RESCUED OF INFECTIOUS SV40 AFTER FUSION BETWEEN DIFFERENT SV40-TRANSFORMED CELLS*

BY BARBARA B. KNOWLES, FRED C. JENSEN, ZENON STEPlewski,† AND HILARY KOPROWSKI

THE WISTAR INSTITUTE OF ANATOMY AND BIOLOGY, PHILADELPHIA, PENNSYLVANIA

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We have previously reported1 activation of infectious SV40 in transformed cell types of various origins after fusion with susceptible African green monkey kidney cells (AGMK). In the same report, we described failure to activate SV40 after fusion of AGMK cells with two sublines of SV40-transformed human cells (W98-VaC and W126VaA) and cultures originating from four clones of SV40-transformed green monkey kidney cells (GMK-EVa).

The present report describes activation of SV40 after fusion of some of these cells with other transformed cells.

Materials and Methods.—Tissue cultures: These were described previously,1 and their characteristics are summarized in Table 1. Except for F5-1, all cell lines were transformed by SV40 in tissue cultures.1 F5-1 is a cell line originally derived from a hamster fibrosarcoma induced by SV402 and maintained in tissue culture for 89 passages. The SV40 pool used for induction of the F5-1 tumor was different from the pool used for transformation of tissue cultures.

For assay of infectious SV40, we used primary AGMK cultures; for fusion purposes, we used secondary cultures of AGMK cells.

Cell fusion technique: The technique was the same as that described previously,3 except that Sendai virus was inactivated by 0.025% β-propiolactone instead of ultraviolet light (UV), and the medium used at the time of fusion contained 2%, instead of 10%, fetal calf serum.

When three instead of two types of cells were to be fused, suspensions of the two transformed cells were exposed to Sendai virus for 5 min at +4°C, and an equal number (5.0×10⁶) of AGMK cells was then added to the cell mixture. After further incubation at +4°C for 5 min, the cell mixture was treated exactly as described before.1

Isolation of infectious SV40: One, two, and three weeks after fusion, the bulk of the medium was removed, and the cells in 6–10 ml of medium (per milk dilution bottle) were frozen and thawed three times before inoculation on AGMK monolayers. Alternatively, the fused cells were scraped off the glass and sonicated in 6 ml of medium; the extract was then inoculated on monolayers of AGMK.

The inoculated AGMK cultures were incubated at 37°C and observed for the presence of cytopathic effect. In order to confirm the presence of infectious SV40, cells of cultures showing cytopathic effect were scraped off the glass by freezing and thawing, and the cell extract was placed on coverslip cultures of AGMK cells. These were stained by immunofluorescence to determine the presence of SV40-induced complement fixation (T) and viral coat (VP) antigens.4

Results.—These are summarized in Table 2. In every experiment, cells of each transformed line were fused with cells of the same line, with AGMK, with other transformed cells, and with other transformed cells and AGMK cells. No infectious SV40 was isolated from cultures of transformed cells fused with cells of the same line or with AGMK cells.1 However, when, within the same experiments, cells of one transformed line were fused with transformed cells of other origin, infectious SV40 was isolated from the heterokaryon cultures. Virus was isolated directly after cells of clone 2A-1 were fused with cells of F5-1 and W98VaC lines.
Table 1. Characteristics of the SV40-transformed cell lines.

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Origin</th>
<th>Recovery of Infectious SV40</th>
<th>Spontaneous</th>
<th>Cocultivation*</th>
<th>Fusion*</th>
</tr>
</thead>
<tbody>
<tr>
<td>F5-1</td>
<td>Hamster tumor</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>GMK-EV₀ clone 2A-1</td>
<td>Transformed AGMK culture</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>W98VaC</td>
<td>Transformed human fibroblast cultures</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>WI26Va₄</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

* With AGMK.

Table 2. Activation of infectious SV40 after fusion of two SV40-transformed cell lines in the presence or absence of AGMK cells.

<table>
<thead>
<tr>
<th>Activated by:</th>
<th>F5-1</th>
<th>W98VaC</th>
<th>WI26Va₄</th>
<th>GMK-EV₀ clone 2A-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>F5-1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>W98VaC</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>WI26Va₄</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>GMK-EV₀ clone 2A-1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* Simultaneous fusion of the two transformed cells and AGMK cells in the presence of Sendai virus.
† Fusion of the two transformed cells in the presence of Sendai virus.

respectively, without additional fusion with AGMK cells. This can be explained by the fact that cells of clone 2A-1 culture, although resistant to superinfection with complete virus, are capable of synthesizing infectious SV40 after exposure to viral DNA. Three other fusions (see Table 2) of cells of two transformed lines with AGMK cells resulted in the isolation of SV40; however, virus was not isolated when the transformed cells were fused in the absence of AGMK.

Cell extracts of heterokaryon cultures usually became infectious one to three weeks after fusion.

In the AGMK assay system, cytopathic effect was first observed within one week after inoculation with undiluted extract of fused cells. The concentration of "rescued" SV40 obtained by fusion of cells from the transformed lines in the absence of AGMK (clone 2A-1 with F5-1 and with W98VaC, respectively) was low, since a 1:10 dilution of the original extract was already not infectious.

Discussion.—In mouse cells transformed by SV40, the virus isolated after cocultivation or fusion with SV40-susceptible cells will bear the genetic marker characteristic of the original "inducing" virus. Since we have no knowledge of the genetic characteristics of the SV40 used for transformation of the cell lines described in this paper, it is impossible to determine whether we are dealing in this study with activation by recombination or complementation of two types of defective virus particles juxtaposed in the same heterokaryocyte or in a synkaryon. Virus particles bearing characteristics of defective mutants were shown to be present in SV40 pools by Sauer et al. In addition, others have shown an increase in the population of defective particles in SV40 populations when the virus was passaged in susceptible cells at high multiplicities of infection.

In Table 3, we have listed categories of SV40-transformed cells classified ac-
cording to their capability of yielding infectious SV40. The 3T3 mouse cells transformed by SV40 yield infectious SV40 spontaneously on occasion and always after cocultivation with susceptible cells. Although we were unable to isolate infectious SV40 from two cloned populations of mKs-A mouse cells kept in cultures by themselves, SV40 was always isolated when cells of these cultures were cocultivated with AGMK cells. Fusion of SV-3T3 and mKs-A cells with AGMK cells further facilitated isolation of SV40. Isolation of infectious SV40 from transformed mouse cell lines after cocultivation with AGMK cells also seems to occur as a rule in the hands of other investigators.9, 10

**Table 3. Rescue of infectious SV40 from various transformed cell lines.**

<table>
<thead>
<tr>
<th>Cocultivation with AGMK</th>
<th>Fusion with AGMK</th>
<th>Fusion of two transformed cell types</th>
</tr>
</thead>
<tbody>
<tr>
<td>SV-3T3: clone 101</td>
<td></td>
<td>W98VaC (GMK-EVa clone 2A-1)</td>
</tr>
<tr>
<td>mKs-A: clone 4-2</td>
<td></td>
<td>F5-1 (W98VaC, WI26Va4, WI26Va4)</td>
</tr>
<tr>
<td>clone 4-3</td>
<td></td>
<td>WI18Va2 (GMK-EVa clone 2A-1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>W98Va†</td>
</tr>
</tbody>
</table>

* Four clones yielded virus and four did not. (Clone 2A-1 did not yield virus.)
† Only one of the eight sublines (W98VaC) did not yield virus.

The SV40-induced hamster ependymomas11 and some cell lines derived from clones of hamster embryo kidney cultures transformed by SV4012, 13 also belong to the category of cultures in which SV40 can be activated by cocultivation with cultures of SV40-susceptible cells. However, other transformed hamster cell lines such as F5-1 and TSV-1114 did not yield SV40 even after these cells were fused with AGMK cells.

Green monkey kidney cell lines and human cell lines transformed by SV40 differ from the SV-3T3, mKs-A, and some hamster cell lines in the sense that activation of SV40 in these cells did not occur either spontaneously or after cocultivation with susceptible cells. Activation took place only after fusion either with AGMK cells alone or with cells of other transformed cell lines that also did not yield virus after fusion with AGMK cells. The activation of SV40 in the cultures of this last category did not seem to be related to the origin of the transformed cells, since virus was isolated from heterokaryon cultures obtained by fusion of hamster cells with either human or green monkey kidney cells or by fusion of human cells with other human cells or green monkey kidney cells.

Activation of SV40 depends on the condition of the viral genome in the transformed cell as well as on the presence or absence of factors in the host cell affecting the status of the viral genome. In cells from which the virus could be recovered after cocultivation or fusion with susceptible cells, the viral genome has to be complete, but expression of its late function is repressed. The nature of the "repressor" is currently under study.

Conversely, cells that yield SV40 only after fusion with other transformed cells may contain SV40 genomes defective at different loci in the two types of cells. In this case, recombination of or complementation between the defective particles in a heterokaryocyte would explain the appearance of complete virus. It is im-
possible to state at this time whether a host factor also plays a role in this “rescue” process.

Summary.—Cells of some SV40-transformed cultures do not yield virus after fusion with susceptible AGMK cells in the presence of Sendai virus. When cells from SV40-transformed cultures are fused with cells of another of these cultures, SV40 is rescued either by recombination or by complementation.

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† Permanent address: Department of Tumor Biology, Institute of Oncology, Gliwice, Poland.

1 Koprowski, H., F. C. Jensen, and Z. Steplewski, these PROCEEDINGS, 58, 127 (1967).
4 Unpublished observations.
6 Sauer, G., H. Koprowski, and V. Defendi, these PROCEEDINGS, 58, 599 (1967).
8 Yoshiike, K., Virology, 34, 391 (1968).
10 Watkins, J. F., and R. Dulbecco, these PROCEEDINGS, 58, 1396 (1967).