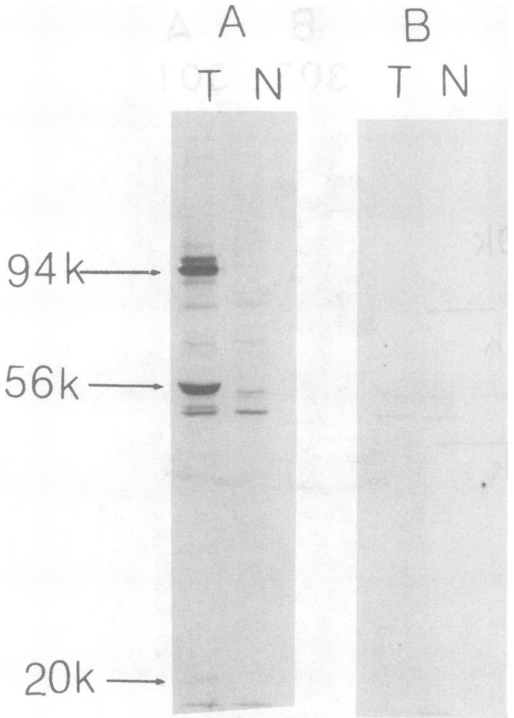

FIG. 4. *Polyacrylamide gel electrophoresis of labeled proteins precipitated from transformed AL/N mouse cells. SV40-transformed AL/N mouse cells (subclone 1) carrying one copy of integrated SV40 DNA and its spontaneously transformed parent (T AL/N, clone 3) were labeled with [^{35}S]methionine and analyzed for immunoprecipitable antigens as described in the legend to Fig. 1. (A) Proteins from the SV AL/N (subclone 1) line; (B) proteins from the T AL/N line.*

small T Ag of SV40 (27). The similarity between the elution profiles of the methionine peptides of the 130K and 94K antigens strongly suggests that the 130K protein is predominantly virus specified. Similar results have been obtained by Kress et al. (13).

DISCUSSION

Analysis of the methionine-labeled tryptic peptides of the 130K immunoprecipitable protein isolated from some lines of SV40-transformed mouse cells indicated that the majority of the sequences in this protein were related to an extensive portion of the large T Ag of SV40. We interpret these results as indicating that the 130K protein is mostly virus coded. Since the maximum coding capacity of the early region of SV40 DNA is 90K to 100K daltons of protein, it is possible that a portion of the 130K molecule is specified by the host genome. Other possibilities are that this protein is extensively modified from large T Ag, or is a product of more than one tandemly integrated virus chromosome or of a single integrated copy of SV40 DNA which has undergone internal duplications and rearrangements. In support of this last model, we noticed that peptides 13, 14, and 19 in the 130K protein (Fig. 8) contained approximately twice as much [^{35}S]methionine radioactivity as the other peptides common to the 130K and 94K antigens.

The origin of Tau antigens remains puzzling. These proteins were isolated from a wide variety of SV40-transformed cells and therefore appear to be universal to these transformants. All T Ag-positive transformants examined to date contained a 56K-dalton Tau antigen, and all T Ag-negative revertant or non-SV40-transformed cell lines apparently lacked Tau antigens. Similarly sized (56K daltons) immunoprecipitable proteins have recently been isolated from a variety of SV40-transformed cells by Kress et al. (13). In our hands, Tau antigens isolated from two

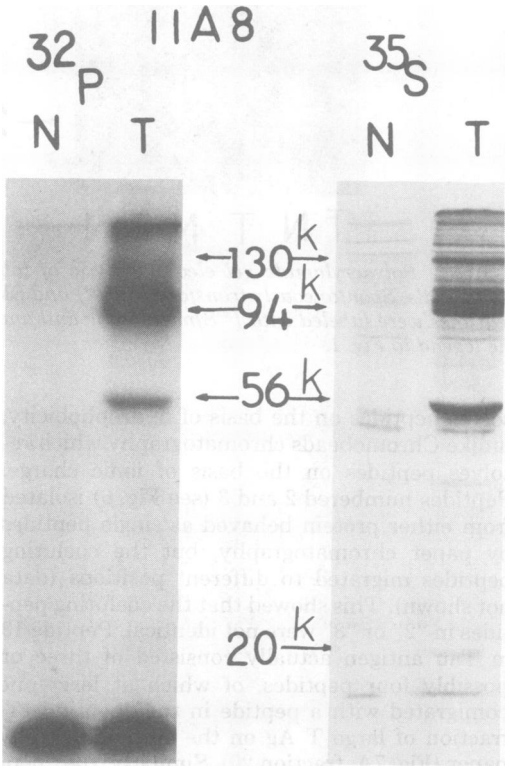

FIG. 5. *Polyacrylamide gel electrophoresis of $H_3{}^{32}PO_4$- or [^{35}S]methionine-labeled proteins immunoprecipitated from SV40-transformed 11A8 cells. SV40-transformed BALB/c mouse cells (11A8) were labeled with either $H_3{}^{32}PO_4$ or [^{35}S]methionine. Extracts of these cells were reacted with either normal (N) or anti-T serum (T), and the labeled proteins in the precipitates were examined by gel electrophoresis.*

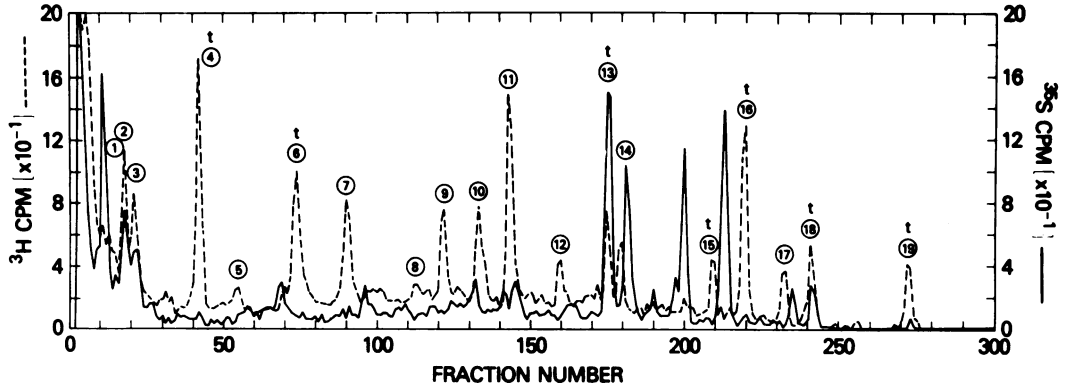

FIG. 6. *Relationship between the methionine-labeled tryptic peptides of the 56K Tau antigen and the large T Ag of SV40. [^{35}S]methionine-labeled 56K Tau antigen was isolated from SV40-transformed cells (line 315), and [^{3}H]methionine-labeled 94K T Ag was isolated from infected monkey cells, by immunoprecipitation and gel electrophoresis. Tryptic peptides of these proteins were chromatographed on columns of Chromobeads. Peaks labeled "t" consist of peptides present in the small T Ag of SV40 (27).* (——) *[^{35}S]methionine-labeled tryptic peptides of the 56K Tau antigen;* (-----) *[^{3}H]methionine-labeled tryptic peptides of the 94K T Ag.*

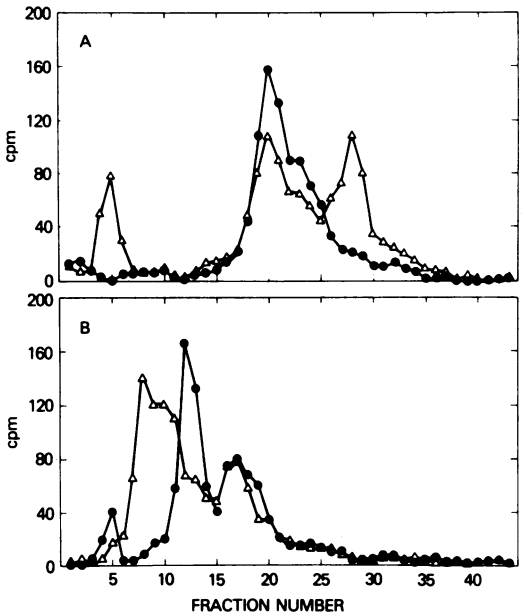

FIG. 7. *Descending paper chromatography of methionine-labeled tryptic peptides. [^{35}S]methionine-labeled tryptic peptides of T and Tau antigens were separated by ion-exchange chromatography. The "peptide 13" and "peptide 18" fractions were subjected to descending paper chromatography. Paper strips (1 cm) were counted for radioactivity. (A) "Peptide 13" fraction of large T Ag* (●) *and 56K Tau antigen* (△); *(B) "peptide 18" fraction of large T Ag* (●) *and 56K Tau antigen* (△).

independently transformed BALB/c mouse cell lines contained indistinguishable methionine-labeled tryptic peptides as determined by ion-exchange chromatography. Furthermore, these proteins contained two methionine-tryptic peptides which appeared to be present in the large T Ag of SV40 as determined by a combination of ion-exchange and paper chromatographies. Since peptides were subjected to two methods of chromatography based on totally different principles (hydrophobicity and ionic charge), the comigration of peptides from T and Tau antigens in these two systems is an argument that the common peptides have similar, perhaps identical, amino acid compositions. Of course, no peptide separation method can prove that the sequence in each peptide is identical. Only a direct analysis of their amino acid sequence can provide this information.

Several possibilities exist to explain the presence of Tau antigens in SV40-transformed cells. First, Tau antigens could be entirely virus coded and extensively modified after their synthesis such that only two methionine-tryptic peptides remain unmodified. The alternate suggestion, that T and Tau antigens are translated from early SV40 RNA in two different codon frames, seems very unlikely due to the presence of multiple stop codons in two of the three possible reading frames throughout most of the early transcript (8, 24). The second possibility is that these proteins are coded entirely by the cell genome. The two similar methionine peptides in T and Tau antigens would therefore be encoded in the virus and cell DNAs, respectively, and their presence in these two proteins would only be a coincidence. If this were the case, the gene for Tau antigens could be derepressed by the virus or the transforming event to explain the

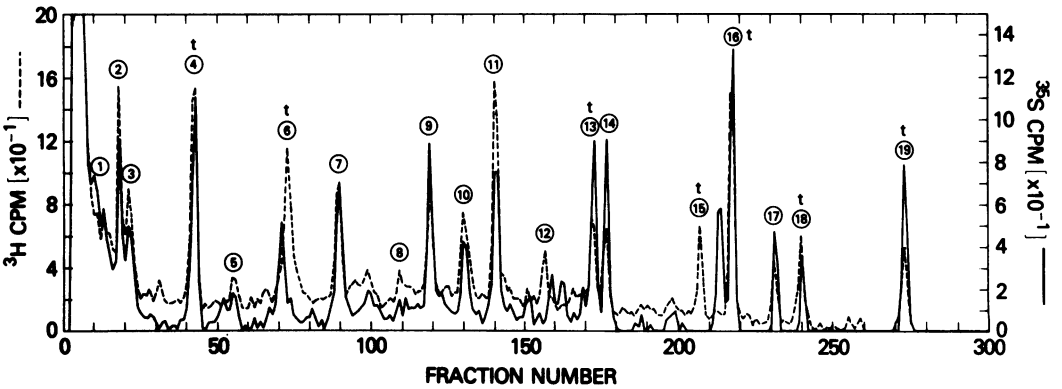

FIG. 8. *Relationship between the methionine-labeled tryptic peptides of the 130K antigen and 94K T Ag. [^{35}S]methionine-labeled 130K antigen and [^{3}H]methionine-labeled 94K T Ag were isolated from SV40-transformed mouse cells (line 315) and infected monkey cells, respectively, by immunoprecipitation and gel electrophoresis. Tryptic peptides of these proteins were subjected to ion-exchange chromatography.* (——) *[^{35}S]methionine-labeled peptides of the 130K antigen;* (-----) *[^{3}H]methionine-labeled peptides of the 94K T Ag.*

observation that these proteins could not be isolated from spontaneously transformed cells. The third possibility is that Tau antigens are partially virus coded and partially cell coded and that the two similar methionine peptides in T and Tau antigens are specified by SV40 DNA. One piece of evidence consistent with this hypothesis is that these two proteins are apparently related immunologically (18). One of the methionine tryptic peptides in T Ag (Fig. 7A, fraction 20), which might be present in Tau antigen, is coded by SV40 DNA mapping between 0.59 and 0.66 on the standard SV40 DNA map, since this peptide was also present in the small T Ag species. If this peptide and the second possible common peptide to T and Tau antigens (Fig. 7B, fraction 17) represent part of a virus-encoded component in Tau antigens, it would be interesting to determine the locations of these peptides in the Tau antigen molecule and the mechanisms involved in producing a hybrid protein of this sort.

A protein similar in size to the SV40 Tau antigen (56K daltons) was isolated from polyoma virus-transformed hamster cells by immunoprecipitation with serum directed against polyoma T Ag (26). None of the methionine-labeled tryptic peptides of this protein coincided with peptides from the large T Ag of polyoma, and, in this context, it may be related to the SV40 Tau antigens. Polyoma-infected or -transformed cells synthesize another protein (middle T Ag; molecular weight, 55K to 63K), which, unlike the 56K-dalton protein described above, contains extensive peptide homologies with the large and small T Ag species of polyoma (10, 26, 29).

The transformation of mammalian cells by SV40 is accompanied by the presence of several newly synthesized antigens. In addition to T, U, and the tumor-specific transplantation antigens, it now appears that Tau antigens make up a new class. The interaction of Tau antigens with anti-T serum, their apparent peptide homology with the SV40 T Ag's, and their presence in all SV40-transformed cells tested so far make these antigens an interesting class of proteins. The elucidation of the structure and origin of these antigens will help us understand virus-cell interactions and the expression of the viral genome in papovavirus-transformed cells.

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