Comparison of T Antigen-associated Host Phosphoproteins from SV40-infected and -transformed Cells of Different Species

By ELLEN FANNING,1* CHRISTA BURGER1 AND ELIZABETH G. GURNEY2

1 Fakultät für Biologie, Universität Konstanz, D-7750 Konstanz, Federal Republic of Germany and 2 Department of Biology, University of Utah, Salt Lake City, Utah 84112, U.S.A.

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SUMMARY

Simian virus 40 (SV40)-infected and -transformed cells contain, in addition to the virus-coded tumour antigens, one or more 48K to 56K host tumour antigens. At least part of this class of host proteins exists as a fast-sedimenting complex with the SV40 large T antigen. The host proteins associated with the large T antigen in SV40-transformed monkey, mouse and human cells and SV40-infected monkey cells were compared by two-dimensional gel electrophoresis and V8 partial proteolysis peptide mapping. Although these proteins differed slightly in apparent mol. wt. and peptide pattern, they migrated identically in isoelectric focusing gels. These results suggest that the cellular proteins associated with large T antigen in different hosts are very closely related to each other. Despite their similarities, the 55K proteins from different host cells form complexes of different stabilities with large T antigen, as judged by spontaneous dissociation of the complexes during storage, and the fractions of the 55K cellular protein and large T antigen found free and in the complexed form in each different host cell.

INTRODUCTION

Cells infected or transformed by simian virus 40 (SV40) contain two virus-coded early proteins called the large and small tumour antigens (T antigens) (for review, see Rigby, 1979; Crawford, 1980). Recently, SV40-transformed cells were shown to contain one or more additional tumour antigens of 48000 to 56000 mol. wt. which are coded by the host cell (Chang et al., 1979; Kress et al., 1979; Lane & Crawford, 1979; Linzer & Levine, 1979; Melero et al., 1979; Smith et al., 1979). These proteins have been referred to as non-viral tumour antigens (NVT), Tau antigens, and 53K, 54K, 55K etc. proteins. Regardless of their apparent mol. wt. they will be referred to here as 55K proteins. Similar tumour antigens have been observed in mouse cells transformed by chemicals and RNA tumour viruses (DeLeo et al., 1979), in uninfected mouse embryonal carcinoma cells (Linzer & Levine, 1979) and in Epstein-Barr virus-transformed human cells (Luka et al., 1980). These 55K proteins are also present in much smaller amounts in SV40-infected and uninfected mouse 3T3 and monkey kidney cells (Linzer et al., 1979; Gurney et al., 1980; Simmons, 1980). In SV40-transformed and -infected cells, at least part of this host protein is phosphorylated and rather tightly associated with a highly phosphorylated class of large T antigen to form a fast-sedimenting complex (Lane & Crawford, 1979; McCormick & Harlow, 1980; Fanning et al., 1981). The biological functions of the 55K host proteins and the complex of 55K protein with SV40 T antigen are unknown.
The purpose of this study was to compare the 55K host proteins associated with the large T antigen in SV40-infected and -transformed cells of different species, to compare the stabilities of the 55K protein–T antigen complexes from different host cells and to compare the fraction of the total intracellular T antigen and 55K phosphoprotein found in these complexes in different host cells.

**METHODS**

**Cells and virus.** The following cell lines were used: SVT2, an SV40-transformed Balb/c mouse cell line (Aaronson & Todaro, 1968) kindly provided by Frank O’Neill; SV80, an SV40-transformed human cell line (Todaro et al., 1966) obtained from A. J. Levine; C2, a CV-1 monkey kidney cell line transformed by u.v.-inactivated SV40 (Gluzman et al., 1977); CV-1P and TC-7 monkey kidney cell lines. Cells were cultured in monolayers in Dulbecco’s modified Eagle’s medium with 10% newborn calf serum (Gibco). Subconfluent CV-1P and TC-7 cells were infected with 1 to 5 p.f.u./cell of SV40, strain SV-S, as described previously (Baumgartner et al., 1979).

**Labelling and preparation of cell extracts.** Cell cultures to be labelled with [35S]methionine were starved for 1 h in methionine- and serum-free medium and then labelled with 100 μCi/ml [35S]methionine (Amersham Corporation) for the desired time period. Similarly, cells to be labelled with 32PO4 were incubated for 1 h in phosphate- and serum-free medium and then labelled for 2 h with 50 to 100 μCi/ml or for 14 to 15 h with 40 to 50 μCi/ml 32PO4. In some experiments, cells were labelled in leucine-, phosphate-, serum-free medium with 30 μCi/ml [3H]leucine (Amersham Corporation) and 40 to 50 μCi/ml 32PO4. At the end of the labelling period, the cultures were rinsed 3 times with cold Hanks’ balanced salt solution, scraped from the plates and pelleted. They were resuspended (107 cells/ml) in 50 mM-tris-HCl pH 8, 120 mM-NaCl, 0.5% Nonidet P40 (Smith et al., 1978), extracted for 30 min at 0°C and centrifuged again. The supernatant was used directly for immunoprecipitation, except where stated otherwise.

**Immunoprecipitation.** Immunoprecipitation with anti-SV40 hamster tumour serum and formaldehyde-fixed *Staphylococcus aureus* (Kessler, 1975) was carried out as reported previously (Smith et al., 1978; Fanning et al., 1981). The hamster tumour sera used in these experiments were able to react with native and denatured large T antigen and small T antigen, but failed to react with the denatured 55K protein from SV80 cells (Fanning et al., 1981). The clone 122 monoclonal antibody directed against 55K host proteins has been described previously (Gurney et al., 1980).

**SDS–polyacrylamide gel electrophoresis.** SDS–PAGE was carried out according to Laemmli (1970), using proteins of known mol. wt. as markers (Fanning et al., 1981).

Two-dimensional gel electrophoresis was performed essentially as described elsewhere (O’Farrell, 1975) with the following exceptions. The final bacterial pellet containing immune complexes was resuspended in 30 μl of 2% SDS, 20 mM-dithiothreitol, incubated for 1 h at room temperature, then boiled for 3 min and centrifuged. The supernatant was boiled for another 3 min and then 30 μl SDS sample buffer (Laemmli, 1970) was added. This procedure was required to completely denature the immune complexes and allow migration of the denatured proteins into the focusing gels. The SDS apparently dissociates from the denatured proteins during focusing without affecting their pI (Manrow & Dottin, 1980). Alkylation of the denatured proteins with N-ethylmaleimide or iodoacetamide did not improve migration of the proteins into the gels or the sharpness of the focused bands (not shown). The sample was then adjusted to 9 M-urea, layered on to the alkaline end of the isoelectric focusing gel and overlaid with 6 M-urea. The gels were focused using pH 3.5 to 10 or pH 6 to 8 ampholytes (LKB) according to O’Farrell (1975) at 400 V for 14 h and then 800 V for 1 h at room temperature. The gels were embedded in 1% agarose on top of 12.5% SDS–polyacrylamide
gels and electrophoresed in the second dimension without equilibration (O'Farrell, 1975). After isoelectric focusing, the pH gradient, monitored in a parallel gel according to Righetti & Drysdale (1976), was approximately linear between pH 4.5 to 8.5 (pH 3.5 to 10 ampholytes) and pH 5.5 to 7.5 (pH 6 to 8 ampholytes).

Partial proteolysis peptide mapping. This was performed with V8 protease as described earlier (Cleveland et al., 1977).

RESULTS

Immunoprecipitation of tumour antigens from SV40-transformed and -infected cells used in this study

SV40-transformed human, monkey and mouse cells and productively infected monkey cells were labelled with $^{32}$PO$_4$ for 15 h. Proteins extracted from each cell line were immunoprecipitated with anti-SV40 hamster tumour serum and normal serum and separated by SDS-PAGE. The large T antigen and the 55K host phosphoproteins were specifically immunoprecipitated from all four cell extracts. The apparent mol. wt. of the 55K protein from the SVT2 mouse cells was smaller than that from SV40-transformed human and monkey cells (Fig. 1). The 55K protein from productively infected monkey cells co-migrated with that from transformed human and monkey cells. Since the tumour antiserum used for immunoprecipitation did not react with the isolated denatured 55K protein (Fanning et al., 1981), it is likely that these proteins co-precipitate as a complex with the large T antigen (Lane & Crawford, 1979).

Partial proteolysis peptide mapping of the T antigen-associated 55K proteins from different host cells

The peptide patterns of the T antigen-associated host proteins from SV40-transformed mouse, human and monkey cells were compared by V8 protease digestion according to Cleveland et al. (1977). Extracts of $[^{35}S]$methionine-labelled cells were immunoprecipitated with anti-SV40 hamster tumour serum. The 55K proteins were separated on SDS-polyacrylamide gels, excised and placed on a second gel for V8 digestion (Fig. 2). The pattern of methionine-containing peptides from the C2 55K protein closely resembled the pattern of those from the SV80 55K protein, but they were not identical. The SVT2 55K peptide pattern differed distinctly from the others, although several peptides did migrate identically with those from human and monkey cells. While this work was in progress, tryptic peptide maps of the 55K proteins from SV40-transformed mouse, rat and human cells (Simmons et al., 1980) and from productively infected and uninfected monkey cells (Simmons, 1980) were published. These reports confirm that all of these 55K proteins have closely related peptide patterns, as might be predicted from their common property of associating specifically with the large T antigen.

Two-dimensional gel electrophoresis

The 55K phosphoproteins associated with SV40 large T antigen in SV40-transformed mouse, monkey and human cells and in monkey cells productively infected with SV40 were compared by two-dimensional electrophoresis. The 55K proteins from $^{32}$PO$_4$-labelled cells were immunoprecipitated as a complex with large T antigen using anti-SV40 tumour serum. The immunoprecipitates were dissociated and then applied to isoelectric focusing gels containing pH 3.5 to 10 or pH 6 to 8 ampholytes. After focusing, the gels were electrophoresed in the second dimension in SDS-polyacrylamide gels. In pH 3.5 to 10 ampholytes the phosphorylated large T antigen migrated as a broad band between approx.
Fig. 1. SDS-polyacrylamide gel autoradiograms of \(^{32}\)PO\(_4\)-labelled T antigens from SV40-infected and -transformed cells. Proteins were immunoprecipitated from extracts of SV40-infected CV-1P (39 h after infection), C2, SV80 and SVT2 cells with normal hamster serum (N) and anti-SV40 hamster tumour serum (T) as indicated. The positions of marker proteins of known mol. wt. are shown on the left.

Fig. 2. Partial peptide mapping of T antigen-associated 55K proteins from human, monkey and mouse cells transformed by SV40. C2, SV80 and SVT2 cells were labelled for 4 h with \(^{35}\)S-methionine as described in Methods. Cell extracts were immunoprecipitated with anti-SV40 tumour serum and the immunoprecipitated proteins were electrophoresed on a 7.5% SDS-polyacrylamide gel. Each of the 55K protein bands, localized by autoradiography of the wet gel, was excised and placed on a 15% gel with 1 μg V8 protease (Miles Laboratories). The digestion was carried out during electrophoresis (Cleveland et al., 1977). Methionine-containing peptides were visualized by fluorography (Bonner & Laskey, 1974). (a) SVT2, (b) SV80, (c) C2.

pH 6·5 and 7·3 (not shown), as observed previously (Crawford & O'Farrell, 1979; Greenspan & Carroll, 1979; Lanford & Butel, 1980; Palme & Henning, 1980). Occasionally, the large T antigen focused more sharply, sometimes as a doublet (Fig. 3a), but attempts to correlate this behaviour with the source of T antigen, sample preparation, cell density, time after infection or differential phosphorylation have so far been unsuccessful. The 55K phosphoproteins from mouse, monkey and human cells transformed by SV40 and CV-1P cells infected with SV40 migrated in pH 6 to 8 ampholytes at about pH 6 ± 0·2 (e.g. Fig. 3) and in pH 3·5 to 10 ampholytes at pH 6·6 ± 0·3 (not shown). No reproducible difference among them could be distinguished.

Both the large T antigen and the 55K host phosphoproteins from immunoprecipitates focused over the same broad pH range. This behaviour could be caused by formation of aggregates of 55K protein with large T antigen during focusing. Thus, isolated denatured 55K
**SV40 T antigen-associated host proteins**

Fig. 3. Two-dimensional gel autoradiograms of T antigens from SV40-transformed mouse and human cells. Extracts of (a) SVT2 and (b) SV80 cells labelled with 50 μCi/ml of $^{32}$PO$_4$ for 2 h were immunoprecipitated with anti-SV40 tumour serum and electrophoresed on two-dimensional gels as described in Methods (pH 6 to 8 ampholytes).

Fig. 4. Two-dimensional gel autoradiogram of T antigen-associated 55K protein partially purified by SDS–PAGE. Cell extract from $10^7$ SVT2 cells labelled with 50 μCi/ml $^{32}$PO$_4$ for 15 h was immunoprecipitated with anti-SV40 hamster tumour serum. The immunoprecipitated proteins were separated by SDS–PAGE, the band of 55K phosphoprotein was localized by autoradiography of the wet gel and excised. The 55K band was macerated, placed on top of an isoelectric focusing gel and overlaid with 6 M-urea buffer (O'Farrell, 1975). The gel was focused using a pH 3.5 to 10 ampholyte gradient and electrophoresed in a 12.5% SDS–polyacrylamide gel in the second dimension.
Fig. 5. Zone velocity sedimentation of extracts from SV40-transformed and -infected monkey cells. C2 cells (a) were labelled with 30 μCi/ml of [3H]leucine and 40 μCi/ml of 32PO₄ for 15 h. SV40-infected CV-1P cells were labelled from 26 to 41 h after infection (b) and 49 to 64 h after infection (c) with 30 μCi/ml [3H]leucine and 40 μCi/ml 32PO₄. The cell monolayers were washed three times with Hanks' balanced salt solution, the cells were scraped from the plates with a silicon policeman and pelleted. The cells were resuspended (10⁶ cells/ml) in 50 mM-NaCl, 0.5% Nonidet P40, 1 mM-dithiothreitol, 1 mM-phenylmethylsulphonyl fluoride, extracted for 30 min at 0 °C and then pelleted again. The supernatant (0.2 ml) was loaded directly on to 5 to 30% sucrose gradient in 10 mM-Hepes pH 7.8, 150 mM-KCl, 0.5 mM-MgCl₂, 1 mM-dithiothreitol, 1 mM-phenylmethylsulphonyl fluoride and centrifuged in the SW55 rotor at 45,000 rev/min and 0 °C for 3 h. SV40 form II DNA (16S) was run in a parallel gradient as a sedimentation marker. Fractions were analysed by immunoprecipitation with anti-SV40 tumour serum, SDS–PAGE and autoradiography. The peak of form II DNA was in fraction 16.
Table 1. Fraction of $[^{3}H]$leucine-labelled T antigen associated with host 55K phosphoproteins in SV40-infected and -transformed cells*

<table>
<thead>
<tr>
<th></th>
<th>Total $[^{3}H]$-labelled T antigen (ct/min)</th>
<th>23−25S</th>
<th>14−16S</th>
<th>5−6S</th>
</tr>
</thead>
<tbody>
<tr>
<td>CV-1P: SV40</td>
<td>60 221</td>
<td>6</td>
<td>76</td>
<td>18</td>
</tr>
<tr>
<td>26−41 h.p.i.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CV-1P: SV40</td>
<td>15 487</td>
<td>16</td>
<td>45</td>
<td>39</td>
</tr>
<tr>
<td>49−64 h.p.i.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C2</td>
<td>12 149</td>
<td>35</td>
<td>39</td>
<td>26</td>
</tr>
<tr>
<td>SV80†</td>
<td>52 342</td>
<td>34</td>
<td>53</td>
<td>13</td>
</tr>
<tr>
<td>SVT2‡</td>
<td>18 354</td>
<td>62</td>
<td>28</td>
<td>10</td>
</tr>
</tbody>
</table>

* Extracts from infected and transformed cells labelled for 15 h with $[^{3}H]$leucine and $^{32}$P0$_4$ were analysed by zone velocity sedimentation, immunoprecipitation, SDS–PAGE and autoradiography (Fig. 5). The bands of large T antigen were excised from the gels and counted. The radioactivity in each peak of large T antigen was summed and expressed as a percentage of the total $[^{3}H]$leucine label incorporated in T antigen during the 15 h period.

† SV80 and SVT2 cells were labelled, extracted and analysed in the same fashion as the C2 and infected CV-1P cells shown in Fig. 5.

protein was prepared from extracts of $^{32}$PO$_4$-labelled SVT2 cells by immunoprecipitation with anti-SV40 tumour serum, SDS–PAGE and excision of the 55K protein band. The macerated gel band was placed on top of the focusing gel and electrophoresed in two dimensions (Fig. 4). The isolated 55K protein focused as a spot in the same pH range as the 55K proteins from immunoprecipitates (pH 6.6 ± 0.3). These results suggest that complex formation between the 55K protein and the large T antigen during focusing was negligible. The slightly sharper focusing pattern of the isolated 55K protein was presumably due to the smaller amount of protein loaded on the gels (O'Farrell, 1975).

The 55K phosphoprotein–large T antigen complexes in productively infected and transformed monkey cells

SV40-transformed mouse (Lane & Crawford, 1979; McCormick & Harlow, 1980) and human cells (Fanning et al., 1981) contain a fast-sedimenting form of highly phosphorylated large T antigen associated with a 55K host phosphoprotein. Despite the similarities among the 55K proteins from human, monkey and mouse cells, the amounts of the 55K protein and the fast-sedimenting T antigen–55K protein complex in productively infected monkey cells were much smaller than in the transformed mouse and human cells (Gurney et al., 1980; Fanning et al., 1981). Therefore, it was of interest to investigate the large T antigen–55K protein complexes in SV40-transformed monkey cells and in productively infected cells at different times after infection.

Extracts from $[^{3}H]$leucine–, $^{32}$PO$_4$-labelled SV40-transformed monkey cells (Fig. 5a) and SV40-infected monkey cells labelled with $[^{3}H]$leucine and $^{32}$PO$_4$ from 26 to 41 h after infection (Fig. 5b) or 49 to 64 h after infection (Fig. 5c) were analysed by zone velocity sedimentation in sucrose density gradients. Each fraction of the gradient was immunoprecipitated with anti-SV40 tumour serum. The immunoprecipitated proteins were separated by SDS–polyacrylamide gel electrophoresis and visualized by autoradiography.

C2 cells contained two prominent peaks of phosphorylated large T antigen, one at 14S to 16S and another at 23S to 25S, which co-sedimented and co-immunoprecipitated with a peak of 55K phosphoprotein (Fig. 5a). When the bands of large T antigen were excised from the gels and counted, about a third of the $[^{3}H]$-labelled large T antigen was found in the 23S to 25S form (Table 1). The rest of the soluble large T antigen was found in the 14S to 16S form and the apparently underphosphorylated 5S to 6S form described recently (Fanning et al., 1981). In extracts of SV80 cells analysed in parallel for comparison, the fraction of large T antigen in
the 23S to 25S complex was similar to that in C2 cells (Table 1). A considerably larger fraction of the \(^3\text{H}\)-labelled large T antigen from SVT2 mouse cells was found in the 23S to 25S form (Table 1), in agreement with an earlier report (McCormick & Harlow, 1980).

At 41 h after infection, productively infected monkey cells contained one major peak of phosphorylated large T antigen at 14S to 16S and a heavy shoulder associated with a trace of 55K phosphoprotein (Fig. 5b), as reported earlier (Fanning et al., 1981). Later after infection, however, a 23S to 25S peak of phosphorylated large T antigen associated with larger amounts of the 55K phosphoprotein could be clearly distinguished (Fig. 5c). Double-labelling experiments showed that about 15 to 20% of the \(^3\text{H}\)leucine-labelled large T antigen was found in the 23S to 25S form at 64 h after infection, as compared with 5 to 10% at 41 h after infection (Table 1). These results suggest that the fraction of large T antigen which associates with the 55K protein increased at late times after infection.

It is interesting to note that the amount of \(^3\text{H}\)leucine incorporated in large T antigen is higher early in infection (Table 1), but the amount of \(^32\text{P}\) incorporated, especially in the 23S to 25S and 5S to 6S forms, is higher during the late labelling period (Fig. 5; E. Fanning et al., unpublished data). These results suggest that the large T antigen, and perhaps the 55K protein, may be more highly phosphorylated at late times after infection. Experiments are in progress to test this possibility.

Free and T antigen-associated 55K host phosphoproteins in SV40-infected and -transformed cells of different species

To estimate the fraction of the 55K phosphoprotein which is associated with large T antigen, sequential immunoprecipitation experiments were performed. Cell extract was immunoprecipitated first with anti-SV40 hamster tumour serum, the precipitate was set aside for analysis by SDS–PAGE and the supernatant was immunoprecipitated a second time with hamster tumour serum. Since this tumour serum reacted with the large T antigen but not with the 55K host proteins (Fanning et al., 1981), this treatment was expected to precipitate free large T antigen and 55K protein–T antigen complexes but not free 55K protein. A third round of immunoprecipitation was carried out using culture medium from hybridoma clone 122, which has been shown to react specifically with a determinant common to the 55K proteins from human, monkey and mouse cells (Gurney et al., 1980).

Most of the large T antigen and a distinct band of 55K host protein were precipitated from each of the extracts in the first round of immunoprecipitation (Fig. 6). A small remaining fraction of large T antigen was found in the second immunoprecipitate. The third immunoprecipitate contained varying amounts of free 55K phosphoprotein, depending upon the host cell. SVT2 mouse cells reproducibly contained little or no detectable free 55K protein. In SV80 human cells, a significant fraction of the total 55K phosphoprotein was found in a form not associated with large T antigen. In productively infected monkey cells, little 55K phosphoprotein was observed at 43 h after infection, but at 67 and 91 h after infection, more 55K phosphoprotein was found, both free and associated with the large T antigen.

**Stability of the 55K phosphoprotein–large T antigen complex**

The 55K protein–T antigen complex was first observed in SV40-transformed mouse cells (Lane & Crawford, 1979) and thought to be present only in transformed cells. Some of the difficulties in reproducibly detecting this complex in productively infected cells could conceivably result from variation in the stability of the complex, depending upon the source of the 55K phosphoprotein.

Extracts from SV40-infected TC-7 cells and SVT2 mouse cells were immunoprecipitated directly (Fig. 7a) and after storage for 24 h at \(-20^\circ\text{C}\) (Fig. 7b), using anti-SV40 hamster
Fig. 6. Sequential immunoprecipitation with anti-SV40 hamster tumour serum and anti-55K protein monoclonal antibody. SVT2 and SV80 cells were labelled with 100 μCi/ml $^{32}$PO$_4$ for 2 h. SV40-infected TC 7 cells were labelled with 100 μCi/ml $^{32}$PO$_4$ from 41 to 43, 65 to 67 and 89 to 91 h after infection. Cell extract from $5 \times 10^6$ cells was sequentially immunoprecipitated with (1) 10 μl of tumour serum, (2) 10 μl of tumour serum, and (3) 200 μl of culture supernatant from hybridoma clone 122, as described earlier (Gurney et al., 1980). The immunoprecipitated proteins were analysed by SDS–PAGE and autoradiography.

Fig. 7. Effect of storage on large T antigen–55K phosphoprotein complexes. SV40-infected TC-7 cells were labelled with 100 μCi/ml $^{32}$PO$_4$ for 2 h. Extract from $5 \times 10^6$ cells was immunoprecipitated with tumour serum immediately (a), or after storage at $-20$ °C for 24 h (b). Immunoprecipitated proteins were analysed by SDS–PAGE and autoradiography.
tumour serum. Immunoprecipitates from fresh cell extracts contained 55K phosphoprotein associated with large T antigen. After storage, the mouse 55K phosphoprotein was still associated with large T antigen, but the monkey 55K protein was absent. The 55K phosphoprotein–T antigen complexes from SV80 human and C2 monkey cells were partially dissociated upon storage for 24 h at -20 °C (results not shown). In some experiments it was possible to recover the dissociated 55K phosphoprotein from the supernatant remaining after immunoprecipitation by a second round of immunoprecipitation with clone 122 monoclonal antibody (not shown). However, the amount of 55K phosphoprotein recovered from these samples was usually smaller than that which dissociated from large T antigen during storage. It seems reasonable to assume that the dissociated 55K phosphoprotein is labile and was either degraded during storage, or denatured to such a degree that the monoclonal antibody could not recognize it.

As a control, fresh cell extracts from SVT2, SV80 and productively infected monkey cells were twice frozen at -20 °C and thawed, and then immediately immunoprecipitated. The amount of 55K phosphoprotein precipitated with tumour serum was identical to that in immunoprecipitates from fresh untreated cell extract. These data suggest that freezing alone did not affect the stability of the 55K protein–T antigen complexes.

**DISCUSSION**

Cells infected and transformed by SV40 contain one or more host-coded 55K phosphoproteins associated with a highly phosphorylated form of the large T antigen (Lane & Crawford, 1979; McCormick & Harlow, 1980; Gurney et al., 1980; Fanning et al., 1981). Although these proteins differ in mol. wt. and peptide pattern between host cells of different species (Chang et al., 1979; Kress et al., 1979; Linzer & Levine, 1979; Melero et al., 1979; Smith et al., 1979), these differences are slight. Tryptic peptide maps, Cleveland peptide mapping and isoelectric focusing of SDS-denatured 55K proteins from mouse, rat, human and monkey cells suggest that the basic structure and composition of these polypeptides are very similar (Melero et al., 1980; Simmons et al., 1980; Simmons, 1980; Fig. 2, 3).

The 55K proteins from non-permissive, semi-permissive and permissive cells all appear to form a fast-sedimenting complex with SV40 large T antigen (Lane & Crawford, 1979; McCormick & Harlow, 1980; Gurney et al., 1980; Fanning et al., 1981). However, we attempt here to distinguish among these different types of host cells with respect to the fraction of the large T antigen and 55K phosphoprotein observed in complexes and the stability of the complexes. Mouse cells, which are non-permissive for SV40 infection, contained the least free 55K phosphoprotein and large T antigen of the cell lines studied (Table 1, Fig. 6). Most of the 55K protein and T antigen were associated with each other in a complex which appeared to be more stable than the analogous complexes from SV80 and TC-7 cells (Fig. 7). In the human cell line SV80, larger amounts of free 55K phosphoprotein and large T antigen were observed. In cells permissive for SV40, TC-7 and CV-1P, most of the large T antigen was not associated with the 55K protein and significant amounts of free 55K phosphoprotein were observed at late times after infection (Table 1, Fig. 6). The complex between the 55K protein and large T antigen appeared to dissociate during storage (Fig. 7).

Thus, the interaction between the 55K host protein and large T antigen appears to be correlated with the virus–host cell interaction and could play a critical role in determining whether a particular host cell is permissive for SV40 infection or not. However, a survey of a number of different permissive and non-permissive cell lines will be necessary to establish this correlation.

The amount of free 55K phosphoprotein and the fraction of large T antigen complexed with the 55K phosphoprotein in productively infected monkey cells increased with time after infection (Fig. 5, 6, Table 1). A similar conclusion was reached from a study of the rates of
synthesis of large T antigen and 55K protein in abortively infected mouse cells (Linzer et al., 1979). Since the 23S to 25S complex in productively infected cells was most prominent very late after infection, it seems unlikely that this complex is involved in conventional viral DNA replication. The T antigen--55K protein from mouse cells was the most stable of those investigated, yet conventional viral DNA replication is not observed in mouse cells. The large T antigen from the C2 transformed monkey cell line aggregates with 55K protein into an apparently normal 23S to 25S form (Fig. 5), but is incapable of complementing the tsA defect in initiation of SV40 DNA replication (Gluzman et al., 1977). These data would also suggest that the 23S to 25S complex is not active in initiation of SV40 DNA replication.

However, a role for the 55K protein--T antigen complex in rolling circle replication of the viral DNA cannot be excluded (Bjursell, 1978). Large amounts of oligomeric SV40 DNA, presumably the result of rolling circle replication, appear in infected monkey cells starting around 60 h after infection (Rigby & Berg, 1978). Thus, the appearance of increased amounts of 55K protein--T antigen complexes and oligomeric SV40 DNA coincide temporally. Oligomeric viral DNA has also been observed in SV40-infected mouse cells (Rigby et al., 1980; W. Chia & P. W. J. Rigby, personal communication).

In summary, two-dimensional gel electrophoresis and V8 peptide mapping of the T antigen-associated 55K host phosphoproteins from SV40-infected and -transformed cells of different species indicate that these proteins are very similar. However, the stability of the complexes formed between the large T antigen and 55K proteins from different host cells may be correlated with the non-permissiveness of the host cell for SV40 replication.

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