Simian Virus 40 (SV40)-Specific Isoelectric Point-4.7-94,000-M,
Membrane Glycoprotein: Major Peptide Homology Exhibited
With the Nuclear and Membrane-Associated
94,000-M, SV40 T-Antigen in Hamsters 1,2,3

Rupert Schmidt-Ullrich, 4 W. Scott Thompson, 4 Stuart J. Kahn, 4 Maureen T. Monroe, 4 and
Donald F. H. Wallach 4

ABSTRACT—Tryptic peptide maps of electrophoretically purified
94,000-molecular weight (relative) (M,) nuclear and membrane-associated
simian virus 40 (SV40) T-antigens, TN and TM, respectively,
were compared to those of the SV40-specific isolectric point (pl)-
4.7-94,000-M, plasma membrane component reactive with anti-T-sera
from Syrian golden hamsters. Bidimensional thin-layer electrophoresis
and chromatography of TN labeled with 3H revealed about 27 tryptic
peptides. A similar number of peptides was identified for TN and the pl-
4.7-94,000-M component. A peptide homology between TN and TM or
TN and the pl-4.7-94,000-M protein exists and indicates that the
previously described pl-4.7-94,000-M, membrane component represents
TM. Only 4 of 27 peptides were labeled when TM was subjected
to lactoperoxidase-catalyzed radiiodination from the outer surface
of the plasma membrane. One of these TM peptides was metabolically
labeled with 14C-glucosamine. The data indicate that TM is partially
exposed on the cell surface and represents a glycosylated form of TN.
Closely associated with TM is a pl-4.5-55,000-M, membrane compo-
ment. This component does not exhibit significant peptide homology
with the 94,000-M, SV40 protein and, therefore, appears to be coded
for by the host cell genome.—JNCI 1982; 69:839-849.

Mammalian cells neoplastically transformed by SV40
express virus-specific proteins on their surface membrane.
These proteins are defined as TSTA or TSSA (1-5). Recent
evidence indicates that TSTA and TSSA may represent identical
antigens that are monitored by in vivo and in vitro
test systems, respectively (6). The two antigens also exist in
a close relationship with the 94,000-M, SV40 T-antigen.
This relationship is indicated by copurification of
TSTA-TSSA and T-antigen during biochemical fractiona-
tion (4), similar subcellular distribution of the 2 groups of
antigens (2,7), and their concordant expression at permi-
sive and nonpermissive temperatures in cells transformed by
nondefective tsA mutants (8,9). Other data indicate that
purified T-antigen can induce a TSTA response in vivo
(10), and this finding is concordant with recent findings
that a 94,000-M, T-antigen, immunochromically indistin-
guishable from nuclear T-antigen, can be immunoprecipi-
tated from membranes of SV40-transformed cells (7, 11,
12). That SV40 T-antigen is expressed at the plasma mem-
brane of SV40-transformed cells is further suggested by
studies in which rabbit immune serum against purified T-
antigen reveals a specific indirect immunofluorescence re-
action at the surface of SV40-transformed cells (11,13).
In previous work we have identified and partially characterized
two SV40-specific proteins in membranes of SV40-trans-
formed cells (14-16). Using highly purified plasma mem-
branes of SV40-transformed GD248 hamster lymphoid cells
(14, 15) and SV80 human fibroblasts (15), we presented
evidence that the SV40-specific pl-4.7-94,000-M, membrane
glycoprotein of these cells represents a host-modified, gly-
cosylated T-antigen (15, 16). We have now obtained tryptic
peptide maps of 3H-labeled SV40 proteins showing that the
SV40-specific pl-4.7-94,000-M, glycoprotein exhibits a ma-
jor tryptic peptide homology with the TN. This similar
peptide homology exists when the TM or pl-4.7-94,000-M,
protein is compared with TN. TM represents maximally 1 %
of the total cellular T-antigen. Like the 94,000-M, intracel-
larular T-antigen (12,17-19), TM is associated with a 55,000-
M host cell protein. This protein does not react with anti-T
serum and, on the basis of tryptic peptide analyses, does not
share peptides with TN or TM (20-22).

MATERIALS AND METHODS

Chemicals, media, and materials.—Triton X-100, HEPES,
DTT, BSA, and PMSF were obtained from Sigma Chemical
Co. (St. Louis, Mo.); Chloramine-T was from Eastman
Kodak Co. (Rochester, N.Y.); SDS and urea were from
Fisher Chemical Co. (Fair Lawn, N.J.); acrylamide, N,N'-
methylenebisacrylamide, N,N',N'-tetrathymethyleneami-
mine, ammonium persulfate, and Coomassie brilliant blue
were from Bio-Rad Laboratories (Richmond, Calif.); and
ampholites (Ampholine, pH 3.5-10.0) were from LKB In-

Received May 13, 1980; revised April 28, 1982; accepted May 10,
1982.

2Supported by Public Health Service grants CA-23642 and CA-12178
from the National Cancer Institute.

3Animals were maintained under the guidelines set forth in the "Guide
for the Care and Use of Laboratory Animals" by the Institute of Laboratory
Animal Resources, National Research Council.

4Radiobiology Division, Department of Therapeutic Radiology,
Tufts-New England Medical Center, 171 Harrison Ave., Boston, Mass.
02111.

JNCI, VOL. 69, NO. 4, OCTOBER 1982

839

Downloaded from https://academic.oup.com/jnci/article-abstract/69/4/839/902707 by guest on 20 November 2018
standard (29). SV40 T-antigen was extracted from nuclei and plasma membrane purified from [35S]methionine and 0.2 mCi/ml was in EMEM-lO% dialyzed FCS. After a 2-hour pulse the cultures were chased with 2 ml buffered saline (0.13 M NaCl, 0.02 M Tris-HCl, 0.001 M CaCl2, and 0.001 M MgCl2 (pH 9.0), 1% (vol/vol) in Triton X-100 and 0.002 M in PMSF. The antigen (1 ml each) was preincubated with 0.01 ml of normal sheep serum and 0.08 ml protein A-Sepharose at 4°C for 60 minutes (23). The protein A-Sepharose beads were washed three times in 0.5 ml of 0.1 M Tris-HCl, 0.5 M LiCl, and 0.14 M β-mercaptoethanol (pH 9.0) for removal of nonspecifically adsorbed protein. Specifically bound proteins were released by incubation in 0.05 ml of 0.02 M Tris-HCl and 0.7 M β-mercaptoethanol (pH 9.0), 5 and 20% (vol/vol), respectively, in SDS and glycerol at 80°C for 30 minutes.

After SDS–PAGE fractionation of immunoprecipitated SV40 Tm and Tn (31), the antigens were identified by 35S autoradiography of dried gels. Bidimensional IEF–SDS–PAGE was as in (15, 16). The pl 4.7–9.4, 90,000-Mr membrane component was isolated after protein staining of the slab gels with Coomassie blue (31). For peptide mapping the immunoprecipitated 55,000-Mr protein was used. The 94,000- and 55,000-Mr membrane proteins specifically bound to protein A-Sepharose were desorbed from the beads and separated from heavy- and light-chain immunoglobulins by means of IEF (32). The [35S]methionine-labeled 94,000- and 55,000-Mr components focusing near pI 4.5 were isolated from the IEF gel, subfractionated by DS–PAGE, and identified by 35S autoradiography. Gels run in parallel were stained with Coomassie blue to assure quantitative separation of immunoglobulin and the SV40 membrane proteins.

The reactivity of the pl 4.7–9.4, 90,000-Mr and pl 4.5–5.0, 55,000-Mr components with anti-T-sera was investigated by an electrophoretic blotting procedure (33). After IEF–DS–PAGE, plasma membrane proteins from SV80 cells, focusing between the pl 5.5 and pl 4.0 regions, were electrophoretically transferred from the slab gel onto nitrocellulose sheets. These were soaked in NaCl–BSA (0.15 M/3%, wt/vol) and reacted with hamster anti-T-serum (0.02 ml/ml of NaCl–BSA–0.001 M NaNa) for 16 hours at 20°C. The
Nitrocellulose sheets were then soaked in NaCl (0.15 M) as in (33) for removal of unbound serum proteins. Specifically bound hamster immunoglobulin was identified by equilibration with 125I-labeled protein A (5×10⁵ cpm/mg protein) for 6 hours at 20°C followed by autoradiography.

125I labeling of SV40-specific proteins.—The chloramine-T technique (34) was used to achieve iodination of TN and SV40 membrane proteins to specific activities of about 10⁷ cpm/mg protein. TN, TM, the pl-4.7-94,000-M₉ protein, and the pl-4.5-55,000-M₉ protein were localized by [35S]methionine autoradiography and then iodinated in polyacrylamide gel pieces according to the procedure of Elder et al. (35). The protein-containing gel pieces were lyophilized in siliconized test tubes and rehydrated in about 0.05 ml of 0.5 M sodium phosphate (pH 8.5). After an additional 0.02 ml of chloramine-T (1 mg/ml in H₂O) and 0.3 mCi of Na[125]I in 0.005 ml, iodination was allowed to proceed for 30 minutes at 20°C. The reaction was then quenched by addition of 0.02 ml sodium bisulfate (1 mg/ml in H₂O). Unreacted [125]I was removed by extensive dialysis against methanol (10%, vol/vol).

To identify the peptide moiety of TM exposed at the cell surface, we 125I-labeled sealed plasma membranes vesicles by using lactoperoxidase-catalyzed radioiodination (36). In control experiments, plasma membranes were solubilized in Triton X-100 (1%, vol/vol) and subjected to the same iodination procedure. Immunoprecipitation and electrophoretic isolation of 125I-labeled TM were as for metabolically labeled proteins, except that the chloramine-T-mediated iodination prior to trypptic peptide mapping was omitted.

Trypsin treatment of SV40-specific proteins.—For cleavage with TPCK—trypsin, dried gel pieces containing 125I-labeled SV40-specific proteins were hydrated with 0.02-0.05 ml of 0.05 M NH₄HCO₃; so TPCK—trypsin (0.5 mg/ml) was then added in the volume of 0.05 M NH₄HCO₃ required for complete hydration of the gel piece. Trypsinization was for 40 hours at 37°C with a second addition of 0.05 mg TPCK—trypsin after 16 hours. To assure that only peptides of the SV40 proteins were detected, we performed several control experiments under identical conditions: a) TPCK—trypsin was incubated alone in the presence of 125I (2×10⁶ cpm); b) [125]IβSA, a protein completely unrelated to T-antigen, was subjected to cleavage by TPCK—trypsin, and c) a gel piece corresponding to the 94,000-M₉ region, obtained from a sample precipitated with normal hamster serum, was trypsinated. The supernatant containing TPCK—trypsin and 125I-labeled peptides was lyophilized, and the residue was solubilized in acetic acid—formic acid (15%/5%, vol/vol) for peptide mapping by electrophoresis and chromatography on cellulose thin-layer plates (33).

Peptide mapping.—Bidimensional thin-layer peptide mapping was used to define the positions of peptides—glycopeptides in a two-dimensional grid (33). In addition, the “grouping” of peptides and the relative intensities of the peptide spots were evaluated. Finally, we investigated the identities of peptides from TN and TM and the pl-4.7-94,000-M₉ component by mixing peptides of TM with TN, or the pl-4.7-94,000-M₉ component, or all three proteins prior to bidimensional thin-layer electrophoresis and chromatography.

RESULTS

SV40-specific membrane proteins.—The present data were obtained with the use of SV80 cells that produce large quantities of SV40 T-antigen (23). As shown in figure 1A, plasma membranes of SV80 cells, purified and freed of nuclear contaminants according to the procedure described for GD248 hamster lymphoid cells and other SV40-transformed fibroblasts (16, 25), contain two membrane proteins characteristic for SV40-transformed cells—the pl-4.7-94,000-M₉ and pl-4.5-55,000-M₉ components. These proteins correspond to entities identified by us in plasma membranes of other SV40-transformed cells (14, 16, 37). Of SDS-denatured components, only the pl-4.7-94,000-M₉ protein reacts with anti-T-serum after transfer from polyacrylamide to nitrocellulose (fig. 1B).

When Triton X-100-solubilized membrane proteins of SV80 cells are reacted with anti-T-serum, a 94,000-M₉ protein and a 55,000-M₉ protein are specifically precipitated (fig. 2). No proteins of this molecular mass are deposited when normal serum is used for precipitation of membrane-associated antigens (fig. 2). We have previously shown that the 94,000-M₉ protein isolated by SDS—PAGE focuses between pI 5.5 and 4.5, with a major protein peak at pI 4.7 (16). Our data indicate that the 94,000-M₉ protein is associated with the 55,000-M₉ component as has been reported for the total cellular T-antigen (12, 17, 18, 38-40). Whereas with the TN a prominent 55,000-M₉ component is deposited, this protein reproducibly precipitates to a lesser amount when plasma membranes are reacted with anti-T-serum. However, the 55,000-M₉ protein per se does not react with anti-T-antibodies (39, 41). In agreement with others’ results (12), the anti-T-serum does not precipitate possible 20,000-M₉ T-antigen from plasma membranes.

Tryptic peptides of the SV40-specific membrane proteins.—Figures 3A-3F compare the tryptic peptide maps of T-antigen isolated by immunoprecipitation from purified plasma membranes, TM, and from isolated nuclei, TN, with those of the SV40-specific, pl-4.7-94,000-M₉ plasma membrane protein.
The sensitivity of bidimensional thin-layer peptide mapping of \(^{125}\)I-labeled peptide mapping of \(^{125}\)I-labeled peptides is sufficient for the resolution of at least 27 \(T_N\) peptides (figs. 3A, 3D). This peptide resolution appears to be superior to [\(^{35}\)S]methionine ion-exchange peptide mapping, which yielded maximally 19 peptides for the 94,000-M\(r\)T-antigen \((21)\). None of the \(^{125}\)I-labeled peptides are contributed by trypsin itself, because no peptides were revealed when trypsin was incubated in the presence of free \(^{125}\)I. Further, there was no peptide homology between \(^{125}\)I-labeled BSA and \(^{125}\)I-labeled \(T_N\), both digested with identical quantities of TPCK-trypsin; no peptides could be identified when gel pieces of the 94,000-\(M_r\) and 55,000-\(M_r\) regions were processed from gels in which material, precipitated with normal hamster serum, was fractionated.

The numbering of peptides was based on the pattern obtained with SV40 \(T_N\). Peptides with identical position and grouping in \(T_N\), on the one hand, and \(T_M\) or the pl-4.7-94,000-\(M_r\) component, on the other hand, carry the same numbers. Peptides apparently deviating in position were assigned the numbers of the corresponding \(T_N\) peptide plus the letters \(N\) or \(M\), depending on their characteristic appearance in the \(T_N\) or \(T_M\) peptide maps. Peptides from \(T_M\) and the pl-4.7-94,000-\(M_r\) component, not corresponding to those of \(T_N\), were assigned letters (in alphabetical order).

Of the 27 peptides obtained by trypsin cleavage of \(^{125}\)I-labeled \(T_N\) (figs. 3A, 3D), a significant number occur also in the maps obtained from both the 94,000-\(M_r\) \(T_M\), isolated by immunoprecipitation from Triton X-100-solubilized membranes, and the pl-4.7-94,000-\(M_r\) protein. The two membrane proteins exhibit a major peptide homology between each other and with \(T_N\) (figs. 3B, 3C, 3E, 3F). The qualitative peptide pattern and the peptide intensity shown in figures 3A–3C are highly reproducible. On the basis of these observations, peptides 3, 7, 8, 9, and 16 exhibit different migrations and/or intensities when \(T_N\) and the SV40 membrane components are compared. Although a minor peptide of \(T_M\) is found in the area of peptide 7N, the displacement of the major peptide 7N relative to the positions of peptides 3, 8, and 9 is significant. New peptides, A and B, are found only in \(T_M\) and the pl-4.7-94,000-\(M_r\) component (figs. 3B, 3C, 3E, 3F). On the basis of the comparisons of figures 3A–3C, the distinctions between \(T_N\) and \(T_M\) appear to be confined to different migration and/or different intensities of peptides 3, 7, 8, 9, and 16 and the presence of additional peptides, A and B in \(T_M\). Peptides 3, 7, 9, and 16 are investigated in more detail by reelectrophoresis-rechromatography of groups of isolated peptides (figs. 4A–4D). Peptides 6, 14, and 16–19 obtained from \(T_M\) and the pl-4.7-94,000-\(M_r\) component comigrate (fig. 4B). Analyses of peptides 3, 7, 8, 9, and 21–23 (fig. 4C) and peptides 1, 2, 16, 18, and 19 (figs. 4D) reveal apparent identity of peptides 1, 2, 14, 17–19, and 21–23. This identity is confirmed by mixing experiments in which individual peptides from all three 94,000-\(M_r\) proteins are rerun (figs. 5A–5E). As shown in figure 5A, a mixture of peptides, three from \(T_M\) and the pl-4.7-94,000-\(M_r\) component comigrate as essentially one spot. Peptide 3 of \(T_M\) moves with a lower electrophoretic mobility (fig. 5B), which results in a lower intensity peptide from \(T_N\) and the more intense spot due to comigration of peptide 3 from \(T_M\) and the pl-4.7-94,000-\(M_r\) component. Due to some heterogeneity, peptides 7 of \(T_M\) and \(T_N\) migrate as two distinct spots. Peptide 16 (fig. 5F) migrates as three spots, indicating that this protein moiety may be different in \(T_N\), and the pl-4.7-94,000-\(M_r\) component. Peptides 8 and 9 (figs. 3D, 5E) are identical in \(T_N\) and \(T_M\).

**Exposure of \(T_M\) at the membrane surface.**—The peptide maps of the 94,000-\(M_r\) \(T_M\), subjected to vectorial lactoperoxidase-catalyzed radiiodination \((36)\), are shown in figure 6. Selective iodination of peptides 3, 7, 8, 9, and possibly 16 indicates that only these portions of \(T_M\) are exposed at the surface of the plasma membrane and accessible to lactoperoxidase-catalyzed radiiodination. Peptides 3 and 7 are characteristic for \(T_M\). A peptide pattern closely resembling that of \(T_M\) (fig. 3B) is obtained when \(T_M\) is isolated from plasma membranes that have been iodinated in the presence of 1% Triton X-100.

**Glycosylation of \(T_M\).**—\(T_M\) isolated from SV80 cells pulse labeled with \([^{14}\text{C}]\)glucosamine and subjected to trypsin peptide mapping reveals five peptides that selectively incorporate this sugar. The most prominent labeling occurs in peptide 7 (fig. 7). Peptides 9, 13, and 15 are weakly labeled. The different mobility of the prominent peptide 7 from \(T_N\) and \(T_M\) is at least in part due to glycosylation of this portion of \(T_M\). These data indicate that some of the differences between \(T_N\) and \(T_M\) are due to posttranslational glycosylation of the membrane-associated SV40 T-antigen and suggest that peptide 7 represents a glycosylated polypeptide moiety. The persistence of a low-intensity peptide in the area of 7N of \(T_M\) may indicate microheterogeneity in the carbohydrate residues or glycosylation of only a part of \(T_M\).

**\(T_M\)-associated 55,000-\(M_r\) membrane protein.**—The peptide maps of \(T_N\), \(T_M\), and the pl-4.7-94,000-\(M_r\) protein reveal no apparent homology with that of the pl-4.5-55,000-\(M_r\) protein (fig. 8). Our data are in agreement with findings by others indicating that the 55,000-\(M_r\) protein represents a protein associated with T-antigen but encoded for by the host cell genome \((42, 43)\).

**DISCUSSION**

Tryptic peptide mapping of the SV40 94,000-\(M_r\) T-antigen associated with the nucleus \(T_N\), surface membrane \(T_M\), and the pl-4.7-94,000-\(M_r\) membrane component, all labeled with \(^{125}\)I, revealed at least 27 \(^{125}\)I-labeled peptides for \(T_N\) and a similar number for the membrane protein. Significant peptide homology was observed between \(T_N\) and \(T_M\) or the pl-4.7-94,000-\(M_r\) protein and an apparently higher homology was observed between the two latter proteins. This analysis and our immunochemical analyses indicate that the pl-4.7-94,000-\(M_r\) glycoprotein \((14)\) represents the membrane-associated T-antigen \(T_M\). The glycosylation of \(T_M\) might easily be missed, unless purified plasma membranes are investigated, because \(T_M\) represents less than 1% of the total cellular T-antigen.

At least three major peptides, peptides 3, 7, and 16 (figs. 3, 4, 5), occupy different positions in peptide maps of \(T_N\) or \(T_M\). The differences in peptides 3 and 16 could mean that the two proteins differ in their primary protein structure. Such a possibility is suggested by data of Mark and Berg \((44)\) according to which a third splice site in the early
messenger RNA would result in synthesis of a 94,000-M<sub>t</sub> T-antigen with a more hydrophobic region near its carboxy terminus. Such a protein might qualify as a protein with high membrane affinity.

Posttranslational modification of T<sub>M</sub> is indicated by our finding that T<sub>M</sub> represents a glycosylated T<sub>N</sub>. Data of others (7) and our own unpublished data indicate that T<sub>M</sub> is also phosphorylated. It remains to be shown, however, whether the phosphate residue(s) in T<sub>M</sub> are bound to the same residues as in T<sub>N</sub> (45). A combination of translational and posttranslational modifications is conceivable.

Changes in the immunologic reactivity of the p14.7–94,000-M<sub>t</sub> component after trypsin and neuraminidase treatment of intact isolated membranes from GD248 cells (14) and surface iodination of plasma membranes of SV80 cells indicate that portions of the T<sub>M</sub> are exposed at the membrane surface. Serologic analyses by Soule et al. (11) have led to similar conclusions. Using immunofluorescence analyses and radioimmunoassays with sera against denatured isolated total cellular T-antigen, sera of tumor-bearing animals and surface-reactive anti-T-sera (all sera precipitated a 96,000-M<sub>t</sub> T-antigen), these workers found differential reactivities of the membrane-associated T-antigen and the nuclear T-antigen. Soule et al. (11) concluded that membrane-bound T-antigen expresses antigenic sites absent on T<sub>M</sub> or that, due to the conformation of T-antigen in the nucleus, certain antigenic sites are masked (11). The data on antigenicity and immunogenicity of T<sub>M</sub> fit our own findings, which indicate that the heterologous guinea pig anti-GD248 membrane sera recognize neuraminidase-sensitive antigenic sites on the T<sub>M</sub> molecule and that these sites are distinct from sites that react with anti-T-serum (16). Conversely, the anti-GD248 membrane serum does not react with T<sub>N</sub> antigen (14).

The peptide homologies between T<sub>N</sub> and T<sub>M</sub> are in accord with previous data showing biochemical and immunologic similarities between T<sub>N</sub> and the surface membrane-associated SV40 protein(s) described as TSTA–TSSA (1–6). However, the full relationship between TSTA and/or TSSA and T<sub>M</sub> cannot yet be established, because there is another group of proteins that appear to be associated with SV40-induced transformation but not encoded by the SV40 genome. This group represents the 53,000- to 56,000-M<sub>t</sub> phosphoprotein (e.g., 19, 46) that do not react with anti-T-immunoglobulin intrinsically but can be immunogenic in some hosts bearing SV40-induced tumors (47). While our peptide analyses suggest, in agreement with data of others (39, 40), that this protein represents a host cell protein, data from other groups (20, 22) seem to indicate that this category of proteins exhibits partial peptide homology with SV40 T-antigen. A functional relationship between T<sub>M</sub> and the 55,000-M<sub>t</sub> protein is suggested because the two proteins are associated in the plasma membrane and the intracellular space (12, 17, 18, 20–22, 38–40, 42) and because they selectively associated in vitro (19).

REFERENCES

(39) LANE DP, CRAWFORD LV. T antigen is bound to a host protein in SV40-transformed cells. Nature 1979; 278:261–263.
Figure 1.—Bidimensional IFF-SDS-PAGE of plasma membranes from SV80 cells. Triton X-100-solubilized protein (≈0.4 mg) was separated by IEF in the first dimension and then, at right angles, fractionated according to $M_r$ in the presence of SDS and DTT. A) Coomassie blue protein staining. The SV40-specific pI-4.7-94,000-$M_r$ and pI-4.5-55,000-$M_r$ membrane components are identified by arrows. B) Electrophoretic blot of the slab gel in the pI-5.5-4.0 range after reaction with anti-T-serum and $^{125}$I-labeled protein A. $^{125}$I autoradiogram. The abscissa gives the pH gradient, and the ordinate gives the $M_r \times 10^3$ (K).

Figure 2.—SDS-PAGE of $^{35}$S-labeled membrane-associated SV40 T-antigen, $T_m$, immunoprecipitated from Triton X-100-solubilized SV80 plasma membranes. $^{35}$S autoradiogram. Right lane: Hamster anti-T-serum reacted with SV80 membrane proteins. Left lane: Normal hamster serum reacted with an identical quantity of membrane protein. $M_r \times 10^3$ (K).
FIGURE 3.—Bidimensional tryptic peptide maps of the 94,000-M₆ SV40-specific proteins, TN, TM, and the p1-4.7-94,000-M₆ component were labeled with ¹²⁵I, digested with TPCK-trypsin, and, in the first dimension (horizontal), separated electrophoretically (E) on thin-layer chromatography plates in acetic acid-formic acid (15%/5%, vol/vol). Fractionation in the second dimension (vertical) was by chromatography (C) in butanol-pyridine-acetic acid-water (32.5/25/5/20, vol/vol). A) Peptide map of TN; ¹²⁵I autoradiogram. B) Peptide map of TM; ¹²⁵I autoradiogram. C) Peptide map of p1-4.7-94,000-M₆ protein; ¹²⁵I autoradiogram. D) Schematic of map A. E) Schematic of map B. F) Schematic of map C. Peptides are numbered from 1 to 27. N and M behind the numbers indicate the peptide characteristic for TN and TM, respectively. Peptides found only in TM are assigned by letters. Results represent four independent experiments.
FIGURE 4.—Bidimensional mapping of tryptic peptides isolated from maps of $^{35}$S-labeled $T_N$, $T_M$, and the $p_l$-4.7-94,000-$M_r$ component. Separation in the first dimension (horizontal) was electrophoretically (E) on thin-layer chromatography plates in acetic acid-formic acid (15%/5%, vol/vol). Fractionation in the second dimension (vertical) was by chromatography (C) in butanol-pyridine-acetic acid-water (32.5/25/5/20, vol/vol). $^{35}$S autoradiograms. A) Peptides 6, 14, and 16-19 combined of $T_N$, $T_M$, and the $p_l$-4.7-94,000-$M_r$ component. B) Peptides 6, 14, and 16-19 combined of $T_M$ and the $p_l$-4.7-94,000-$M_r$ component. C) Peptides 3, 7, 8, 9, and 21-23 combined of $T_N$, $T_M$, and the $p_l$-4.7-94,000-$M_r$ component. D) Peptides 1, 2, 16, 18, and 19 combined of $T_M$ and the $p_l$-4.7-94,000-$M_r$ component.
**FIGURE 5.**—Bidimensional mapping of tryptic peptides isolated from maps of $^{125}$I-labeled TN, TM, and the pl-4.7-94,000-M$_r$ component. Separation in the first dimension (horizontal) was electrophoretically (E) on thin-layer chromatography plates in acetic-formic acid (15%/5%, vol/vol). Fractionation in the second dimension (vertical) was by chromatography (C) in butanol-pyridine-acetic acid-water (32.5/25/5/20, vol/vol). $^{125}$I autoradiograms. A) Peptides 3 combined of TM and the pl-4.7-94,000-M$_r$ component. B) Peptides 3 combined of TN, TM, and the pl-4.7-94,000-M$_r$ component. C) Peptides 7 combined of TN and TM. D) Peptides 8 combined of TN, TM, and the pl-4.7-94,000-M$_r$ component. E) Peptides 9 combined of TN, TM, and the pl-4.7-94,000-M$_r$ component. F) Peptides 16 combined of TN, TM, and the pl-4.7-94,000-M$_r$ component.

**FIGURE 6.**—Bidimensional tryptic peptide maps of the SV40 TM, surface iodinated in plasma membranes of SV80 cells with the use of lactoperoxidase-catalyzed radioiodination. After digestion with TPCK-trypsin, the peptides were separated electrophoretically in the first dimension (horizontal) on thin-layer chromatography plates in acetic acid-formic acid (15%/5%, vol/vol). Fractionation in the second dimension (vertical) was by chromatography in butanol-pyridine-acetic acid-water (32.5/25/5/20, vol/vol). $^{125}$I autoradiogram. Numbering of the peptides is as in fig. 3B. Results are of three independent experiments.
Figure 7.—Bidimensional tryptic peptide maps of SV40 TM. After digestion with TPCK-trypsin, the peptides were separated electrophoretically in the first dimension (horizontal) on thin-layer chromatography plates in acetic acid-formic acid (15%/5%, vol/vol). Fractionation in the second dimension (vertical) was by chromatography in butanol-pyridine-acetic acid-water (32.5/25/5/20, vol/vol). A) Peptide map of TM; 125I autoradiogram. B) Peptide map of TM; [14C] autoradiogram of TM metabolically labeled with [14C]glucosamine. Results of two independent experiments.

Figure 8.—Bidimensional tryptic peptide maps of SV40 TM and the 55,000-Mr membrane component. After digestion with TPCK-trypsin, the peptides were separated electrophoretically (E) in the first dimension (horizontal) on thin-layer chromatography plates in acetic acid-formic acid (15%/5%, vol/vol). Fractionation in the second dimension (vertical) was by chromatography (C) in butanol-pyridine-acetic acid-water (32.5/25/5/20, vol/vol). 125I autoradiograms. A) Peptide map of TM. B) Peptide map of the 55,000-Mr component. Results are of three independent experiments.