Further Studies on
the Differences in the Interaction of Simian Virus 40 with
African Green Monkey Kidney and Human Diploid Cells

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Infection of human diploid cells with Simian virus 40 (SV40) leads to an abortive virus cycle characterized by little virus production, little or no cell destruction, no inhibition of cell replicating capacity, low efficiency of induction of tumour antigen (T-antigen) and eventual transformation of the morphological, chromosomal, and growth characteristics of the cells (Koprowski et al. 1962; Shein & Enders, 1962; Rabson et al. 1962; Weinstein & Moorhead, 1965). In marked contrast, in stationary-phase primary African green monkey kidney (GMK) cells, SV40 infection leads to high yields of virus, highly efficient induction of T-antigen and extensive cell destruction (Carp & Gilden, 1966). The determination of the cause of these marked differences might clarify the mechanism of the transformation process. Previous results indicated that the virus adsorbed and entered into eclipse phase with equal efficiency for the two cell types (Carp & Gilden, 1966). In the present study, two aspects of the problem were examined: the efficiency of infection with virus DNA was determined in the two cell systems, and the net synthesis of RNA hybridizable with purified SV40 DNA was compared.

SV40 was grown and assayed in GMK cells and GMK and human diploid cells (WI-38) were grown, maintained and infected as described previously (Carp & Gilden, 1966).

For the isolation of infectious DNA, a 100 ml. pool of SV40 was prepared. This pool had an infectivity of $10^{8.3}$ p.f.u./ml. The virus was purified and infectious DNA isolated as described previously (Carp, Sauer & Sokol, 1969).

DEAE-dextran was used to enhance the infectivity of the phenol-extracted virus DNA (McCutchan & Pagano, 1968). A stock solution of DEAE-dextran was maintained frozen at a concentration of 100 mg./ml. in 0.15 M-tris+HCl buffer at pH 7.5. Virus DNA, maintained on 0.1 × SSC (SSC: 0.15 M-NaCl, 0.015 M-sodium citrate), was diluted in Eagle's basal medium (BME) buffered with 0.05 M-tris+HCl at pH 7.4 (without NaHCO₃) and containing 250 or 1000 μg./ml. of DEAE-dextran. Cells maintained on coverslips in 60 mm. Petri dishes were washed twice with phosphate-buffered saline (0.13 M-NaCl, 0.02 M phosphate, pH 7.3) prior to the addition of the diluted DNA. After 15 min. of adsorption at room temperature the cells were washed with phosphate buffered saline and overlaid with 6 ml. of BME containing 2% calf serum. Coverslips were recovered and stained for T-antigen as described previously (Gilden et al. 1965).

Techniques of RNA isolation, of RNA-to-DNA hybridization and the specificity of the virus DNA were established by Carp et al. (1969). In the experiments described here cells were labelled from 2 to 44 hr after infection with 5 μc./ml. of [3H]uridine (Schwarz Bioresearch, Orangeburg, N.Y.; specific activity: 24.7 c/m-mole). Virus entrance into eclipse, a process shown to occur with equal efficiency in GMK

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and WI-38 cells, does not necessarily measure the availability of virus nucleic acid for the processes of transcription and replication. Therefore, it was of interest to compare the ability of whole virus and of virus stripped of its protein coat (i.e. infectious DNA) to initiate processes of virus replication in GMK and WI-38 cells. Cells maintained on coverslips were infected with SV40 or with virus DNA, harvested 44 hr after infection and stained for T-antigen. In a typical experiment (Table 1) the expression of virus DNA in the two cell systems, as determined by T-antigen synthesis, was similar whether virus or infectious DNA was used as the infecting agent. In other experiments the ratio of T-antigen induction in GMK cells to that in WI-38 cells following virus infection varied between 30 and 90 to 1, while with infectious DNA the ratio varied between 44 and 111 to 1. The variation in the ratio when infectious DNA was used was not related to the dosages (250 and 1000 μg.) of DEAE-dextran employed, since both yielded similar ratios. If infectious DNA was incubated at 37° for 30 min. with DNase (20 μg./ml.) in the presence of 5 x 10⁻⁸ m-MgSO₄ the infectivity for both WI-38 and GMK cells was eliminated.

The net synthesis of virus-specific RNA was determined in GMK cells infected at a high or low multiplicity and in WI-38 cells infected at a high multiplicity. The induction of T-antigen was used as a measure of virus-specific messenger RNA translation and this value was compared with the amount of RNA which could be hybridized to SV40 DNA. In human diploid cells an accumulation of large amounts of hybridiz-

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**Table 1. The induction of T-antigen in GMK and WI-38 cells infected with SV40 or with DNA isolated from SV40**

<table>
<thead>
<tr>
<th>Infecting material</th>
<th>% positive GMK</th>
<th>% positive WI-38</th>
<th>Ratio of % positive GMK cells to % positive WI-38 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virus (3 p.f.u./cell)</td>
<td>90</td>
<td>20</td>
<td>45</td>
</tr>
<tr>
<td>DNA* (0.05 p.f.u./cell)</td>
<td>5.3</td>
<td>0.11</td>
<td>48</td>
</tr>
</tbody>
</table>

* The volume of the inoculum for DNA infection was 0.4 ml., and this contained 3.2 μg. of DNA and 100 μg. of DEAE-dextran.

**Table 2. The synthesis of T-antigen and of RNA hybridizable with SV40-DNA in GMK and WI-38 cells infected with SV40**

<table>
<thead>
<tr>
<th>Multiplicity of infection (p.f.u./cell)</th>
<th>T-antigen-positive (%)</th>
<th>Counts/min. of hybridizable RNA*</th>
<th>T-antigen-positive (%)</th>
<th>Counts/min. of hybridizable RNA*</th>
</tr>
</thead>
<tbody>
<tr>
<td>500</td>
<td>100</td>
<td>3200</td>
<td>5</td>
<td>53</td>
</tr>
<tr>
<td>0.05</td>
<td>5</td>
<td>45</td>
<td>Not done</td>
<td>Not done</td>
</tr>
</tbody>
</table>

* The specific activities (counts/min./μg. RNA) of RNA isolated from GMK cells infected at high and low multiplicities were 5.5 x 10⁴ and 4.03 x 10⁴, respectively. The specific activity of RNA isolated from WI-38 cells was 2.86 x 10⁴. RNA was isolated from cells harvested 44 hr after infection. The radioactivity of all RNA samples added to the virus DNA was 2.6 x 10⁶ counts/min. In each instance the RNA was hybridized with excess SV40-DNA (0.43 μg. with RNA from WI-38 cells and with RNA from GMK cells infected at a low multiplicity and 1.7 μg. with RNA from GMK cells infected at a high multiplicity).
zable RNA, in the absence of translation comparable to that found in GMK cells, would indicate that a block at the translation level leads to the reduction in the expression of SV40 previously noted in WI-38 cells (Carp & Gilden, 1966; Carp, 1967). The amount of hybridizable RNA found in the abortive system was the same as that found in cultures of GMK cells with a comparable number of T-antigen-positive cells, but was much less than that found in GMK cells infected at a high multiplicity (Table 2). Thus, the amount of hybridizable RNA found in WI-38 cells was compatible with the small number of cells in which translation was manifested. It should be noted that with the techniques used it is impossible to determine if the hybridizable RNA found in the human diploid cultures occurred only in those cells positive for T-antigen or if it also occurred in cells negative for the antigen.

The ratio of the efficiency of induction of T-antigen-positive cells in GMK cells to that in WI-38 cells was similar whether virus or infectious DNA was used as the infectious agent, supporting the previous finding that the early aspects of the cell-virus relationship, such as adsorption and entrance into eclipse, are not responsible for the differences in the two cell systems (Carp & Gilden, 1966). The differences appear to be related to events occurring after the stripping of the DNA and its being rendered capable of being transcribed.

The data on the induction of SV40-specific RNA in the two cell systems suggest that there is a block in the transcription process in the majority of WI-38 cells. There are however, alternative explanations. The translation of an early function may be blocked, a function that is subsequently required for further transcription of the genome. Thus, a block in translation could express itself as a reduction in the synthesis of virus-specific RNA. A further possibility is that virus-specific RNA is more rapidly destroyed in WI-38 than in GMK cells and is not accumulated even though there is extensive transcription.

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REFERENCES


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