Interaction of Ultraviolet-irradiated Herpes Simplex Virus Type 1 with BSC-1 cells

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SUMMARY

Ultraviolet irradiation of herpes simplex virus (HSV) did not affect the transfer of uncoated virus DNA to the nuclei of infected cells but the synthesis of virus DNA was suppressed. The virus-specific DNA polymerase was synthesized in cells infected with the u.v.-irradiated HF strain of HSV. In cells infected with the u.v.-irradiated KOS strain, the virus DNA polymerase activity was hardly detectable. The two strains of HSV differ in the sensitivity of the virus DNA polymerase gene to u.v.-irradiation.

Infection of cultured mammalian cells with u.v.-irradiated herpes simplex virus (HSV) types 1 and 2 can result in cell transformation (Duff & Rapp, 1973, 1975) or in the induction of a latent oncornaavirus in the infected cells (Hampar et al. 1977). The mechanisms leading to both phenomena are not known, but the observations indicate that the u.v.-irradiated virus is able to alter control mechanisms in the infected cell. The u.v. effect on virus DNA prevents (Rapp & Duff, 1973) or delays (Ross et al. 1972) virus multiplication. Irradiation of HSV has been shown to result in the incorporation of a fragment of virus DNA into L cell DNA (Kraiselburd et al. 1975). The present study was undertaken to study the fate of u.v.-irradiated HSV type 1 DNA in infected cells and the effect of the inactivated virus on the synthesis of the virus-coded DNA polymerases.

The HF and KOS strains of HSV type 1 were propagated in BSC-1 cells under the conditions described by Shlomai et al. (1976). The cells were infected at a multiplicity of infection of 10 p.f.u./cell in Dulbecco's modified Eagle's medium (DMEM; Grand Island Biological Co.). To label the virus DNA, BSC-1 cells were infected in the presence of 25 μCi/ml of ³H-thymidine (sp. act. 16 Ci/mmol, Nuclear Research Centre, Negev, Israel).

Wild type stocks of HSV were irradiated with a Mineralight lamp (Model No. N-52) for 8 min at a distance of 28 cm (42 ergs/mm²). BSC-1 cultures were infected with the irradiated virus diluted to 10 p.f.u./cell (before irradiation). Irradiation of the virus preparation led to over 99.9% inactivation of virus infectivity (not shown).

Infection of BSC-1 cells with ³H-thymidine labelled irradiated or unirradiated virus resulted in a linear uptake of the incoming virus to the same extent (not shown). To determine the fate of the incoming virus DNA, BSC-1 cells were infected with labelled u.v.-irradiated HF virus, harvested at 4 h after infection and the nuclei separated from the cytoplasm by Dounce homogenization. The DNA was extracted from the nuclei and centrifuged in CsCl density gradients as described by Shlomai et al. (1976). Fig. 1(a) shows that all the radioactivity was situated at the density of the virus DNA (1.718 g/ml). The u.v.-irradiated virus DNA is therefore transported to the nucleus of the infected cell.

To determine whether DNA from u.v.-irradiated virus can act as a template for DNA synthesis, cells were infected with unlabelled u.v.-irradiated HF virus and incubated in the presence of ³H-thymidine. The DNA was isolated 4 h after infection and centrifuged in
Fig. 1. CsCl density gradient centrifugation of DNA from HSV infected cells. Cell monolayers were infected with u.v.-irradiated HSV labelled with $^3$H-thymidine (a). Four h after infection, the cells were washed, fractionated and the DNA was extracted from the nuclei. The DNA was centrifuged in CsCl gradients at 35000 rev/min for 48 h at 20 °C in the R50 Ti rotor of the Beckman ultracentrifuge using $^{14}$C-HSV DNA as a marker. BSC-1 cells were also infected with unlabelled u.v.-irradiated (b) and unirradiated (c) HSV. Two h after infection, $^3$H-thymidine (60 µCi/ml) was added to the cultures and 2 h later the cells were harvested, fractionated and the DNA was centrifuged in CsCl gradients. The density and the $^{14}$C-(○—○) and $^3$H-(●—●) trichloroacetic acid (TCA) precipitable radioactivity were determined.

CsCl density gradients (Fig. 1b). Virus DNA synthesis was suppressed by u.v. irradiation although some radioactivity was incorporated into HSV DNA. It is not known whether the incorporation of $^3$H-thymidine into the virus DNA is due to replication, repair or recombination events. The incorporation of $^3$H-thymidine into cellular DNA (density 1.700 g/ml) occurred in cells infected with both u.v.-irradiated and active virus (Fig. 1b and c, respectively).

The DNA polymerases present in cells infected with irradiated and unirradiated virus were investigated. For comparison, the KOS strain of HSV type I was included since this virus strain was reported by Duff & Rapp (1973) to transform cells efficiently when partially inactivated by u.v. irradiation. The cells were harvested at 12 h after infection, homogenized and the nuclei were precipitated with ammonium sulphate and centrifuged in sucrose gradients as described by Hirai & Watanabe (1976). The cellular and virus DNA polymerases were assayed in vitro in the presence of low (10 mM) and high (250 mM) concentrations of KCl, respectively. The reaction mixtures contained 2 mmol MgCl$_2$, 0.5 mmol each of dGTP, dATP and dCTP, 0.01 mmol TTP and 3.75 µCi of $^3$H-TTP (sp. act. 50 Ci/mm; The Radiochemical Centre, Amersham, England), 500 µg/ml of activated calf thymus DNA, 100 mM-tris-HCl, pH 8.1 and 500 µg/ml of bovine serum albumin. The assays for the cellular DNA polymerases (at low salt concentrations) were done with 10 mM-MgCl$_2$.
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Fig. 2. Sucrose gradient analysis of the DNA polymerases in infected and uninfected BSC-1 cells. Nuclear extracts from cells infected with u.v.-irradiated (a) and unirradiated (b) HF virus; u.v.-irradiated KOS virus (c) and uninfected non-confluent BSC-1 cells 48 h after seeding (d) were centrifuged in 10 to 20 % (w/v) sucrose gradients at 35 000 rev/min for 15 h at 4 °C in the SW 50.1 rotor of the Beckman ultracentrifuge. The tubes were fractionated and portions from each sample were assayed for DNA polymerase activity in vitro in the presence of 250 mM-(O--O) and 10 mM-(O--O) KCl. Note the difference in scales in the left and right panels.

Infection of BSC-1 cells with u.v.-irradiated virus (HF strain) resulted in the synthesis of the virus DNA polymerase (active at high salt concentration) (Fig. 2a). The level of DNA polymerase isolated in sucrose gradients was about 25 % of that in BSC-1 cells infected with active virus (Fig. 2b). In these cells the level of the cellular DNA polymerase activities (α and β) was much lower than that of the virus DNA polymerase.

A marked difference was noted in BSC-1 cells infected with u.v.-irradiated KOS virus. In these cells the virus DNA polymerase activity was hardly detectable in sucrose gradients, whereas the cellular DNA polymerases α and β were easily demonstrable (Fig. 2c). A virus coded DNA polymerase activity similar to that shown in Fig. 2(b) was found in BSC-1 cells infected with unirradiated KOS virus. The profiles of both the virus and cellular DNA polymerases in cells infected with u.v.-irradiated KOS virus (Fig. 2c) resembled that obtained with actively growing cells at low salt concentration (Fig. 2d). The cellular
DNA polymerase $\alpha$ was not active in growing cells at high salt concentration (Fig. 2d). The slight virus DNA polymerase activity at high salt concentration, in the region of the cellular DNA polymerase $\alpha$ in Fig. 2(c), may be expressed by residual virus in the preparation after u.v. irradiation.

The relative sensitivity to u.v. irradiation of the DNA polymerase genes of the HF and KOS virus strains was assessed using preparations of nuclei from infected BSC-1 cells that were isolated and extracted as described by Hiraï & Watanabe (1976). The DNA polymerase activity in each preparation was determined in the presence of 250 mM-KCl as described above. The $^3$H-TMP radioactivity incorporated was calculated per $2 \times 10^7$ cells. The radioactivity incorporated into a nuclear homogenate from HF infected cells was $1.69 \times 10^6$ ct/min whereas incorporation by nuclei from cells infected with u.v.-irradiated HF virus was $0.98 \times 10^6$ ct/min. Thus the virus DNA polymerase activity was reduced by 42%. The total incorporation by a homogenate prepared from cells infected with the KOS strain was $2.18 \times 10^6$ ct/min. However, only $0.19 \times 10^6$ ct/min were incorporated by a nuclear homogenate from cells infected with u.v.-irradiated KOS virus. This confirms the results of the experiment presented in Fig. 2 and shows that only 9% of the virus DNA polymerase activity is expressed in BSC-1 cells infected with u.v.-irradiated KOS virus.

The results of the present study provide information on some of the events that occur in cells early (4 h) after infection with u.v.-irradiated herpes simplex virus. The incoming irradiated parental virus DNA reaches the nucleus of the cell and serves as a template for either very limited DNA synthesis or DNA repair. The extent to which the virus DNA templates are utilized is not yet known, although it was demonstrated that the u.v.-irradiated HF strain codes for its own DNA polymerase. This finding may explain the limited incorporation of $^3$H-thymidine into the parental virus DNA.

This study has shown that the DNA polymerase gene of the KOS strain of HSV is much more sensitive to u.v. irradiation than that of the HF virus strain. Further studies are required to elucidate the nature of the difference between the two virus strains.

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**References**


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