Kinetics of Inhibition of Papovavirus DNA Synthesis by Superinfection with Adenovirus 2 and Non-defective Adenovirus 2–Simian Virus 40 Hybrid Viruses

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SUMMARY

Simian Virus 40 (SV40) DNA synthesis is inhibited in monkey cells by superinfection with adenovirus 2 (Ad2) and various non-defective Ad2–SV40 hybrid viruses. Similarly, BKV (a human papovavirus) DNA synthesis is inhibited in human cells by superinfection with Ad2. Kinetic studies indicate that inhibition begins during the early phase of the Ad2 lytic cycle. Superinfection with Ad2 does not significantly alter the formation of SV40 T antigen. Superinfection with Ad2 late in the SV40 lytic cycle is less efficient in the inhibition of SV40 DNA synthesis, and the onset of Ad2 DNA synthesis is delayed, compared to superinfection early in the SV40 lytic cycle. These findings suggest that the Ad2 and SV40 genomes may compete to bind an early Ad2 protein which is essential for Ad2 replication, but which blocks SV40 replication.

INTRODUCTION

Adenovirus (Ad) and simian virus 40 (SV40) interact with each other in two important ways during dual infection of monkey cells. First, monkey cells, which are only semi-permissive for human Ad, become fully permissive when coinfectected with SV40. Secondly, SV40 replication is largely suppressed in monkey cells coinfectected with Ad. These interactions were first observed by O’Conor et al. (1963, 1965) and Rabson et al. (1964) in electron microscopic studies of monkey cells infected with Ad, SV40, or both viruses. They noted that while few Ad virion particles were formed in singly infected cells, large numbers were present in cells coinfectected with Ad and SV40. On the other hand, very few SV40 virion particles were observed in monkey cells infected by Ad and SV40. These authors concluded that SV40 provided a helper function essential for Ad replication in monkey cells and concomitantly, some Ad function(s) acted to suppress SV40 replication in the same cells. The SV40 helper (or ‘enhancing’) property has been studied extensively and appears to be an early SV40 function that acts to relieve a block in the translation of some late Ad capsid proteins (Eron et al., 1975; Klessig & Anderson, 1975).

In contrast to the many studies carried out on enhancement, the concomitant inhibition of SV40 replication during dual infection has received relatively little attention (Patch et al., 1979). Friedman et al. (1970) showed that SV40 DNA synthesis was inhibited by coinfection with Ad2, and that the degree of inhibition was dependent on the multiplicity of Ad2 infection. More recently Van Roy & Fiers (1978) have confirmed that inhibition of SV40 DNA synthesis increases with increasing multiplicity of Ad2 infection. They also showed that
inhibition was attenuated with increasing multiplicity of SV40 infection, and that inhibition was less efficient when superinfection with Ad2 was delayed until the SV40 infection was well established. Moreover, they showed that temperature-sensitive mutants of Ad5 which do not replicate or express late Ad2 genes at non-permissive temperatures nevertheless inhibit SV40 DNA synthesis under non-permissive conditions. These same mutants also suppress cellular DNA synthesis (Wilkie et al., 1973) and one of them blocks Ad5 replication (Galos et al., 1979) at non-permissive temperatures.

In the work reported here we have extended the results of Friedman et al. (1970), and Van Roy & Fiers (1978). We have performed a kinetic analysis of suppression of SV40 DNA synthesis and the onset of Ad2 DNA synthesis in dually infected monkey cells by labelling viral DNA with short pulses of radioactive thymidine at different times after Ad2 superinfection. These kinetic studies confirm that inhibition of SV40 DNA synthesis begins early in the Ad2 lytic cycle. Moreover, our results suggest that there is competition between Ad2 and SV40 genomes for factors essential for Ad2 DNA synthesis.

**METHODS**

*Viruses and cells.* BSC-1, CV-1, Vero and HEK (Flow Laboratories) cells were grown in minimal essential media (MEM) containing 10% foetal bovine serum (FBS). KB cells were grown in suspension MEM containing 5% horse serum. Plaque-purified BKV (Dun strain) was propagated in HEK cells. SV40 (777), Ad2, Ad2+ND3, Ad2+ND4, and Ad2+ND6 were obtained from Dr A. M. Lewis, Jr. Working stocks of SV40 were grown in Vero cells, and Ad2 and Ad2−SV40 hybrids in KB suspension cultures.

*Radiolabelling of viral DNA.* In experiments on viral DNA synthesis, cells were grown to confluence in 60 mm plastic dishes. Infections were carried out by adsorption of virus (0.5 ml) for 1 h followed by the addition of 5 ml MEM + 2% FBS. DNA was labelled with [3H]methyl thymidine (40 to 60 Ci/mmol) (Amersham Corp.) and/or [14C]methyl thymidine (50 mCi/mmol) (Amersham Corp.).

*Hirt extraction of viral DNA.* Virus DNA was selectively extracted from infected cells by the method of Hirt (1967), modified as follows: after radioactive labelling, the infected cells were washed with cold PBS (0.85 % NaCl, 0.005 M-PO4 pH 7.2) and lysed with 5 ml 0.6% SDS, 0.01 M-EDTA pH 7-5. High mol. wt. DNA was precipitated with 1 M-NaCl (18 h, 4 °C) and centrifuged at 17000 g for 30 min. The supernatant was removed and extensively dialysed against 1 × SSC (0.15 M-NaCl, 0.015 M-sodium citrate pH 7.2). The supernatant (DNA, RNA and protein) was precipitated by adding 2 vol. cold ethanol (18 h, -20 °C) and centrifuging at 1600 g for 10 min, and dissolved by incubation (30 min, 37 °C) with 200 µl nuclease-free Pronase (100 µg/ml in 0-002 M-tris, 0-001 M-EDTA pH 7-5). The final preparation was contained in 400 µl 0.5% Sarkosyl (NL 97) in tris–EDTA. In reconstruction experiments using intact radioactive virus added to cell lysates, it was determined that 80 to 90% of the SV40 DNA could be recovered from the Hirt supernatant. In contrast, 50 to 60% of the Ad2 DNA remained with the Hirt pellet.

*Alkaline sucrose gradients.* Sucrose gradients (5 to 20% in 0.2 M-NaOH, 0.7 M-NaCl, 0.15% Sarkosyl) were prepared. Aliquots (50 to 100 µl) of DNA solutions were layered on to gradients and centrifuged at 40000 rev/min for 2.5 h at 5 °C in an SW50.1 rotor (Beckman). The gradients were collected from the bottom (100 µl fractions) and counted in 10 ml acidified Aquasol (New England Nuclear).

*SV40 T antigen staining.* CV-1 cells were grown on microscope coverslips in 35 mm dishes and infected with SV40 or with SV40 and Ad2. At various times, the coverslips were washed twice with cold PBS and fixed for 10 min in cold (−20 °C) acetone. The fixed cells were incubated (30 min, 37 °C) with serum from hamsters bearing SV40 tumours, washed five times with warm PBS, and reincubated with fluorescein-conjugated rabbit anti-hamster IgG. The stained coverslips were again washed, mounted with glycerine, and examined and photographed in a u.v. light microscope.
Inhibition of papovavirus DNA synthesis by Ad2

Table 1. Ad2 inhibition of SV40 DNA synthesis: effect of multiplicity and timing of Ad2 superinfection*

<table>
<thead>
<tr>
<th>Ad2 m.o.i. (p.f.u./cell)</th>
<th>SV40 DNA†</th>
<th>Ad2 DNA†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>[3H] (ct/min/μl)</td>
<td>%max</td>
</tr>
<tr>
<td>A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>239</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>107</td>
<td>47-8</td>
</tr>
<tr>
<td>4</td>
<td>35</td>
<td>14-6</td>
</tr>
<tr>
<td>8</td>
<td>22</td>
<td>9-2</td>
</tr>
<tr>
<td>17</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>33</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>66</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>471</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>367</td>
<td>77-9</td>
</tr>
<tr>
<td>4</td>
<td>176</td>
<td>37-4</td>
</tr>
<tr>
<td>8</td>
<td>146</td>
<td>31-0</td>
</tr>
<tr>
<td>17</td>
<td>75</td>
<td>15-9</td>
</tr>
<tr>
<td>33</td>
<td>38</td>
<td>8-1</td>
</tr>
<tr>
<td>66</td>
<td>38</td>
<td>8-1</td>
</tr>
</tbody>
</table>

* BSC-1 cells were infected with SV40 (11 p.f.u./cell) at 37°C. SV40-infected cells were superinfected with Ad2 at the indicated m.o.i. (first column) either at 6 hPI SV40 (A) or 15 hPI SV40 (B). Immediately after Ad2 adsorption (1 h), media containing [14C]thymidine (4 μCi/ml) was added. All cells were labelled with [3H]-thymidine (90 μCi/ml) 40 to 41 hPI SV40. DNA was extracted (Hirt, 1967) and analysed on alkaline sucrose gradients.

† The amount of [3H] and [14C]SV40 DNA (53S) or Ad2 DNA (34S) was determined in each gradient and is expressed as ct/min/μl of DNA solution. For purposes of comparison, SV40 DNA I synthesis is also presented as the percentage of maximum observed (i.e. superinfected with Ad2 m.o.i. = 1).

RESULTS

Inhibition of SV40 DNA synthesis by Ad2 has been reported to depend on the multiplicity of Ad2 infection (Ad2 m.o.i.) (Friedman et al., 1970; Van Roy & Fiers, 1978). The rate of SV40 DNA synthesis in monkey cells superinfected at different Ad2 m.o.i.s is shown in Table 1. In this experiment, SV40-infected cells were superinfected with Ad2 at the indicated m.o.i. (first column) either at 6 hPI SV40 (A) or 15 hPI SV40 (B). Immediately after Ad2 adsorption (1 h), media containing [14C]thymidine (4 μCi/ml) was added. All cells were labelled with [3H]-thymidine (90 μCi/ml) 40 to 41 hPI SV40. DNA was extracted (Hirt, 1967) and analysed on alkaline sucrose gradients.
Fig. 1. Alkaline sucrose gradients of radioactive DNA extracted (Hirt, 1967) from BSC-1 cells infected with SV40 alone (11 p.f.u./cell) (a), or infected with SV40 and superinfected with Ad2 (17 p.f.u./cell) at 15 hPI SV40 (b), or 6 hPI SV40 (c). Infected cells were labelled with [14C]thymidine (4 μCi/ml) (△) from 16 to 40 hPI SV40 (a, b) or 7 to 40 hPI SV40 (c). All dishes were labelled with [3H]thymidine (90 μCi/ml) (○) 40 to 41 hPI SV40. The shaded area in (b) indicates the method used to estimate the amounts of 34S Ad2 DNA (or 53S SV40 DNA) in alkaline sucrose gradients. The area (an isosceles triangle) is assumed to approximate the distribution of DNA in the gradient and is defined by the two points (F) and (P). The total ct/min (area) of each peak was divided by the volume of DNA solution applied to the gradient and expressed as ct/min/μl, which is a measure of the rate of viral DNA synthesis at a given time in a particular cell population.

Fig. 2. Effect of multiplicity and timing of superinfection on Ad2 inhibition of SV40 DNA synthesis (data from Table 2). SV40-infected cells were superinfected with Ad2 at different m.o.i.s (abscissa) at 6 hPI SV40 (○) or 15 hPI SV40 (△). The dashed line is computed from: 100 e^{-(m.o.i.−1)} which is the theoretical (Poisson) percentage of cells uninfected at a given m.o.i. (normalized to m.o.i. = 1) (see text).

infected]. Thus a plot of log P (0) against m.o.i. should be linear (dashed line, Fig. 2). It is evident that when cells are superinfected early (6 hPI SV40), inhibition of SV40 DNA synthesis closely follows the predicted distribution of infected cells. This finding suggests that SV40 DNA synthesis is blocked in any cell superinfected early with one or more Ad2 p.f.u. The efficiency of inhibition of SV40 DNA synthesis by Ad2 is less when superinfection is carried out late (15 hPI SV40) (Fig. 2). Moreover, in both cases there appears to be a decrease in efficiency of inhibition at high Ad2 m.o.i., suggesting that some cells either resist Ad2 superinfection or permit SV40 DNA synthesis concomitantly with Ad2 DNA synthesis. The latter notion is supported by an electron microscopic study (O’Conor et al., 1963) in
Table 2. Kinetics of Ad2 inhibition of SV40 DNA synthesis

<table>
<thead>
<tr>
<th>Duration of Ad2 infection (h)*</th>
<th>Early Ad2 superinfection†</th>
<th>Late Ad2 superinfection†</th>
<th>Superinfection with Ad2+ND3‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SV40 DNA (ct/min/μl)</td>
<td>Ad2 DNA (ct/min/μl)</td>
<td>SV40 DNA (ct/min/μl)</td>
</tr>
<tr>
<td>0</td>
<td>618 ± 63</td>
<td>NA§</td>
<td>630 ± 72</td>
</tr>
<tr>
<td>4</td>
<td>612 ± 58</td>
<td>0</td>
<td>NT</td>
</tr>
<tr>
<td>5</td>
<td>NT</td>
<td>NT§</td>
<td>564 ± 98</td>
</tr>
<tr>
<td>6</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>8</td>
<td>459 ± 52</td>
<td>0</td>
<td>NT</td>
</tr>
<tr>
<td>10</td>
<td>NT</td>
<td>NT</td>
<td>482 ± 102</td>
</tr>
<tr>
<td>13</td>
<td>NT</td>
<td>NT</td>
<td>243</td>
</tr>
<tr>
<td>14</td>
<td>231 ± 20</td>
<td>152 ± 12</td>
<td>NT</td>
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<tr>
<td>16</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
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<tr>
<td>17</td>
<td>120 ± 25</td>
<td>170 ± 52</td>
<td>411 ± 112</td>
</tr>
<tr>
<td>21</td>
<td>41 ± 2</td>
<td>967 ± 66</td>
<td>329 ± 93</td>
</tr>
<tr>
<td>25</td>
<td>NT</td>
<td>NT</td>
<td>145 ± 56</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>NT</td>
</tr>
</tbody>
</table>

* The duration of Ad2 or Ad2+ND3 superinfection is the difference between the time of superinfection and the end of the labelling period.
† BSC-1 cells were infected with SV40 (20 p.f.u./cell) at 37 °C. At various times after SV40 infection, the cells were superinfected with Ad2 (20 p.f.u./cell). The cells were labelled with a 1 h pulse of [3H]thymidine (100 μCi/ml). For early Ad superinfection the first superinfection was at 4 hPI SV40 and the cells were labelled 24 to 25 hPI SV40. For late Ad superinfection the first superinfection was at 12 hPI SV40 and the cells were labelled 36 to 37 hPI SV40. The rate of SV40 and Ad2 DNA synthesis was determined as described in the text. Standard deviations for 3 to 5 determinations are indicated.
‡ BSC-1 cells were infected with SV40 (15 p.f.u./cell) at 40.5 °C beginning at 4 hPI SV40 and at various times thereafter cells were superinfected with Ad2+ND3 (15 p.f.u./cell). All dishes were labelled with [3H]thymidine (100 μCi/ml) at 24 to 25 hPI SV40.
§ NA, Not applicable; NT, not tested.

which it was observed that about 1% of cells dually infected with Ad12 and SV40 contained large numbers of both Ad12 and SV40 virion particles. This observation indicated that all cells within the infected cell population do not respond uniformly to the same viral challenge. However, since inhibition of SV40 DNA synthesis is strongly dependent on the timing of Ad2 superinfection (Fig. 2), it is unlikely that differences in response to a viral challenge can be due to cell factors alone. It is probable that the timing of viral replicative events within superinfected cells is governed by the ratio of Ad2 to SV40 within the individual cells of the infected population. Thus, cells in which SV40 infection is proceeding rapidly (i.e. due to a statistically high SV40 m.o.i.) might support replication of both viruses if, in the same cells, Ad2 infection is proceeding slowly.

Kinetics of Ad2 inhibition of SV40 DNA synthesis

The data shown in Table 1 and Fig. 2, as well as the results of other workers (Van Roy & Fiers, 1978), indicate that Ad2 inhibition of SV40 DNA synthesis is more efficient when Ad2 superinfection is carried out early in the SV40 lytic cycle. Accordingly, we have measured the kinetics of inhibition of SV40 DNA synthesis and the onset of Ad2 DNA synthesis in cells superinfected before and after SV40 DNA synthesis begins (Table 2). The data in Table 2 were normalized to the maximum rates of SV40 or Ad2 DNA synthesis and plotted in Fig. 3.

As can be seen, inhibition of SV40 DNA synthesis begins within 8 h of Ad2 superinfection, indicating that the inhibitory function is expressed early in Ad2 infection. This finding is in agreement with the observation that ts mutants of Ad5, which do not express late Ad5 functions at non-permissive temperatures, nevertheless inhibit SV40 DNA synthesis under these conditions (Van Roy & Fiers, 1978). The data in Fig. 3 show that the kinetics of inhibition of SV40 DNA synthesis are much slower when superinfection is carried out after SV40 DNA synthesis in underway (i.e. 15 to 20 hPI SV40). Moreover, these data show that
not only is SV40 DNA synthesis more resistant to inhibition by Ad superinfection late in the SV40 lytic cycle, but synthesis of Ad2 DNA is delayed when compared to superinfection early.

This apparent delay in the onset of Ad2 DNA synthesis could be due to a general deterioration of the SV40-infected cells with time. However, the data in Table 1B indicate that the cells are capable of supporting high rates of Ad DNA synthesis as late as 40 hPI SV40. Thus, the observed delay in the onset of Ad2 DNA synthesis probably indicates that a factor(s), essential for Ad2 DNA synthesis, is less available late in SV40 infection.

**Inhibition by non-defective Ad2–SV40 hybrids**

We also examined the effect of several non-defective Ad2–SV40 hybrid viruses on the kinetics of SV40 DNA synthesis. The data shown in Table 2 indicate that Ad2⁺ND₃ is an efficient inhibitor of SV40 DNA synthesis, and the kinetics of inhibition are similar to those of Ad2. Moreover, both Ad2⁺ND₄ and Ad2⁺ND₅ are also efficient inhibitors of SV40 DNA synthesis (data not shown). This result is of interest, since all three viruses have large deletions of Ad2 DNA in the E3 region of the Ad2 genome (Kelly & Lewis, 1973). Thus, it appears that while Ad2 inhibition of SV40 DNA synthesis is expressed early in Ad2 infection, the inhibitory function is evidently not mediated by a gene product encoded in the E3 region. With regard to the possibility that the SV40 function(s) in the Ad2–SV40 hybrids might have influenced these results, it should be noted that we have attempted to complement SV40 ts A 58 with Ad2⁺ND₄ (which induces SV40 T antigen), but found that the Ad inhibitory function(s) was dominant and observed no SV40 DNA synthesis (C. Patch & P. Howley, unpublished results).

**SV40 Tag synthesis in dually infected cells**

It is known that inhibition of SV40 DNA synthesis by cytosine arabinoside does not alter the rate of accumulation of SV40 T antigen (Tag) during the first 24 h of infection (Oxman et al., 1971). However, since SV40 early protein(s) (presumably large Tag) are involved in initiation of both SV40 and host DNA synthesis (Martin et al., 1974), it is possible that Ad2 inhibition of SV40 DNA synthesis could be mediated by interference with synthesis of large 

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**Fig. 3.** Kinetics of SV40 (——) and Ad2 (---) DNA synthesis in BSC-1 cells superinfected early (O, □) or late (■, □) in the SV40 lytic cycle. The data from Table 2 are normalized to the maximum rate of SV40 (618 or 630 ct/min/μl) or Ad2 (967 ct/min/μl) DNA synthesis.
Inhibition of papovavirus DNA synthesis by Ad2

Tag. To test this possibility, monkey cells grown on glass coverslips were infected with SV40 alone or coinfected with SV40 and Ad2. At various times after infection, the cells were fixed and stained for SV40 Tag using serum from hamsters bearing SV40 tumours together with fluorescein-conjugated anti-hamster IgG (Fig. 4). Duplicate dishes (either infected with SV40 alone or co-infected with Ad) were pulsed with $[^3]$H]thymidine at 24 to 25 h post-infection. Analysis of the radiolabelled DNA indicated that SV40 DNA synthesis in the co-infected cultures was only 5% of that in cells infected with SV40 alone. Despite the fact that SV40 DNA synthesis is strongly inhibited in dually infected cells, these cells accumulate SV40 Tag in amounts comparable to singly infected cells (Fig. 4). Moreover, it is evident that cells which exhibit extensive Ad2 cytopathic effects (fixed at 42 h post-infection, Fig. 4 F) still contain large amounts of SV40 Tag. Since the quantity of SV40 Tag is not significantly reduced in dually infected cells, the inhibition of SV40 DNA synthesis cannot be mediated by inhibition of SV40 T antigen synthesis. This result is in agreement with recent findings of Goldman &
C. T. Patch and Others

Fig. 5. Kinetics of inhibition of BKV DNA synthesis by superinfection with Ad2. HEK cells were infected with BKV (20 p.f.u./cell) at 37 °C. Beginning at 36 hPI BKV, dishes were superinfected at 4 h intervals with Ad2 (20 p.f.u./cell). At 60 hPI BKV, media were removed and replaced with media containing [3H]thymidine (100 μCi/ml). After 1 h, DNA was extracted (Hirt, 1967) and analysed on alkaline sucrose gradients. The ordinate indicates the amount of DNA recovered expressed as a percentage of maximum; BKV DNA (O, max. = 259 ct/min/μl) and Ad2 DNA (C, max. = 306 ct/min/μl). The abscissa indicates the duration of Ad2 superinfection. Each point represents the average of triplicate determinations.

Khoury (1980) which show that SV40 RNA and protein synthesis remain undiminished in dually infected cells.

Ad2 inhibition of BKV DNA synthesis in human cells

To determine whether Ad2 inhibition is specific for SV40 in monkey cells or whether other papovaviruses might be inhibited in other cell types, we have examined the effect of Ad2 superinfection on BKV (a human papovavirus) DNA replication in human cells. In the experiment shown in Fig. 5, BKV-infected HEK cells were superinfected with Ad2 at 36 hPI BKV and at 4 h intervals thereafter; the cells were radiolabelled at 60 to 61 hPI BKV (a time of maximum BKV DNA synthesis) (Howley, 1980). It is evident that Ad2 superinfection inhibits BKV DNA synthesis, and the kinetics of inhibition are similar to the kinetics of inhibition of SV40 DNA synthesis in monkey cells.

Discussion

In this study, we confirmed that superinfection with Ad2 inhibits SV40 DNA synthesis in monkey cells, and have determined the kinetics of inhibition of SV40 DNA synthesis, as well as the onset of Ad2 DNA synthesis, in such dually infected cells. The results of these experiments indicate that inhibition of SV40 DNA synthesis is mediated by an early Ad2 protein(s) or a host protein(s) induced early in Ad2 infection. This finding is in agreement with the results of Van Roy & Fiers (1978). In addition, we have measured the kinetics of inhibition of BK virus DNA synthesis in human cells co-infected with Ad2 (Fig. 5). The similarity between the kinetics of inhibition of papovavirus DNA synthesis in two different cell types (human and monkey) may imply that the inhibitory factor(s) is not of host origin, although direct evidence for such a conclusion is lacking.

When SV40-infected cells were co-infected with Ad2 at different multiplicities (m.o.i.), we observed that SV40 DNA synthesis was blocked when the Ad2 m.o.i. was equal to or greater
Inhibition of papovavirus DNA synthesis by Ad2

than 1 p.f.u./cell; the efficiency of inhibition was decreased if superinfection was delayed until late in SV40 infection (Fig. 2). A more detailed study of these kinetics in cells superinfected at early and late times in the SV40 lytic cycle revealed not only that the inhibition of SV40 DNA synthesis is decreased when superinfection is effected late, but that the onset of Ad2 DNA synthesis is delayed as well (Fig. 3).

The mechanisms of Ad and papovavirus DNA synthesis are quite different (Salzman & Khoury, 1974; Lavelle et al., 1975; Lechner & Kelly, 1977). However, both types of virus depend on host proteins for the replication of their genomes, and both induce virus-encoded proteins early in lytic infection which are essential for viral DNA synthesis. The Ad genome contains at least six early template regions (Ross et al., 1980). Approx. 15 proteins are induced early in Ad infection and at least half of these have been demonstrated to be encoded in the early viral templates. While precise functions for all of the early Ad-induced proteins have not been established, it is assumed that many play an essential role in Ad DNA synthesis (Winnacker, 1978; Wold et al., 1978). Replication of the SV40 genome is initiated when a 90K early SV40 protein (large T antigen, Tag) binds to the viral genome near the origin of DNA replication (Tjian, 1978). All of the other molecular components of the SV40 DNA-synthesizing apparatus are of host origin (Klempnauer et al., 1980). Tag begins to accumulate within 3 to 5 hPI SV40, but viral DNA synthesis is not detected until 15 to 20 h post-infection. In the interim, Tag(s) and other factors essential for replication must accumulate to critical concentrations. Moreover, Tag undergoes post-translational modification (e.g. phosphorylation; Edwards et al., 1979) which may be essential for its role in DNA replication. Presumably if any of these processes is interrupted, initiation of SV40 DNA synthesis will not occur. We have examined the induction of Tag in cells coinfected with SV40 and Ad2 (Fig. 4). Our results, although not quantitative, indicate that the accumulation of SV40 Tag in singly infected cells or in cells dually infected with Ad is very similar, although SV40 DNA synthesis is almost entirely suppressed in the dually infected cells. This finding is in agreement with the results of Goldman & Khoury (1980) who detected no change in SV40 mRNA or protein synthesis during dual infection with Ad5.

The results of our kinetic studies suggest that there is competition between replicating SV40 and Ad genomes in dually infected cells. Thus, late in SV40 infection, when more SV40 genomes and/or more SV40 proteins (early and late) are present, factors essential for Ad replication appear to be less available than is the case when superinfection is effected early in the SV40 lytic cycle. Since host factors (e.g. DNA polymerases) participate in the replication of both viral genomes, it is possible that independently replicating viral genomes compete for factors required by both viruses. However, the almost total suppression of SV40 DNA synthesis in dually infected cells probably could not result from passive competition. Moreover, our evidence indicates that inhibition begins early, before Ad DNA synthesis could compete for host factors. Goldman & Khoury (1980) suggest that Ad DNA could successfully compete to bind SV40 Tag, thus making that protein unavailable for initiation of SV40 DNA synthesis. However, the absence of dependence of inhibition on Ad DNA synthesis argues against this possibility (Van Roy & Fiers, 1978).

Another mechanism consistent with the notion of competition requires that the factor(s) which inhibits SV40 DNA synthesis be essential for Ad replication. This model suggests that a protein which is induced early in Ad infection can bind to either Ad2 or SV40 DNA. This protein would be essential for Ad2 DNA synthesis, but binding to SV40 genomes would lead to inhibition of SV40 DNA synthesis. If the inhibitory protein is of viral origin, it cannot be either the 19K (glycosylated) or the 14K proteins encoded in the early Ad template region E3, since Ad2+ND3, which is an efficient inhibitor of SV40 DNA synthesis (Table 2), induces neither protein (Storch & Maizel, 1980). At non-permissive temperatures, the temperature-sensitive (ts) mutants Ad5 ts 36 and Ad5 ts 125, which have defects in the early regions E1
and E2, inhibit SV40 DNA synthesis (Van Roy & Fiers, 1978). Ad5 \( t_s \) 125 induces a temperature-sensitive DNA-binding protein (DBP) (Van der Vliet et al., 1975), and Ad5 \( t_s \) 125, but not Ad5 \( t_s \) 36, interferes with wild-type (wt) Ad5 replication at non-permissive temperatures (Galos et al., 1979). Although the mechanism of this interference with wt Ad5 replication is not known, it is likely to involve viral DNA synthesis since this is the main function of the DBP (Gilead et al., 1975). It should be noted, however, that at non-permissive temperatures the DBP of Ad5 \( t_s \) 125 is synthesized in reduced quantities and has a reduced capacity to bind to single-stranded DNA (Van der Vliet et al., 1975). Nevertheless, it is reasonable to speculate that \( t_s \) DBP could interfere with wt Ad5 DNA synthesis, and wt DBP could interfere with SV40 DNA synthesis, by binding to the viral genome and either preventing initiation of DNA synthesis or chain elongation.

To summarize, the data presented here suggest strongly that Ad inhibition of papovavirus DNA synthesis in dually infected cells is mediated by factors induced early in Ad infection, in agreement with the results of Van Roy & Fiers (1978). Although our data do not exclude other mechanisms (e.g. interference with modification of SV40 Tag which may be necessary to initiate viral DNA synthesis), the simplest interpretation of our results is that replicating papovavirus and Ad genomes compete to bind a protein which is inhibitory for papovavirus DNA synthesis, but is an essential factor in Ad DNA replication. We have not identified the protein(s) involved or determined how it acts to effect this inhibition. However, further elucidation of the molecular events involved in this viral interaction should provide insight into the functions of early viral proteins and the process of viral DNA synthesis.

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Inhibition of papovavirus DNA synthesis by Ad2


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