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Simian Virus 40 Production After Viral Uncoating in the CV-1 Cell Nucleus

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A normal yield of infectious simian virus 40 was produced by a CV-1 cell culture after inoculation of a few cells with an average of one particle per nucleus by microinjection.

To expand our knowledge of the early events associated with the production of simian virus 40 (SV40), the fate of virions injected into host cell nuclei has been investigated. We reported that SV40 virions were uncoated after injection into the cell nucleus and subsequently directed the synthesis of T antigen (3). Now we present evidence that, as a result of intranuclear injection, infectious virus is produced in amounts considered normal for our subcloned African green monkey kidney CV-1 cell line and plaque-purified virus strain. These findings establish the biological importance of the uncoating of virus within the nucleus.

These results also support the earlier experiments of others (1, 4, 5) which suggested that SV40 virus, once adsorbed to the plasmalemma, is vesiculated and transported to the nuclear envelope by pinocytosis. The vesicles then fuse with the nuclear envelope, releasing intact virions which are then uncoated within the cell nucleus.

Our methods for cell culture and for the preparation and purification of SV40 have been described in detail (3). Cells from a subclone of the CV-1 cell line, grown to subconfluent density, were infected at low multiplicity. At 17 days after infection, virus stocks were collected after freezing and thawing. Before injection, virions were purified by precipitation with polyethylene glycol 6000, sequential incubation with DNase and RNase, centrifugation through a sucrose gradient, and then centrifugation in CsCl at a density, ρ, of 1.34. The criteria for the intactness of virus have also been discussed in detail (3). One virus particle on the average was required for the infection of an individual cell when the particle was injected directly into the cell nucleus (3).

Cells from the same subclone in which the virus was propagated were grown on acid-washed glass cover slips. When the cells were 50% confluent, the cover slip was transferred to a microsurgical chamber (2). A circle approximately the size of a microscope field as seen with a 10× objective at the magnification of 200 was scribed in the center of the cover slip. SV40 was injected into the nucleus of each of 17 cells in the center of this circle. Inocula contained 0.04 infectious unit, an average of one virus particle, in 2×10^{-10} ml. The tips of the micropipettes used for injection were of about 0.5-μm outer diameter. Intranuclear injections of virus and the intact condition of the cells so injected were monitored at a magnification of 2,000 with phase-contrast optics.

The cover slip was transferred to a petri dish containing Eagle medium enriched with 2% fetal bovine serum and incubated at 37°C in a humidified incubator.

Cells were monitored daily for evidence of cytopathic effect. At 24 h after infection, the monolayer reached confluency. By 7 days post-infection, some cytopathic effect was evident in the center of the cover slip. By 10 days a single circular area containing only about 20% of the cells present before injection could be seen in the center of the cover slip within the area of the scribed circle, the result of virus-induced cytopathic effect. Cell density was highest at the periphery of the cover slip and decreased progressively toward the center of the circle where the cells had been injected. This observation paralleled the extent to which cytopathic effect was evident in cells.

The medium containing the released virus and detached cells was collected at this time, frozen and thawed three times, and centrifuged at 6,000 × g for 20 min to remove cell debris. Cells remaining on the cover slip were washed with phosphate-buffered saline, fixed in absolute ethanol at −70°C as described (R. M. D'Alisa and E. L. Gershey, J. Histochem. Cytochem., in press) and stained for T antigen by an immunofluorescence technique (6). An end point titration was performed 48 h after infection of fresh monolayers of CV-1 cells with the conditioned medium by using the immunofluorescence tech-

nique described by Robb (6). Hamster anti-T serum was obtained from Flow Laboratories and used at a 1:50 dilution. Fluorescein isothiocyanate-conjugated goat immunoglobulin G anti-hamster immunoglobulin G was obtained from Antibodies Inc., Davis, Calif., and used at a 1:20 dilution. The cells on the cover slip were observed with phase-contrast optics and ploem epifluorescent optics with excitation at 495 nm and suppression at 525 nm (Leitz, Diavert) at a magnification of 40. Gradation in fluorescence intensity varied inversely with cell density, confirming that cells near the periphery were either uninfected or not yet producing T antigen. Uninfected cells growing on a cover slip over the same period of time appeared as a nonfluorescent, intact monolayer. From these observations it is clear that infection proceeded outward from the center of the scribed circle and was not yet complete 10 days after virus injection.

A titer of 3.3×10^7 T antigen-forming units per ml per a volume of 3 ml indicated that a total of 10^8 T antigen-forming units had been produced. It has been demonstrated by others (7) and confirmed with our virus culture system (3) that T antigen-forming units are equal to PFU at low input multiplicities, and it was obvious from the increase in number of T antigen-positive nuclei in the culture in which 17 cells received virus by injection that spread of newly produced infectious virus was occurring. If one assumes from the cell density of a confluent monolayer grown on glass that 3×10^5 cells were present on the cover slip and that 10^3 PFU are produced per cell, which we have calculated from our standard conditions for virus replication, then, theoretically, 3×10^8 PFU could be produced. At a time when not all of the cells were releasing virus, we were able to recover 30% of this amount.

These findings provide further evidence that SV40 can be uncoated in the cell nucleus and that infectious progeny is produced after intranuclear uncoating of the virus.

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