Replication of Simian Virus 40 in Permissive Cells: assembly of Virus Components

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SUMMARY

Incubation in medium lacking arginine of monkey kidney cells infected with simian virus 40 resulted in a marked inhibition of production of both infectious virus and empty capsids. Virus DNA synthesis and formation of a virus DNA-protein complex were unaffected. All the virus structural polypeptides were made, although in reduced amounts (42%) when compared with infected cells incubated in medium containing arginine. Protein synthesis was inhibited in uninfected cells deprived of arginine. All the newly synthesized virus proteins were found in the nuclei of arginine-deprived cells. Addition of arginine to arginine-deprived cells resulted in the assembly of pre-synthesized virus components into infectious virus and empty capsids, as well as the production of virus from virus proteins and DNA synthesized after the addition of arginine. Studies with inhibitors of protein and DNA synthesis suggested that two late virus functions were affected in the absence of arginine: the assembly of capsids and the encapsidation of DNA by capsids to form infectious virus.

INTRODUCTION

Cells infected with a variety of viruses containing DNA such as adenovirus (Rouse & Schlesinger, 1967; Russell & Becker, 1968), simian virus 40 (SV40) (Goldblum, Ravid & Becker, 1968), polyoma (Winters & Consigli, 1971), vaccinia (Archard & Williamson, 1971), pseudorabies (Mark & Kaplan, 1971) and herpes simplex viruses (Tankersley, 1964; Becker, Olshevsky & Levitt, 1967; Inglis, 1968; Courtney, McCombs & Benyesh-Melnick, 1970) failed to yield infectious virus when maintained in culture medium lacking arginine. In the case of vaccinia virus, which replicates and matures in the cell cytoplasm, the inhibition of virus yield in arginine-deprived (arg−) cells resulted from a lack of both virus specific RNA (early virus function) and virus DNA synthesis. In addition, the presence of arginine is also required for a late function involving virus assembly (Archard & Williamson, 1971). In contrast, polyoma, adeno, pseudorabies and herpes simplex viruses, viruses which mature in the cell nucleus, synthesized both virus DNA and protein in arg− cells (Becker et al. 1967; Rouse & Schlesinger, 1967; Russel & Becker, 1968; Olshevsky & Becker, 1970; Courtney, McCombs & Benyesh-Melnick, 1971; Mark & Kaplan, 1971; Winters & Consigli, 1971). In cells infected with these viruses, a late virus function(s) was affected in the absence of arginine, resulting in either the failure of transport of virus proteins synthesized in the cytoplasm into the nucleus or, once inside the nucleus, a lack of assembly of virus proteins and DNA into infectious particles. In all these cases, the addition of arginine to the culture medium resulted in the production of infectious virus.

* Dr F. Sokol died suddenly on 26 May 1974.
In arg- cells infected with SV40, there was a marked inhibition of infectious virus production. Only 38% of the infected cells synthesized virus structural proteins ('V' antigen), whereas the number of cells producing 'T' antigen was unaffected (Goldblum et al. 1968). Little is known about the mechanism of inhibition of SV40 production in arg- permissive cells or the effects of arginine deprivation on the host cell metabolism. We found that the major effect of arginine deprivation was in the inhibition of virus maturation rather than the suppression of the synthesis of virus DNA and proteins. In the absence of arginine, protein synthesis was inhibited in uninfected but not in infected cells. We also show that investigations on the interaction of SV40 with arginine-deprived permissive cells have shed light on the mode of replication of this virus.

METHODS

**Cells and virus.** Secondary cultures of African green monkey kidney (AGMK) cells prepared from cells obtained from Flow Laboratories (Rockville, Md.) were grown in Eagle's basal medium containing 10% calf serum. Cells were seeded in Blake bottles to form confluent monolayers in 3 days at 37 °C and were used 4 to 6 days after reaching confluency (2 x 10^7 cells per monolayer) (Tan & Sokol, 1973a). Stock suspension of SV40 was prepared in CV-1 cells as described previously (Tan & Sokol, 1972).

**Infection and processing of cells.** The cell monolayers were washed twice with Earle's balanced salt solution and incubated a further 24 h with Earle's salt solution containing vitamins and 2% dialysed calf serum to partially deplete the intracellular amino acid pools. The cells were then infected with 50 p.f.u. of SV40 per cell in the presence of 0.04 M-MgSO4 as described previously (Tan & Sokol, 1973a). After 60 min at 37 °C, the cells were washed twice with Earle's salt solution and incubated at 37 °C with 7 ml of 'arg-' medium consisting of Earle's salt solution containing vitamins, 2% dialysed calf serum and the following amino acids (mg/litre): cystine (24), glutamine (292), histidine (31), isoleucine (52.5), leucine (52.4), lysine (58), methionine (15), phenylalanine (32), threonine (48), tryptophan (10), tyrosine (36), and valine (46). For comparison, arginine-HCl (200 mg/litre) was added to some cultures (arg+ cells). Details of labelling of infected cells with [3H]-thymidine, [14C]-amino acid mixture, or [3H]-lysine, are given in the text and figure legends. The uptake of lysine from the medium into cells is not affected after incubation of the cells in amino acid deficient medium lacking arginine (Piez & Eagle, 1958). At times indicated in the text, the culture medium was decanted and the cells were scraped off the glass with a rubber policeman into NT buffer (0.13 M-NaCl, 0.05 M-tris-hydrochloride, pH 7.8), collected by low speed sedimentation and frozen in NT buffer.

**Virus purification.** The method of purification of cell-associated virus is a modification of that previously described (Tan & Sokol, 1972). Cells from one Blake bottle were frozen and thawed three times in 3 ml of NT buffer, further disrupted by ultrasonic vibration, incubated with 1% sodium deoxycholate for 30 min at 37 °C, and centrifuged (1000 g, 15 min). The clarified supernatant fraction was layered over 1.5 ml of NT buffer containing 1 mg purified bovine serum albumin per ml and saturated with KBr at 20 °C and centrifuged (Spinco SW50.1 rotor; 27000 rev/min; 2.5 h; 13 °C) to separate virus particles from unassembled virus structural proteins and cellular proteins. The two sharp bands of virus in the KBr solution were collected (0.6 ml), mixed with 1.8 g CsCl (vol. adjusted to 4.5 ml with NT buffer), and centrifuged (Spinco SW50.1 rotor; 40000 rev/min; 18 h; 4 °C). Fractions (0.15 ml) were collected from the bottom of the tubes and samples were mixed with a water miscible scintillation fluid (TXT solution; Tan & Sokol, 1973b) for determination of radioactivity.
Table 2. Uptake of SV40 by arg+ and arg- cells

<table>
<thead>
<tr>
<th>Growth condition</th>
<th>Cytoplasm (~ input virus uptake) by acid-precipitable radioactivity</th>
<th>Nucleus (~ input virus uptake) by acid-precipitable radioactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ arginine</td>
<td>39.7</td>
<td>13.5</td>
</tr>
<tr>
<td>- arginine</td>
<td>40.6</td>
<td>12.7</td>
</tr>
</tbody>
</table>

Secondary cultures of AGMK cells were infected with purified [3H]-thymidine-labelled SV40 (100 p.f.u./cell or 15000 ct/min/culture).

**Extraction of virus DNA from infected cells.** Virus DNA was extracted from infected cells by a modification of the method described by Hirt (1967). Cells were washed three times with chilled NT buffer, scraped off the glass into NT buffer and centrifuged (800 g, 5 min). The cell pellet (from one Blake bottle) was suspended in 1 ml of 0.01 M-EDTA, 0.01 M-tris-hydrochloride, pH 7.5, and 1% SDS. After 15 min at 20 °C, 0.1 ml of pronase (20 mg/ml, pre-incubated at 37 °C for 30 min) was added and the mixture was incubated at 37 °C for 3 h. The mixture was made 1 M in NaCl, kept overnight at 4 °C and centrifuged (36000 g, 30 min). The supernatant fraction, containing virus DNA, was analysed in a 5 to 20% (w/v) sucrose gradient prepared in NT buffer containing 0.001 M EDTA as described in the Results section.

**Extraction of virus DNA-protein complex.** Procedures described elsewhere (White & Eason, 1971; Goldstein, Hall & Meinke, 1973) were modified as follows: infected cultures were washed three times with chilled NT buffer, scraped off the glass and collected by low speed sedimentation. Sedimented cells from two Blake bottles were suspended in 0.8 ml of 0.02 M-tris-hydrochloride, pH 7.9, and mixed with 0.8 ml of 0.5% Triton-X 100 in 0.02 M-EDTA. After 10 min incubation at 20 °C, the suspension was mixed gently with 0.2 ml 2 M-NaCl and centrifuged (10000 g, 5 min). The supernatant fraction, containing the virus DNA-protein complex, was analysed in sucrose gradients prepared in 0.01 M-EDTA, 0.2 M-NaCl, and 0.01 M-tris-hydrochloride, pH 7.4, as described in the Results section.

**Polyacrylamide gel electrophoresis of proteins.** Samples were precipitated with 10% trichloroacetic acid and the precipitates were collected by sedimentation, washed with acetone and air-dried. The dried precipitates were solubilized with SDS and dithiothreitol and analysed in 10% polyacrylamide gels containing 6 M-urea, as described previously (Tan & Sokol, 1972, 1973b). Gels were stained with Coomassie blue or cut up into 1 mm thick slices for determination of radioactivity after overnight incubation of the slices in 8 ml of Protosol (3%) liquid scintillation fluid (New England Nuclear, Boston, Mass.).

**Sources of materials.** [3H]-thymidine (50 Ci/mmol) was obtained from New England Nuclear, Boston, Mass. [14C]-amino acid mixture and [3H]-lysine (55 Ci/mmol) were obtained from Schwarz/Mann, Orangeburg, N.Y. Bovine pancreas DNase I was from Sigma Chemical Co., St Louis, Mo., and bovine spleen phosphodiesterase was from Worthington Biochemical Corp., Freehold, N.J.

**RESULTS**

**Virus uptake by cell nuclei**

In arg- cells infected with herpes viruses, virus proteins which are synthesized in the cytoplasm were not transported into the nucleus, the site of virus assembly (Mark & Kaplan, 1971; Courtney et al. 1971). Therefore, since SV40 replication is thought to be initiated by
virus that has entered the nucleus (Tan & Sokol, 1973a), it is important to determine whether the SV40 particles have penetrated the nuclei of arg⁻ cells. Confluent monolayers of AGMK cells were incubated for 2 days with arg⁻ medium and then infected with purified [³H]-thymidine-labelled SV40 suspended in phosphate-buffered saline, as described previously (Tan & Sokol, 1973a). After 60 min incubation at 37 °C, the infected cells were then fractionated into cytoplasmic and nuclear fractions and the amount of acid-precipitable virus associated with these fractions was determined. No difference was observed in the amount of virus taken into the nuclei of cells grown in either the presence or absence of arginine (Table 1).

Production of virus particles

Purified preparations of SV40 grown in AGMK cells are composed of 3 forms of virus particles, namely, infectious heavy virus (buoyant density 1.35 g/ml in CsCl solution), light
Assembly of SV40

Table 2. Yield of SV40 from cells infected in the absence of arginine and subsequently supplied with arginine

<table>
<thead>
<tr>
<th>Hours incubation of arg- cells with arginine</th>
<th>Virus yield from arg- cells (% of yield from arg+ cells)</th>
<th>Determined from radioactivity of pre-synthesized protein in</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>infectious virus</td>
<td>empty capsids</td>
</tr>
<tr>
<td>0</td>
<td>39</td>
<td>3.5</td>
</tr>
<tr>
<td>4</td>
<td>11.2</td>
<td>9.2</td>
</tr>
<tr>
<td>10</td>
<td>13.7</td>
<td>25.0</td>
</tr>
<tr>
<td>24</td>
<td>60.1</td>
<td>152.5</td>
</tr>
</tbody>
</table>

Infected arg- cells were labelled with [3H]-lysine (2 μCi/ml) from 24 to 48 h after infection. Cells in one culture were harvested while the others were washed with unlabelled medium and incubated with arg+ medium containing an excess of unlabelled lysine (0.1 mg/ml) and were harvested after a further 4, 10 or 24 h incubation at 37 °C. As a control, cells infected in the presence of arginine were labelled with [3H]-lysine (2 μCi/ml) at 24 h and harvested at 48 h after infection. Virus was extracted from infected cells and purified in CsCl gradients as described in Methods. Samples of gradient fractions were assayed for radioactivity to quantitate pre-synthesized proteins. The rest of the gradient fractions containing either infectious virus or empty capsids were pooled and made 10% in trichloroacetic acid. The precipitated proteins were then subjected to electrophoresis in polyacrylamide gels which were stained with Coomassie blue. The intensity of staining of the virus polypeptides, as measured by E_{590}, was taken as a measure of amount of protein (pre-synthesized plus newly synthesized).

particles (1.32 g/ml), and empty capsids (1.30 g/ml) (Sokol et al. 1974). The effect of arginine deprivation on the production of cell-associated infectious virus and empty capsids was investigated. Light virus particles, which represent only a small proportion of the total virus yield, were not studied. About 90% of the total virus yield (cell-associated virus and virus released into the culture medium) in arg+ cultures at the time when the cells showed c.p.e. was cell-associated (K. B. Tan, unpublished data).

Cells infected in the presence or absence of arginine were labelled with [3H]-lysine (1.4 μCi/ml) at 24 h after infection. At 48 h after infection, when arg+ cultures began to show c.p.e., cells from the arg+ and arg- cultures (no c.p.e. observed) were harvested and frozen. Replicate infected arg- cells were washed twice with medium to remove [3H]-lysine and further incubated with arg+ medium containing a large excess of unlabelled lysine (0.1 mg/ml) and [14C]-labelled amino acid mixture (2.4 μCi/ml) to label newly synthesized virus proteins. These cells were then harvested after 4, 10, or 24 h incubation at 37 °C. Arg- cells at 24 h after the addition of arginine showed c.p.e. resembling those of arg+ cells. Harvested cells were frozen and thawed, and virus was extracted and purified as described in Methods.

The results presented in Fig. 1 and Table 2 show that: (1) very little infectious virus or empty capsids were produced in arg- cells when compared with the yield from arg+ cultures, as determined from the incorporation of [3H]-lysine or from the protein content of purified virus particles; (2) unassembled [3H]-labelled virus proteins which were synthesized during the period of arginine deprivation were assembled into virus particles, as indicated by the increased yield of [3H]-labelled virus, when arginine was subsequently added to arg- cells; and (3) addition of arginine to arg- cells also resulted in the production of virus particles from newly synthesized [14C]-labelled proteins. Under these conditions the total yield of infectious virus was markedly increased and even more empty capsids were produced than in arg+ cultures (24 h sample, Table 2).
Fig. 2. Electrophoretic pattern of polypeptides synthesized in SV40-infected and mock-infected cells. (a) Infected arg+, (b) infected arg-, and (c) mock-infected arg- cells. SV40 structural polypeptides are numbered from 1 to 5 (Tan & Sokol, 1972). Electrophoretic migration is from left to right.

Synthesis of virus and cellular proteins in arg- cells

In the absence of arginine, cells produced only 4% of the virus particle yield from arg+ cultures (Table 2). However, when arginine was added to arg- cultures, the subsequent virus yield (infectious virus and empty capsids) contained particles which were assembled from [3H]-lysine-labelled proteins synthesized during the period of arginine deprivation (Fig. 1) and which amount to about 32% of the virus yield from arg+ cultures at 48 h after infection. This observation indicates that arginine deprivation inhibited virus assembly more severely than virus protein synthesis. To determine the extent of virus protein synthesis in arg- cells, the following experiment was performed. Cells infected in the presence or absence of arginine were labelled with [3H]-lysine (2 μCi/ml) at 24 h after infection and at 48 h after infection, the cells were fractionated into cytoplasmic and nuclear fractions which were solubilized for electrophoresis in polyacrylamide gels.

In arg- cells all the virus structural polypeptides were detected in the nucleus but not in the cytoplasm (Fig. 2). From the total amount of radioactivity present in the gels, it was calculated that the amount of virus proteins in the nuclei of arg- cells amounted to 42% of that in the nuclei of arg+ cells. The proportions of the different structural proteins in the nuclei of arg- or control cells are different from those of purified virus (Table 3). Polypeptide 2, a minor component in purified virus, is present in a relatively large amount in infected cells (Table 3, Fig. 2). This observation is not in agreement with those reported by other investigators (Walter, Roblin & Dulbecco, 1972; Kiehn, 1973) and may be peculiar to our strain of SV40. The virus structural polypeptides represented from 70% to 85%
Assembly of SV40

Table 3. Distribution of [³H]-lysine incorporated into virus structural polypeptides present in purified virus and in the nuclei of infected cells

<table>
<thead>
<tr>
<th>Polypeptides in</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purified virus</td>
<td>83.7</td>
<td>2.0</td>
<td>5.2</td>
<td>6.5</td>
<td>2.8</td>
</tr>
<tr>
<td>Nuclei of arg⁻</td>
<td>25.9</td>
<td>22.2</td>
<td>10.8</td>
<td>13.7</td>
<td>12.3</td>
</tr>
<tr>
<td>Nuclei of arg⁺</td>
<td>26.6</td>
<td>17.1</td>
<td>10.0</td>
<td>10.7</td>
<td>5.8</td>
</tr>
</tbody>
</table>

Purified virus was derived from cells infected in the presence of arginine. The corresponding polypeptide profiles are shown in Fig. 2.

Fig. 3. Production of infectious virus containing newly synthesized DNA in arg⁻ cells which subsequently received arginine. At 48 h after the infection of arg⁻ cells, arginine-HCl (200 mg/ml) and [³H]-thymidine (1 μCi/ml) were added to the cultures. After an additional 4 h (---), 10 h (O--O), or 24 h (●●●) incubation at 37 °C, the cells were harvested and cell-associated virus was purified.

of the total proteins synthesized in arg⁻ or arg⁺ cells (Fig. 2). Virus structural proteins were present in the cytoplasm of arg⁺ cells (Fig. 2). They probably represent the protein moiety of virus particles which had leaked out from the nuclei since these cells showed c.p.e. at the time of harvest. In the absence of arginine, protein synthesis was not detected in either the nuclei or cytoplasm of uninfected cells (Fig. 2).
Effects of inhibitors of protein and DNA synthesis on the assembly of virus in arg− cells

When arginine was added to infected arg− cells, there was assembly of pre-synthesized virus protein and DNA into virus particles as well as synthesis of new virus protein (Fig. 1) and DNA (Fig. 3). To determine if the assembly of pre-synthesized proteins into virus particles that resulted after the addition of arginine could occur in the absence of new protein and DNA synthesis, medium containing arginine and cycloheximide (10 µg/ml; Kang et al. 1971) or hydroxyurea (0.01 M; Magnusson, 1973) was added to arg− cells at 48 h after infection. After an additional 24 h incubation at 37 °C, cell-associated virus was purified. Cycloheximide inhibited the assembly of free pre-synthesized proteins into virus particles (Fig. 4). A reduced yield of both virus and empty capsids was consistently observed for cycloheximide-treated cells. Cycloheximide-treated monolayers remained intact and no loss of cells was observed. However, in the presence of hydroxyurea, free pre-synthesized proteins were assembled into empty particles only, and not into infectious virus (Fig. 4) as is evidenced by the accumulation of empty capsids. Similarly, only empty capsids were formed from proteins synthesized in the presence of hydroxyurea (K. B. Tan, unpublished data). Therefore, the assembly of virus requires concomitant virus DNA and virus protein synthesis.
Table 4. Distribution of [3H]-lysine in structural polypeptides of SV40
derived from arg+ or arg- cultures

<table>
<thead>
<tr>
<th>Virus form</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infectious virus</td>
<td>a</td>
<td>77.4</td>
<td>6.3</td>
<td>7.8</td>
<td>5.8</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>81.9</td>
<td>4.7</td>
<td>6.9</td>
<td>4.7</td>
</tr>
<tr>
<td></td>
<td>c</td>
<td>75.0</td>
<td>6.5</td>
<td>7.6</td>
<td>7.0</td>
</tr>
<tr>
<td>Empty capsids</td>
<td>a</td>
<td>83.9</td>
<td>4.0</td>
<td>5.9</td>
<td>5.1</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>86.0</td>
<td>3.3</td>
<td>4.7</td>
<td>4.3</td>
</tr>
<tr>
<td></td>
<td>c</td>
<td>76.2</td>
<td>5.8</td>
<td>5.3</td>
<td>9.1</td>
</tr>
</tbody>
</table>

Infected arg+ or arg- cells were labelled with [3H]-lysine (3.5 μCi/ml) at 24 h after infection. At 48 h after infection cells from the arg+ culture (a) and an arg- culture (b) were harvested. Another arg- culture (c) was washed with unlabelled medium and incubated in arg+ medium containing lysine (0-1 mg/ml) for 24 h at 37 °C before harvesting.

Polypeptide composition of virus from arg- and arg+ cells

Table 3 shows that the structural polypeptides of SV40 are present in similar relative proportions in either arg- or arg+ cells. If one (or more) of the structural polypeptides synthesized in arg- cells is defective, then this may result in an inhibition of virus assembly. However, if the structural polypeptides are non-defective and if the inhibition of virus yield resulted from a lack of virus maturation, then when arginine is added to infected arg- cells the virus that is assembled from polypeptides synthesized during the period of arginine deprivation (i.e. pre-synthesized proteins; see Fig. 1 and Table 4) should contain these proteins in relative proportions similar to those of virus from arg+ cultures. The polypeptide composition (Table 4) and the electrophoretic mobility of the structural polypeptides in polyacrylamide gels of virus particles from arg+, arg- or arg- cultures that subsequently received arginine, were similar although for some unknown reasons, virus particles derived from arg- cultures that subsequently received arginine, contained a higher proportion of pre-synthesized polypeptides 4 and 5. When [14C]-amino acid-labelled purified infectious virus and empty capsids from arg+ cultures were mixed with [3H]-lysine-labelled virus particles from either arg- cells or arg- cells to which arginine was subsequently added (cf. Fig. 1), and centrifuged to equilibrium in CsCl solutions, the infectious virus or empty capsids from all these cultures showed identical buoyant densities (K. B. Tan, unpublished data).

Synthesis of virus DNA in arg- cells

The extent of virus DNA synthesis was determined by labelling infected arg- or arg+ cells with [3H]-thymidine (0.8 μCi/ml) in the presence of 2.5 μM-thymidine at 27 h after infection. Virus DNA was selectively extracted from the cells (see Methods) at 48 h after infection and a 0.2 ml sample was sedimented into a 5 to 20 % (w/v) sucrose gradient (Spinco SW50 L rotor, 45000 rev/min, 3 h, 13 °C). Identical profiles were obtained for virus DNA obtained from either arg- or arg+ cells (Fig. 5). Arg- cells synthesized 63% of the virus DNA which was produced in arg+ cells. However, the specific radioactivity ([H]-radioactivity: E260) of virus DNA from arg- cultures was 1.32 times (average value from three experiments, range 1.18 to 1.58) greater than that from arg- cells. Therefore, in reality, there was only a 16.8 % inhibition of virus DNA synthesis in arg- cells. In the same experiment, duplicate cultures were harvested, disrupted by ultrasonic vibration and incubated with 200 μg of DNase I/ml and 0.125 units of phosphodiesterase/ml at 37 °C.
Fig. 5. Sucrose gradient analysis of SV40 DNA synthesized in infected arg⁺ (●—●) and arg⁻ (○—○) cells. The 21S and 16S DNAs are SV40 components I and II DNA, respectively.

Fig. 6. Sucrose gradient analysis of SV40 DNA-protein complex from arg⁺ and arg⁻ cells. Infected arg⁺ (●—●), arg⁻ (○—○) or mock-infected arg⁻ (----) cells. Sedimentation is from right to left.
for 30 min (Tan & Sokol, 1972). About 69.4% and 70.6% of the DNA in infected arg- and arg+ cells, respectively, were rendered acid-soluble after the enzyme digestion whereas about 92.5% of the DNA from uninfected arg- cells was rendered acid-soluble. Therefore, the virus DNA synthesized in arg- or arg+ cells were ‘coated’ by proteins to a similar extent (see below).

**Virus DNA-protein complex formation**

Recently it was demonstrated that during lytic infection, replicating DNAs of polyoma virus and SV40 are complexed with proteins present in the infected cells (White & Eason, 1971; Goldstein et al. 1973; Hall, Meinke & Goldstein, 1973; Seebeck & Weil, 1974; Shmookler, Buss & Green, 1974). Since such a complex must play an important role in virus replication, we looked for its presence in infected arg- cells. Infected cells were labelled with [3H]-thymidine from 27 to 48 h after infection as described in the experiment shown in Fig. 5. The virus DNA-protein complex was extracted from the cells and a 0.2 ml sample was sedimented into a 5 to 20% (w/w) sucrose gradient (Spinco SW50L rotor, 36000 rev/min, 2.5 h, 4°C). The DNA-protein complex was found in both arg- and arg+ cells,
although in a decreased amount (28.9\% less, value corrected for specific radioactivity of DNA) in arg\textsuperscript{−} cells (Fig. 6).

The protein composition of the peak fraction of the DNA-complex is shown in Fig. 7. In this experiment uninfected cultures pre-labelled for 2 days with \[^{14}\text{C}\] amino acid mixture (0.4 \(\mu\text{Ci/ml}\)) in arg\textsuperscript{+} medium containing half the regular concentration of unlabelled amino acids, were washed with unlabelled medium and incubated overnight with Earle's salt solution containing 2\% dialysed calf serum. The cells were then infected with SV\(_40\), incubated with arg\textsuperscript{−} or arg\textsuperscript{+} medium, and labelled from 24 to 48 h after infection with \[^{3}\text{H}\] lysine (3.5 \(\mu\text{Ci/ml}\)). The virus DNA-protein complex was extracted and a 0.5 ml sample was sedimented in a 10 to 30\% (w/v) sucrose gradient (Spinco SW41 rotor, 36000 rev/min, 165 min, 4 \(\degree\text{C}\)) as described by Goldstein et al. (1973). Fractions of 0.4 ml were collected and 20 \(\mu\text{l}\) samples were mixed with TXT solution for determination of radioactivity to locate the position of the DNA-protein complex. The remainder of the peak fractions of the complex were pooled, precipitated with 10\% trichloracetic acid and processed for electrophoresis in polyacrylamide gel. The DNA-complex from infected arg\textsuperscript{−} and arg\textsuperscript{+} cells were similar in composition, containing all the virus structural proteins (Fig. 7). The virus structural proteins present in the virus DNA-protein complex extracted from arg\textsuperscript{−} cells most probably represent free proteins and not contamination by assembled virus particles because (a), very little assembled virus particles are formed in arg\textsuperscript{−} cells (Fig. 1), and (b), in a reconstruction experiment, when purified \[^{14}\text{C}\]-amino acid-labelled infectious virus was mixed with the Triton extract from \[^{3}\text{H}\]-lysine-labelled infected cells and analyzed in a sucrose gradient, purified virus was clearly separated (in a position corresponding to fraction 7 or 8 of the gradient shown in Fig. 6) from the DNA-complex (K. B. Tan, unpublished data). Also present in the complex were several cellular proteins which were synthesized before (\[^{14}\text{C}\]-labelled) and after (\[^{3}\text{H}\]-labelled) virus infection. It is interesting to note that the cellular histones present in the complex represented mostly proteins synthesized after virus infection.

**DISCUSSION**

A previous study (Goldblum et al. 1968) showed that in the absence of arginine there was a marked inhibition of production of infectious SV\(_40\) but not of 'T' or 'V' antigens. We have confirmed that virus production was inhibited in arg\textsuperscript{−} cells and have extended the study to show that nuclear uptake of the infecting virus, virus DNA and protein synthesis, and virus DNA-protein complex formation occurred in these cells.

In arg\textsuperscript{−} cells we observed a 60\% inhibition of protein synthesis. All the virus structural proteins were located in the nuclei of arg\textsuperscript{−} cells since virus proteins are rapidly transported into the nucleus soon after synthesis (Ozer & Tegtmeyer, 1972; Kiehn, 1973). Addition of arginine to arg\textsuperscript{−} cells resulted in virus assembly and new virus DNA and protein synthesis. Our data indicate that virus assembly requires concomitant virus DNA and protein synthesis. When cycloheximide was added simultaneously with arginine to arg\textsuperscript{−} cells, pre-synthesized virus components were not assembled into either infectious virus or empty capsids. Therefore, in arg\textsuperscript{−} cells a maturation factor (protein) is either not synthesized, or if synthesized, is non-functional. Similarly, Russell & Becker (1968) suggested that the arginine-requiring step in adenovirus type 5 replication involved a component of the 'P' antigen, which is probably an internal maturation factor.

Simultaneous addition of hydroxyurea and arginine to arg\textsuperscript{−} cells resulted in the production of empty capsids only. Although the relationship between empty capsids and infectious virus has not been firmly established, the available data (Ozer, 1972; Ozer & Tegtmeyer,
Assembly of SV40

1972) showed that empty capsids are not breakdown products of infectious virus and that they are synthesized independently and they suggest that empty capsids are precursors of infectious virus. Our results are in agreement with these findings. If empty capsids are indeed precursors of infectious virus, then the hydroxyurea experiment suggests that a second arginine requiring step may be affected, namely, the encapsidation of virus DNA by empty capsids. Poliovirus particles have been shown to be formed by the encapsidation of virus RNA by an empty protein shell or procapsid (Jacobson & Baltimore, 1968). SV40 maturation may occur by a similar mechanism. We have previously shown that SV40 empty capsids are permeable to macromolecules (Tan & Sokol, 1973b). Replicating (25S) and mature superhelical (component I DNA) DNAs of both SV40 (White & Eason, 1971; Goldstein et al. 1973; Hall et al. 1973) and polyoma virus (Goldstein et al. 1973; Seebeck & Weil, 1974) are always complexed with proteins in the infected cells. Since the SV40 DNA-protein complex contains unassembled virus structural polypeptides, such a complex may represent a virus ‘factory’ in which both virus DNA maturation and empty capsid formation occur. It is envisaged that both the formation of empty capsids and the encapsidation of virus DNA by the empty capsids occur rapidly because assembled virus is not found in the complex.

Alternatively, an obligatory chemical modification of the virus structural proteins prior to virus maturation, such as phosphorylation and/or dephosphorylation (Tan & Sokol, 1972), may be affected in arg- cells.

Depriving uninfected cells of arginine leads to an inhibition of cellular macromolecular synthesis (Archard & Williamson, 1971) and replication (Henle & Henle, 1968). Most investigators studying the effects of arginine deprivation on virus replication have failed to relate their observations to the inhibition of host cell replication. Thus, an inhibition of cellular replication could lead to an inhibition of virus yield as well. However, the yield of EB virus (a herpes-type virus) was enhanced when persistently-infected cells were deprived of arginine (Henle & Henle, 1968). In view of our finding that arginine deprivation inhibited cellular but not virus protein synthesis, the enhancement of EB virus production could have resulted from an inhibition of synthesis of cellular repressors. In the absence of arginine, the synthesis of SV40 structural proteins was not markedly inhibited. In agreement with the published data (Anderson & Gesteland, 1972; Fischer & Sauer, 1972; Walter et al. 1972; Kiehn, 1973), we detected the synthesis of several virus-induced proteins.

Several investigators reported that both polyoma virus and SV40 contain three histone-like proteins (Estes, Huang & Pagano, 1971; Roblin, Harle & Dulbecco, 1970) which are probably derived from the host cell (Frearson & Crawford, 1972). However, our strain of SV40 contains only two histones (Tan & Sokol, 1972; Sokol et al, 1974). Although proof is lacking, our data suggest that most of the histones incorporated into SV40 are derived from cellular proteins synthesized after virus infection. Frearson & Crawford (1972) have shown that pre-formed histones are also incorporated into polyoma virus. SV40 polypeptides 1 and 3 are probably primary gene products (Walter et al. 1972; Kiehn, 1973). Polypeptide 3 is a minor component in our infected cells, whereas polypeptide 2, which accounts for only about 2% of the proteins of purified virus, is a major component in infected cells. Since it does not correspond in electrophoretic mobility to any cellular protein, it is probably a virus gene product (K. B. Tan & F. Sokol, unpublished observations).

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REFERENCES


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