Stimulation of DNA Synthesis and Enhancement of Murine Sarcoma Virus Replication in Mouse Embryo Cells by SV 40

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Mouse embryo cells maintained on a rigid subculture regime undergo a rapid decline in growth rate leading to a period of negligible growth. Eventually the cells recommence growth and become established in vitro (Todaro & Green, 1963; Aaronson & Todaro, 1968; Baker & Simons, 1971). A very small percentage of cells in the non-dividing phase incorporate [3H]-thymidine and cells in this phase are not transformed by murine sarcoma virus (Baker & Simons, 1971). Treatment with colchicine does not result in the detection of metaphases and cells in this phase have been termed 'amitotic' (Baker & Simons, 1971).

Cellular DNA synthesis has been shown to be induced in resting cells by SV 40 (Girardi, Jensen & Koprowski, 1965; Coggin, 1969). Consequently it was possible that SV 40 would be able to induce DNA synthesis in the amitotic mouse cells rendering them susceptible to MSV.

The standard method of cultivating the cells has been described in detail previously (Baker & Simons, 1971). Briefly BALB/C mouse embryo cells were trypsinized every 3 days and passed at a concentration of 3 x 10⁴ cells per cm.² of plastic dish. The growth medium was Eagle's Minimum Essential Medium (Gibco Powder Medium F 15) supplemented with 10% inactivated calf serum. Cell growth was monitored by counting the cells on days 1 and 3. Passage 8 cells shown to be in the amitotic phase were used throughout.

Wild type simian virus 40 (SV 40) was obtained from Dr J. S. Rhim, U.S. National Institutes of Health, and was grown in BSC-1 cells. The stock virus used in this study contained 10⁸ TCD₅₀/ml., when assayed in BSC-1 cells by the end-point method. SV 40 nuclear T-antigen was stained with a specific fluorescein-conjugated antiserum (Microbiological Associates, Inc.) produced in hamsters bearing SV 40 induced tumours. The percentage of nuclei containing T-antigen was determined in preparations counterstained with 0.01% Evans Blue.

Murine sarcoma virus-HARVEY (MSV-H) preparations consisted of filtered (450 nm.) fluids from cultures of MSV-H induced tumour cells (Simons & McCully, 1970).

Replicate cultures, some containing cover-slips, of cells were prepared by plating 5 x 10⁵ cells into 60 mm. Falcon plastic dishes. The cells were allowed to settle for 24 hr at 37°C then each culture was infected with 1 ml. of SV 40 suspension containing 1.2 x 10⁸ TCD₅₀ (m.o.i. of 250). After 2 hr incubation at 37°C the inoculum was replaced with fresh growth medium.

Duplicate dishes were trypsinized and the cells counted immediately and then at 24 hr intervals. Cells on cover-slips were placed in medium containing [³H]-thymidine (0.5 μCi/ml., specific activity 5 c/m-mole) at the same time intervals. After 24 hr incubation in [³H]-thymidine the cells were processed for autoradiography and the proportions of cells synthesizing DNA were determined.

For the simultaneous infection of amitotic cells with SV 40 and MSV, 10⁵ cells were plated in 35 mm. Falcon plastic Petri dishes in 2 ml. growth medium. The dishes were incubated for 24 hr to allow the cells to settle and then treated with DEAE-dextran solution (25 μg./ml. of growth medium) for 1 hr at 37°C. After 3 washes with growth medium the cultures were infected with 0.2 ml. of SV 40 (10⁸ TCD₅₀) and 0.7 ml. of MSV (10⁴ f.f.u.). Replicate cultures...
were infected with either SV40 or MSV, both with 0.7 ml. of growth medium. Two ml. of growth medium was added to each dish after 2 hr incubation.

After infection of amitotic cells with SV40 there was a rapid increase in the number of cells incorporating [3H]-thymidine. Concomitantly, there was an increase in the cell numbers.

Confluent primary (P1) cells and passage eight cells were infected with SV40 and examined for the presence of SV40 T-antigen after 48 hr. Less than 5% of P1 cell nuclei contained T-antigen, whereas 25% of the P8 nuclei showed specific fluorescence. The infected amitotic cells had fluorescent nuclei in 95% of cells by the fourth passage after SV40 infection.

Cells which had been passaged 4 times after infection with SV40 were inoculated intracerebrally into newborn Balb/c mice (10^6 cells/mouse). No lesions of any kind developed in the animals, which were kept for a period of 6 months.

Six days after double infection with SV40 and MSV the cultures showed extensive transformation to densely staining spindle cells. Cells treated with SV40 alone showed considerable growth but cultures infected with MSV alone could not be distinguished from mock-infected cultures.

### Table 1. Enhancement of MSV production by simultaneous infection of amitotic cells with MSV+SV40

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<th>Infecting Virus(es)</th>
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<tr>
<td></td>
<td>After 2 days</td>
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<tr>
<td>SV40+MSV</td>
<td>1.2 × 10^3*</td>
</tr>
<tr>
<td>MSV</td>
<td>o</td>
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<tr>
<td>SV40</td>
<td>o</td>
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<td>Control</td>
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* Focus-forming units per ml., assayed on secondary P.H. embryo fibroblasts.

Fluids were collected from all cultures 2 and 4 days following infection. After filtration, the fluids were assayed for MSV using Prince Henry secondary embryonic fibroblasts. The results are shown in Table 1.

Kit et al. (1966) have shown that when BALB/c kidney cells are infected by SV40 at a m.o.i. of 230 to 620, a maximum of 20% of the cells are stimulated into DNA synthesis. In this study, 60% of the mouse embryo cells were synthesizing DNA 48 hr after infection and this was clearly not due to cell growth. This finding is in line with previous reports of the increased sensitivity to SV40 of aged hamster cells (Coggin, 1969) and of aged human cells (Todaro, Wolman & Green, 1963). After four subcultures the SV40 infected cells did not show signs of altered morphology nor were they tumorigenic in syngeneic mice. Similar findings have been reported for other SV40 infected mouse cells (Eagle et al. 1970).

Amitotic cells are not susceptible to transformation by MSV-ß, although very small amounts of virus are produced (Baker & Simons, 1971). Simultaneous infection with SV40 results in extensive transformation with a resulting increase in MSV production. The mechanism appears to be clear. MSV requires host cell DNA synthesis for the successful infection of a cell (Buck & Bather, 1969; Hirschman et al. 1969) and this is provided by the SV40 infection. This is a further example of the interaction of a DNA tumour virus and a RNA tumour virus. Rhim et al. (1971) have described the increased susceptibility of Rauscher virus infected rat embryo cells to SV40 transformation. The properties of the SV40+MSV-transformed cells are of interest and are being investigated.
The factors responsible for the entry of mouse embryo cells into the amitotic phase are not clear. Unpublished experiments showed that enrichment of the media did not affect entry into the amitotic phase and repeated subculture with trypsin or versene had no specially deleterious effect. The non-dividing phase is probably intrinsic to the cells, being dependent primarily on the degree of inter-cell contact during subculture (Todaro & Green, 1963). The amitotic cells retain their potential for division but do not have the capacity until stimulated by SV 40. Furthermore, the stimulus by SV 40 is not transitory and the cells enter DNA synthesis and division not much later than other cells stimulated by monolayer wounding or medium changes (Todaro et al. 1967; Gurney, 1969; Vasiliev et al. 1969; Yoshikura, 1970).

Cells in the amitotic phase are easily prepared and can be stored in liquid nitrogen without loss of viability. Apart from the intrinsic interest in the amitotic phase itself, the cells provide an excellent system for studying the induction of host cell DNA synthesis by SV 40.

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**REFERENCES**


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