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Cellular and Cell-Free Synthesis of Simian Virus 40 T-Antigens in Permissive and Transformed Cells

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mRNA extracted from a variety of simian virus 40 (SV40)-infected monkey cell lines directs the cell-free synthesis of viral T-antigen polypeptides with molecular weights estimated as 90,000 and 17,000. However, the size, abundance, and distribution of these T-antigens synthesized in vivo vary greatly over a range of permissive and transformed cell lines. To establish whether differences in the size of T-antigen polypeptides can be correlated with the transformed or lytic state, recently developed lines of SV40-transformed monkey cells that are permissive to lytic superinfection were analyzed for T-antigen. In these cells, regardless of the state of viral infection, the size and pattern of T-antigen are the same. However, species differences in the largest size of T-antigen do exist. In addition to the 90,000 T-antigen, mouse SV3T3 cells contain a 94,000 T-antigen polypeptide as well. Unlike the size variations in monkey cells, which are due to modification of T-antigen polypeptides, the 94,000 SV3T3 T-antigen results from an altered mRNA, since the cell-free products of SV3T3 mRNA also contains the 94,000 T-antigen polypeptide.

The *A* gene product of simian virus 40 (SV40), responsible for initiation of viral DNA replication (18) and malignant cell transformation (3, 8, 11, 12, 19), has been identified in several ways as the viral tumor (T-) antigen. Through one approach, it has been shown that viral E-strand-specific mRNA directs the cell-free synthesis of polypeptides with molecular weights estimated as 90,000 and 17,000. These cell-free products can be immunoprecipitated with serum from SV40 tumor-bearing hamsters (anti-T serum), and they comigrate electrophoretically with similarly immunoprecipitated polypeptides from extracts of lytically infected BSC-1 monkey cells (16). Several different estimates of the monomer molecular weight of the largest T-antigen polypeptides have been reported, ranging from 70,000 (5) to 100,000 (21). The possibility exists that different cell lines used for extraction of T-antigen may exhibit varying sizes and distributions of T-antigen polypeptides, and that these variations may be related to the biological function of T-antigen in lytically infected and transformed cells.

To assess T-antigen synthesis in these two biological states, we analyzed the T-antigen polypeptides in a variety of permissive and nonpermissive cell lines, including a recently developed series (7) containing lines that are both transformed and fully susceptible to lytic superinfection. In many cases, the cellular T-antigen-specific polypeptides were compared with the T-antigen-specific cell-free products of mRNA purified from the corresponding lines. In this way we were able to assess the monomer size of the largest possible T-antigen polypeptide in each line. Although no consistent differences between the T-antigen in the transformed versus the lytic state of the virus were established, we have observed variations in the largest size of T-antigen as well as in the distribution of T-antigen-specific polypeptides over the range of different cell lines that we examined.

MATERIALS AND METHODS

Cells and viruses. All cell lines were grown in Dulbecco modified Eagle medium (GIBCO) with 10% calf serum. BSC-1, CV-1, and Vero monkey lines and SV40-transformed hamster cells were obtained from Flow Laboratories. AGMK cells were purchased from GIBCO. The T22 line of SV40-transformed AGMK cells was a gift from H. Shimojo. SV80-transformed cells were generously provided by David Livingston, and SV3T3 cells were provided by H. Green. A pair of tumorigenic and non-tumorigenic SV40-transformed Chinese hamster lines were provided by Nava Shacter.

The standard wild-type SV40 used was a plaque-purified stock of strain 777 grown as described previously (10). SV40 virus rescued from SV3T3, prepared according to published procedures (9), was kindly provided by Barbara Hoffman.

Extraction and immunoprecipitation of T-antigen from infected and transformed cells. Procedures were generally as described by Tegtmeyer et al. (20). Cultures of 2.0×10^6 cells were labeled for 2

h with [^{35}S]methionine (40 μCi/ml) in methionine-free medium prior to lysis in 0.3 ml of HIP buffer (pH 8.2) containing 0.14 M NaCl, 0.05 M *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES), 0.001 M $CaCl_2$, 0.001 M $MgCl_2$, 0.5% Nonidet P-40, 0.001 M 2-mercaptoethanol, and 15% glycerol. α-Toluene sulfonyl fluoride was added to a final concentration of 0.25 mg per ml of extract. Nuclei were removed by centrifugation at 2,000 rpm for 2 min, and cytoplasmic extracts were centrifuged at 36,000 rpm for 40 min in a Beckman 40 rotor at 0°C. Anti-T serum (0.005 to 0.01 ml) was added to 0.3 ml of the supernatant. After incubation at room temperature for 1 h, goat anti-hamster serum was added at equivalence, and the mixture was incubated at 0°C for several hours. Immunoprecipitates were washed three times by suspension in Tris-saline buffer (0.01 M Tris, pH 7.5–0.14 M NaCl) followed by centrifugation in a Beckman Minifuge for 2 min. The pellet was finally suspended in electrophoresis sample buffer (14) and heated at 90°C for 10 min.

Cell-free synthesis of T-antigen-specific polypeptides. Generally, RNA from the equivalent of 10^8 infected or transformed cells was extracted and subjected to oligo(dT)-cellulose chromatography (1); 1 to 2 μg of poly(A)-containing RNA was then added to the [^{35}S]methionine-containing wheat germ translation system (200 μl of reaction mixture) as previously described (15, 16). Cell-free products were centrifuged at 36,000 rpm prior to the immunoprecipitation, which was carried out as for labeled cellular extracts and similarly subjected to polyacrylamide gel electrophoresis.

Polyacrylamide gel electrophoresis. [^{35}S]methionine-labeled immunoprecipitates in electrophoresis sample buffer were heated at 95°C for 10 min prior to electrophoresis in 10 to 20% gradient slab polyacrylamide gels as described (15). Gels were dried and exposed to Kodak SB-5 film to obtain autoradiographs of labeled polypeptides.

Materials. Hamster anti-SV40 T serum and goat anti-hamster serum were obtained through the courtesy of J. Gruber, National Cancer Institute, Bethesda, Md. Wheat germ was obtained from the Bar Rav Mills, Jaffa, Israel. [^{35}S]methionine was purchased from The Radiochemical Centre, Amersham, England.

RESULTS

T-antigen synthesis in permissive cells. Variations in size and pattern of T-antigen polypeptides may be related to either the extraction procedure or the properties of the host cells. Since the extraction techniques used were always the same and frequently in parallel, differences observed were attributed to cell line variations. Three different permissive lines were analyzed for their T-specific polypeptides: CV-1, BSC-1, and Vero (Fig. 1a, e, and g) as well as AGMK primary cells (see Fig. 7). It was found that there were variations among the pattern of polypeptides immunoprecipitated from extracts of the different lines. However, the mRNA from each line directed the synthesis of two major T-antigen-specific products with molecular weights estimated as 90,000 and 17,000 (Fig. 1b, d, and f). Generally, immunoprecipitates from BSC-1 cells contained the 90,000 polypeptide as the most prominent T-antigen-specific component (Fig. 1a), whereas CV-1 cells contained, in addition, approximately equal amounts of other T-antigen-specific polypeptides with estimated molecular weights of 86,000 and 80,000 (Fig. 1e). Extracts of Vero cells produced only a smaller-sized T-antigen polypeptide estimated as 80,000 (Fig. 1g). AGMK primary cells, from which BSC-1 and CV-1 lines are derived, generally exhibited a polypeptide pattern somewhat similar to that of BSC-1 cells (see Fig. 7).

Evidence that smaller forms of T-antigen in the size range of 80,000 to 90,000 are due to cellular modification such as proteolytic cleavage was observed after co-incubation of T-antigen synthesized in vitro with an extract of infected BSC-1 cells. The largest cell-free product was reduced in size after incubation and comigrated with the 80,000 minor polypeptide seen in extracts of [^{35}S]methionine-labeled and immunoprecipitated BSC-1 cells (Fig. 2). It is thus likely that smaller forms of T-antigen may result from cellular cleavage enzymes either present or activated during the extraction procedure. Evidence for this has been previously reported (4, 20). The considerable number of extra immunoprecipitated products in Fig. 2c, which should be similar to Fig. 1b, is not clearly understood, but may result from an unusually great amount of prematurely terminated polypeptide chains, a feature of some wheat germ protein-synthesizing systems. The appearance of anti-T immunoprecipitable polypeptides in sizes ranging from 17,000 to 40,000 occurs very rarely and only with cell-free products, never with cell extracts. The absence of these extra products in Fig. 2b may be related to their co-incubation with cell extracts. Despite a size alteration in the 90,000 product, there was no apparent effect on the 17,000 product, indicating that it is more resistant to cleavage under these conditions.

SV40 T-antigen synthesis in transformed cells. The synthesis of T-antigen-specific polypeptides was analyzed in several SV40 transformants which were labeled and extracted as with permissive lines (Fig. 3). In all observed transformed lines, there were far fewer polypeptides smaller than 90,000 than observed in any permissive cells, and generally the major T-antigen comigrated electrophoretically with the 90,000 T-antigen-specific cell-free product of SV40 mRNA from monkey cells. However, in the SV3T3 mouse line (Fig. 3), an additional polypeptide larger than the 90,000 product, estimated as 94,000, was specifically immunoprecipitated. To assess whether the 94,000 poly-

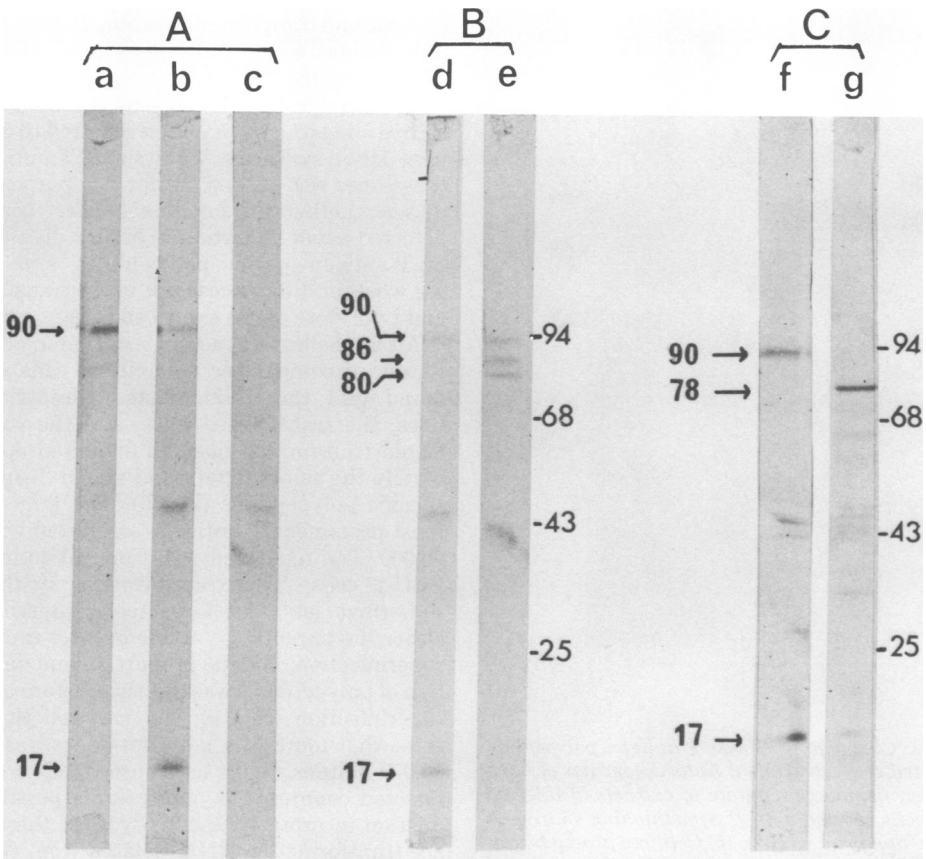


FIG. 1. *Cellular and cell-free synthesis of T-antigen polypeptides from permissive cells. Infected cultures were labeled with [^{35}S]methionine 46 to 48 h postinfection, and immediately thereafter extracts were prepared and subjected to immunoprecipitation and polyacrylamide gel electrophoresis. Groups A, B, and C are autoradiograms of [^{35}S]methionine-labeled polypeptides from experiments with BSC-1, CV-1, and Vero cells, respectively. Group A: (a) anti-T-antigen immunoprecipitate of polypeptides extracted from BSC-1 cells; (b) anti-T-antigen immunoprecipitate of polypeptide products synthesized in response to mRNA from BSC-1 cells; (c) hamster control serum immunoprecipitate of polypeptides extracted from BSC-1 cells. Group B: (d) anti-T-antigen immunoprecipitate of polypeptide products synthesized in response to mRNA from CV-1 cells; (e) anti-T-antigen immunoprecipitate of polypeptides extracted from CV-1 cells. Group C: (f) anti-T-antigen immunoprecipitate of products synthesized in response to mRNA from Vero cells; (g) anti-T-antigen immunoprecipitate of polypeptides extracted from Vero cells. Groups A and B were subjected to electrophoresis in the same slab gel, whereas group C was run in a separate gel. Numbers on the right-hand side of (e) and (g) refer to kilodalton molecular weights of marker proteins from top: phosphorylase (94), bovine serum albumin (68), actin (43), and chymotrypsinogen (25). Numbers on the left of (a), (d), and (f) refer to estimated molecular weights of T-antigen-specific polypeptides by comparison with markers.*

peptide was the result of cellular modification such as glycosylation, which may produce reduced electrophoretic migration, mRNA was extracted from SV3T3 cells and translated in the wheat germ system. The products were immunoprecipitated with anti-T serum and analyzed electrophoretically in comparison with SV3T3 cellular T-antigen and T-antigen from permissive cells. Immunoprecipitates of SV3T3 mRNA cell-free products contained both the 94,000 and 90,000 T-antigen polypeptides observed in SV3T3 cell extracts (Fig. 4). Thus, the size variation in SV3T3 T-antigen is directly related to an alteration in the viral mRNA rather than a cellular modification. Two other T-antigen polypeptides directed by SV3T3 mRNA with estimated molecular weights of 30,000 and 17,000 were immunoprecipitated with anti-T serum. The 17,000 polypeptide comigrated electrophoretically with the 17,000 polypeptide immunoprecipitated from extracts of SV40-infected monkey cells. A subclone of the SV3T3 cells used herein also gave rise to the 94,000 and 90,000 polypeptides, although there was propor-

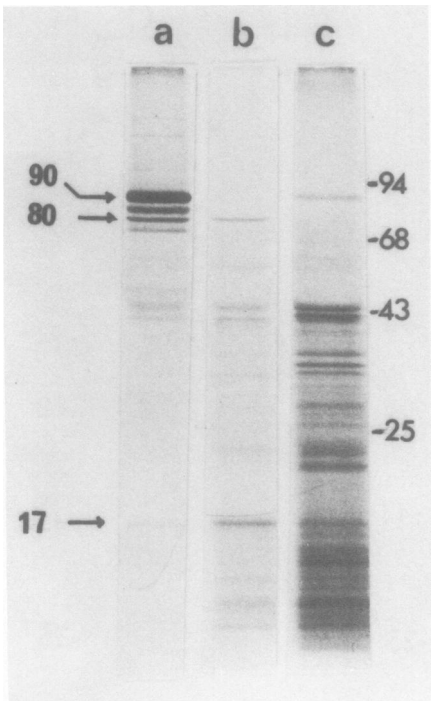


FIG. 2. *Cleavage of 90,000 T-antigen polypeptide by infected cell extracts. (a) Autoradiograms of anti-T-antigen immunoprecipitate of extracts of infected BSC-1 cells labeled with [^{35}S]methionine 46 to 48 h postinfection prior to lysis. (b) Cell-free products synthesized in response to 3 μg of mRNA from infected BSC-1 cells which were incubated with 0.3 ml of an extract of unlabeled, infected cultures for 1 h at 0°C. The extract was prepared from 4 × 10^6 BSC-1 cells 48 h postinfection (parallel cultures to those in [a]) by washing the cells three times in Tris-saline and suspending in 0.3 ml of S buffer containing 0.1 M NaCl, 0.003 M HEPES, pH 7.5, and 0.001 M EDTA before sonic treatment in a Raytheon sonic oscillator for 1 min at half-maximum intensity. (c) Cell-free products as in (b) after similar incubation in 0.3 ml of S buffer alone. Numbers at the right of (c) refer to migration of markers as in Fig. 1.*

tionally more of the 90,000 polypeptide (not shown). It is noteworthy that virus rescued from SV3T3 cells produced only the 90,000 T-antigen polypeptide when used to infect permissive cells (Fig. 4).

T-antigen synthesis in transformed permissive cells. Recently, Gluzman et al. (7) have developed a series of SV40-transformed monkey cells (CV-1 lines) which are fully permissive to lytic superinfection. The properties and development of these lines have been described elsewhere (7); briefly, the cells are considered to be transformed because of their growth properties and because they contain SV40 T-antigen as measured by both immunofluorescent antibody staining and complement fixation. Because these cells do not support the growth of SV40 *tsA* mutants at the restrictive temperature, it was concluded that their SV40 gene *A* product is defective and lacks the function required to initiate viral DNA synthesis. Analysis of T-antigen in these lines was carried out for two purposes: (i) to see whether the functional defect could be detected as an alteration in size or distribution of T-antigen-specific polypeptides, and (ii) to see whether differences exist in the transformed and lytic state of the same cell lines.

After labeling, extraction, and immunoprecipitation of comparable amounts of cells, it was found that the SV40-infected parental CV-1 lines, the transformed lines, and the superinfected transformed lines all exhibited approximately the same pattern and size of T-antigen-specific polypeptides (Fig. 5). The largest and most prominent T-antigen comigrated with the 90,000 T-antigen isolated from SV40-infected BSC-1 cells. The transformants synthesized somewhat less anti-T-reactive material than either the parental CV-1 line or after their own superinfection, but the proportion and distribution of polypeptides was the same before or after superinfection (Fig. 6); the parental line had somewhat more of a polypeptide, estimated as 80,000 daltons, than the transformed or superinfected counterparts, indicating a possible reduction in proteolytic activity after these cells are transformed. mRNA isolated from the C-2 transformed variant of CV-1 cells directs the cell-free synthesis of the 90,000 and 17,000 T-antigen polypeptides as observed with all other permissive lines (data not shown).

A variant of the independently established (17), transformed monkey T22 cell line (T-1), which was selected for increased ability to support lytic superinfection, was also tested by the same techniques for T-antigen synthesis. As seen with the transformed CV-1 lines developed by Gluzman et al. (7), it was found that the T-antigen-specific polypeptide pattern was unchanged in the parental lytic, transformed, and superinfected state (Fig. 7).

DISCUSSION

We have presented a comparative study of SV40 T-antigen polypeptides extracted from cells and synthesized in the wheat germ cell-free translation system. It is unlikely that products synthesized in wheat germ extracts are modified in a manner which would affect electrophoretic mobility. Known glycoproteins such as the G protein of vesicular stomatitis virus (2) or the capsid protein of measles virus (S. Rozenblatt and C. Prives, unpublished data) have lower molecular-weight estimates when synthesized in

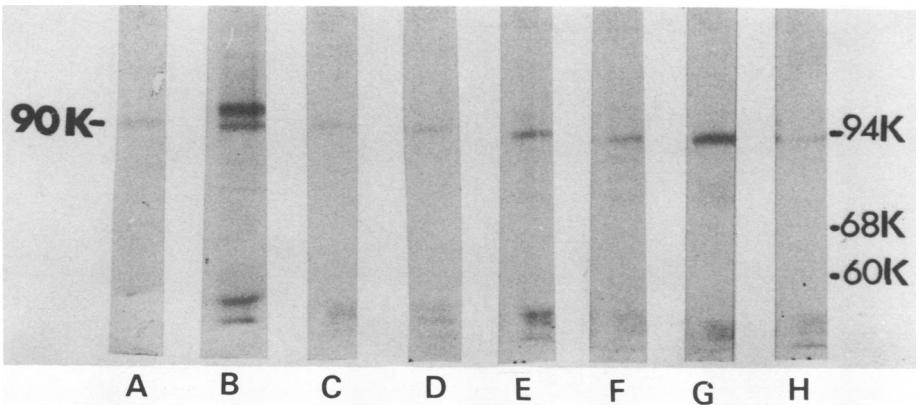


FIG. 3. *T-antigen synthesis in various SV40-transformed cell lines. Autoradiograms of anti-T-antigen immunoprecipitate of [^{35}S]methionine-labeled (A) cell-free products of mRNA isolated from BSC-1 cells; (B–F) extracts of transformed SV3T3 mouse cells (B), SV80 human cells (C), tumorigenic Chinese hamster cells (D), non-tumorigenic subclone of Chinese hamster cells (E), and BSC-1 monkey cells (F); (G) extracts of infected BSC-1 cells as described in Fig. 1; and (H) extracts of transformed Syrian hamster cells. Cultures (2 × 10^6 cells) were labeled for 2 h with [^{35}S]methionine (40 μCi/ml) in methionine-free medium prior to extraction in 0.3 ml of HIP buffer and immunoprecipitation as described in the text. Only upper-molecular-weight-range T-antigen-specific polypeptides are shown in these autoradiograms. Numbers at the right of (H) refer to migration in the same slab gel of markers whose kilodalton molecular weights, from the top, are: phosphorylase (94), bovine serum albumin (68), and tubulin (60).*

the wheat germ system, presumably due to lack of glycosylation of newly synthesized polypeptides in vitro. Although phosphorylation of products synthesized in wheat germ extracts is not excluded, we have not succeeded in demonstrating this activity. It is therefore suggested that the largest possible T-antigen in any cell line would be the largest cell-free product primed by SV40 mRNA from the same given line. Variations in the estimated upper molecular weight of T-antigen, all derived from polyacrylamide gel electrophoresis analysis, have been reported, including 90,000 (16), 94,000 (4), and 100,000 (21). Tegtmeyer et al. have shown that varying the polyacrylamide gel buffer system results in altered electrophoretic mobility of T-antigen with respect to the 94,000 phosphorylase marker (20). It is therefore important to compare all T-antigens in the same slab gel. For example, the 90,000 T-antigen polypeptide which we have immunoprecipitated from extracts of infected BSC-1 cells comigrates electrophoretically with the T-antigen estimated by Tegtmeyer as 100,000 (P. Tegtmeyer, personal communication).

Despite variations in T-antigens extracted from different permissive cell lines, their mRNA directs the cell-free synthesis of only two major T-antigen polypeptides, with molecular weights estimated in this gel system as 90,000 and 17,000. These two proteins contain common sequences as determined by tryptic peptide mapping, but differ with respect to their antigenic specificities and DNA binding properties (C. Prives and Y. Beck, INSERM Colloq., in press). It is not yet definitely established whether they are encoded by the same species of viral mRNA. The detection and identification of the 17,000 polypeptide in cellular extracts is unpredictable, as can be seen in the autoradiograms shown herein—sometimes it is not observed, and occasionally it is obscured by the appearance of cellular polypeptides in the same size range. However, it is consistently observed as a major immunoprecipitable cell-free product of mRNA isolated from all cells transformed or lytically infected by wild-type SV40 that we have examined. The fact that the ratio of the 17,000 to the 90,000 T-antigen is considerably greater in the cell-free products than in cellular extracts is most likely due to inefficient translation of the larger product in the wheat germ system. In a recently developed reticulocyte translation system with reduced tendency to prematurely terminate polypeptide chains (14), the proportions of 90,000 and 17,000 T-antigens are closer to that observed in cellular extracts (H. Shure and C. Prives, unpublished observations).

Considerable variations in the size and distribution of T-antigen in different permissive cell lines were observed in this study. Tegtmeyer et al. (20) have previously described differences in T-antigen polypeptides among various cell lines and have presented evidence that the lower-molecular-weight components are generated during the extraction procedure. Rozenblatt

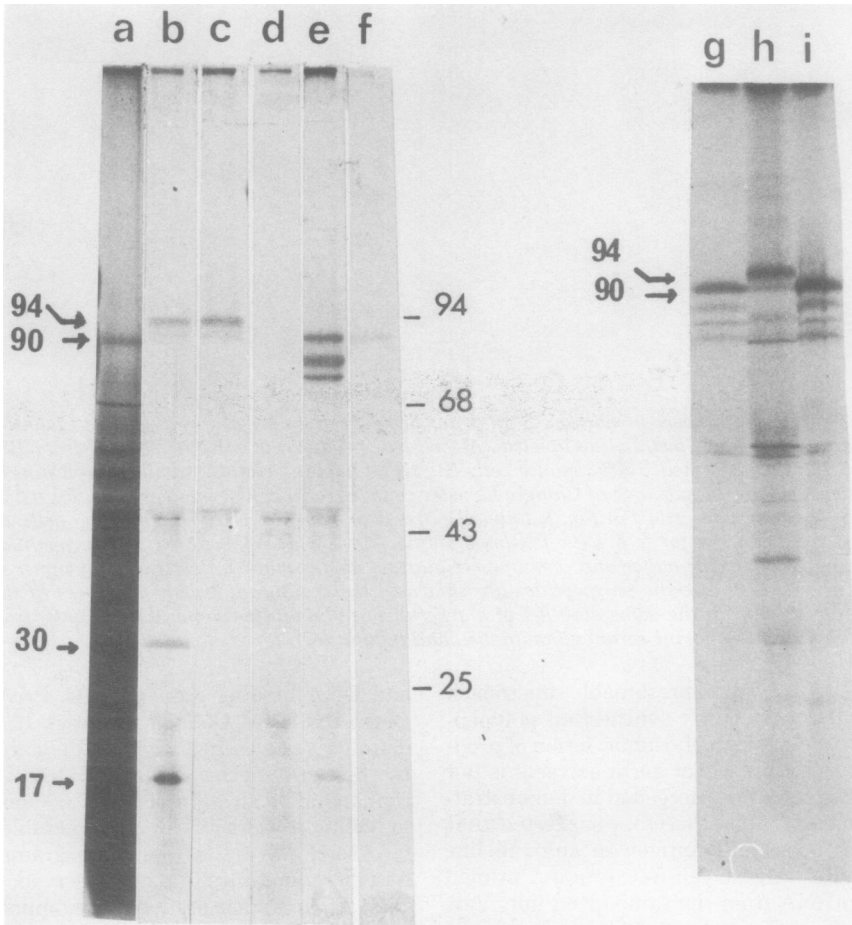


FIG. 4. *T-antigen synthesis in SV3T3 cells. (a–f) and (g–i) are autoradiograms of separate slab gels. (a–f) Autoradiograms of [^{35}S]methionine-labeled cell-free products directed by 0.2 μg of mRNA from SV3T3 cells (a); anti-T-antigen immunoprecipitate of products directed by 3 μg of mRNA from SV3T3 cells (b); anti-T-antigen immunoprecipitate of SV3T3 cells as in Fig. 3 (c); hamster control immunoprecipitate of cell-free products directed by 3 μg of mRNA from SV3T3 cells (d); anti-T-antigen immunoprecipitate of extracts of SV40-infected CV-1 cells as in Fig. 1 (e); and anti-T-antigen immunoprecipitate of extracts of infected BSC-1 cells as in Fig. 1 (f). Numbers at the right of (f) refer to migration positions of marker proteins as in Fig. 1. (g–i) Autoradiograms of anti-T-antigen immunoprecipitates of [^{35}S]methionine labeled extracts of BSC-1 cells infected by SV40 virus rescued from SV3T3 cells as described in the text and Fig. 1 (g); SV3T3 cells (h); and BSC-1 cells infected by SV40 virus strain 777 as used in all experiments (i).*

(personal communication) has shown that repeated freezing and thawing of cell extracts increases the proportion of smaller T-antigen polypeptides; Carroll and Smith (4) have reported that T-antigen extracted from one permissive line has a molecular weight estimated as 84,000, while that isolated from another transformed line is 94,000. The results of the survey presented herein suggest that either their choice of extraction procedure or their choice of a permissive line that is analogous to the Vero line, which we have used, may have resulted in the reported size discrepancy.

In contrast to the permissive lines analyzed, the different SV40 transformants exhibited far fewer T-antigen polypeptides smaller than 90,000. Although it is likely that the monkey kidney cell lines are particularly abundant in proteases, it is also possible that transformed cells have relatively reduced amounts. This is suggested by a comparison of the T-antigen polypeptides in the infected CV-1 cells and their transformed and superinfected transformed counterparts. CV-1 cells have greater quantities of the smaller 80,000 T-antigen polypeptide than the other two cases, indicating a reduction in

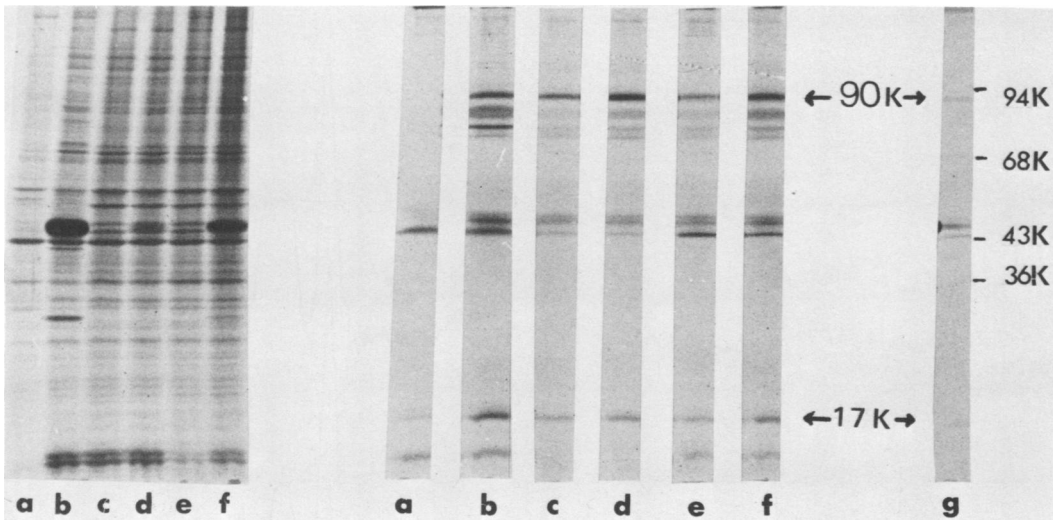


FIG. 5. *T-antigen polypeptides synthesized in transformed CV-1 cells. (Right) Autoradiograms of [^{35}S]methionine labeled anti-T-precipitate of (a) uninfected CV-1 cells, (b) SV40-infected CV-1 cells, (c) uninfected SV40-transformed C-2 lines of CV-1 cells, (d) SV40-infected C-2 cells, (e) uninfected SV40-transformed C-6 line of CV-1 cells, (f) SV40-infected C-6 cells, and (g) SV40-infected BSC-1 cells. Labeling, extraction, and immunoprecipitation of cultures (2 × 10^6 cells) were as described in the text and Fig. 1 and 4. (Left) (a–f) represent 10 μl of [^{35}S]methionine-labeled cell extracts from which immunoprecipitates in (a–f) on the right were prepared.*

proteolytic activity after the cells become transformed. The similarity in pattern of T-antigen polypeptides in transformed and superinfected CV-1 cells shows that, under the extraction and immunoprecipitation conditions used, there was no difference in their distribution. This suggests that by using this method of extraction and identification of T-antigen, it would be unlikely that a specifically cleaved T-antigen polypeptide could be identified as the functionally active species. It also shows that, as with T-antigen polypeptides isolated from cells infected with *tsA* mutants at the nonpermissive temperature (2), the lesion in function of the transformed monkey cells is not reflected in their size or distribution. Deppert et al. (6) have described experiments demonstrating the existence of a class of T-antigen-specific polypeptides in cells infected with adenovirus-SV40 hybrids, which are stabilized by their association with cell membranes. As the procedures described herein involved solubilization of membranes and associated material with the detergent Nonidet P-40, it is possible that an alternate class of unstable, membrane-associated T-antigen polypeptides exists in these cells and that variations in different functional forms of T-antigen may be detected in that class. Experiments to test this possibility are in progress. In the absence of any detectable difference in T-antigen polypeptides, it may be suggested that the lesion in gene *A* function in CV-1 transformed cells may be the same as or analogous to a point mutation resulting in an amino acid substitution affecting one but not all functions of T-antigen. Such a difference may be detected by tryptic peptide mapping, amino-terminal sequencing, or sequencing the viral DNA integrated in these lines.

A point of interest arising from this study was the observation that, in SV3T3 cells, both the 90,000 T-antigen polypeptide and another, more prominent 94,000 T-antigen polypeptide are immunoprecipitated. The presence of similar proportions of these two polypeptides among the immunoprecipitated cell-free products of SV3T3 mRNA indicates that these T-antigen polypeptides observed in SV3T3 cell extracts are a function of altered mRNA rather than cellular modification of the protein. It is intriguing that the size of the SV3T3 17,000 T-antigen remained unchanged. The presence of the smaller, 90,000 polypeptide among the mRNA-directed cell-free products indicates that it is not likely to be a proteolytic product of the 94,000 protein and may be either the result of a separate initiation of the altered SV40 mRNA or a second class of normal viral mRNA coding for the typical 90,000 product. Transformed mouse cells generally have been shown to contain more than one viral DNA copy per cell genome (13), which may result in more than one class of virus-specific mRNA. The presence of a larger T-antigen poly-

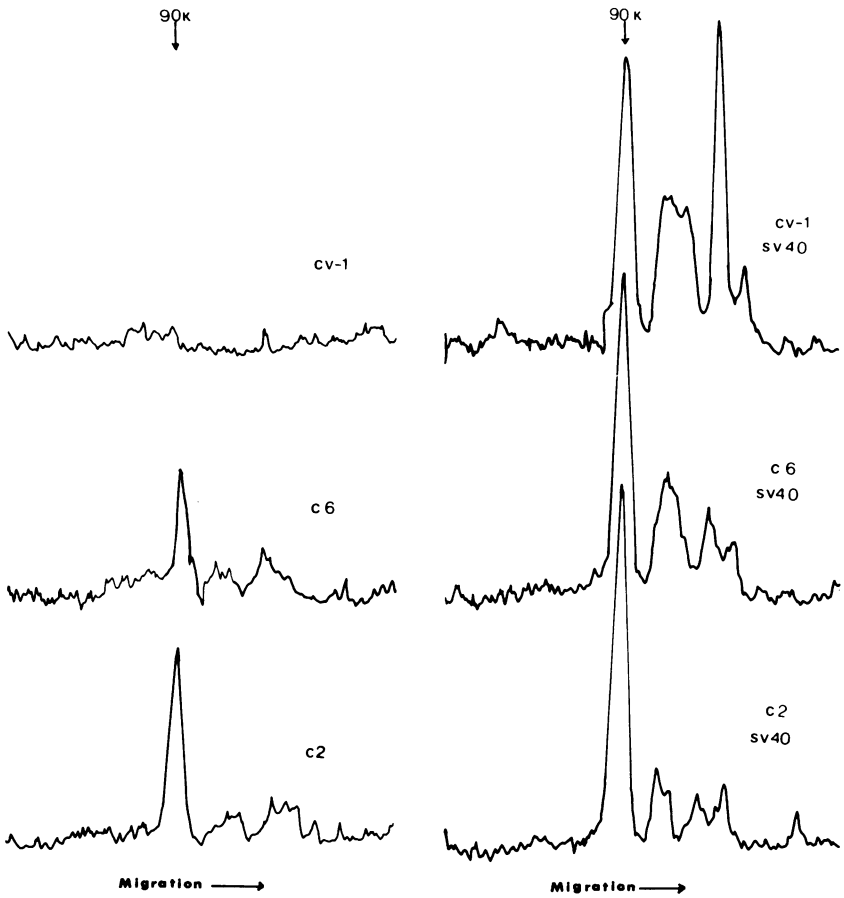
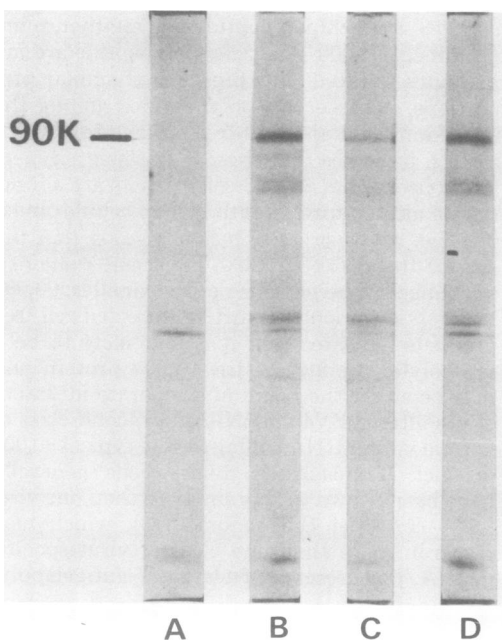


FIG. 6. *T-antigen polypeptides synthesized in transformed CV-1 cells. Densitometer tracings of anti-T-antigen immunoprecipitates in the 90,000 region of autoradiograms (a–f) shown in Fig. 5.*



peptide is not exclusive to mouse SV3T3 cells; we have also observed one additional but proportionally less prominent 94,000 component in similar immunoprecipitates from an SV40-transformed human cell line (data not shown) and trace amounts in immunoprecipitates from SV40-transformed golden hamster cells. It will be of interest to compare the amino-terminal sequences of normal and larger T-antigens. If they differ and cannot be aligned with the DNA sequence of the integrated SV40 genome, this may provide information about possible integration sites in host chromosomes. The observation that only the 90,000 polypeptide is expressed following infection of monkey cells with SV40 that was rescued from SV3T3 cells may indicate

FIG. 7. *T-antigen polypeptides synthesized in transformed AGMK cells. Immunoprecipitation of extracts of (A) AGMK cells, (B) SV40-infected AGMK cells at 48 h postinfection, (C) T-1 subclone of transformed T22 cells, and (D) SV40-infected T1 subclone of transformed T22 cells. Labeling, extraction, and immunoprecipitation of cultures (2×10^6 cells) were as described in the text and Fig. 1 and 4.*

that monkey cells cannot synthesize a T-antigen that is larger than 90,000, that the integrated genome representing the 94,000 protein is incomplete in this particular cell line, or that during lytic infection only the 90,000 T-antigen is functional.

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