Ultraviolet Irradiation Inhibits Encapsidation of Simian Virus 40 Chromatin

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During normal maturation the majority of pulse-labeled simian virus 40 DNA progresses from chromatin to previrions and virions within 5 h. UV light inhibits this progression. In heavily irradiated cultures (108 J m^{-2}) most of the simian virus 40 DNA synthesized immediately before irradiation remains as chromatin for at least 5 h. This inhibition of maturation seems to be a result of the inhibition of protein synthesis. The data suggest that the pool of proteins required for maturation is sufficient to convert one-third of the simian virus 40 DNA molecules labeled in a 10-min pulse (at 33 h postinfection) from chromatin to previrions and virions and is exhausted within 1 h.

Effects of UV irradiation on the replication and repair of simian virus 40 (SV40) DNA have been examined (21, 25, 26), but without regard for the viral maturation process. Pulse-chase experiments suggest that in unirradiated cells labeled SV40 DNA progresses along a maturation pathway from chromatin (NPC-I) to previrions (NPC-II) to mature virions (1, 7, 9). NPC-I contains SV40 DNA, cellular histones, and cellular and viral proteins involved in DNA and RNA synthesis (2, 6, 7, 16, 17, 20, 23). NPC-II is a heterogeneous set of complexes containing SV40 DNA, histones with a greater degree of acetylation, and viral structural proteins (2, 3, 7, 10, 13). Mature virions contain histones (except H1) and viral structural proteins (2, 14, 18). The majority of pulse-labeled DNA is converted from NPC-I to NPC-II or virions within 4 to 6 h after its synthesis (1, 7, 9, 24).

Maturation places constraints upon SV40 DNA that could affect both replication and repair. The accessibility of the SV40 DNA in different nucleoprotein complexes to cellular enzymes may differ greatly. DNA in virions, for example, is inaccessible to micrococcal nuclease digestion (7) and presumably also inaccessible to cellular enzymes. To provide the information on maturation essential for analyzing SV40 DNA replication and repair in UV-irradiated cells, we have examined the progression of SV40 DNA along the maturation pathway by quantitating the fraction of pulse-labeled DNA in previrions and virions in both unirradiated and UV-irradiated cells. We have found that UV inhibits progression from NPC-I to NPC-II and virions (NPC-II + V).

MATERIALS AND METHODS

Cells and virus. BSC-1 cells were obtained from M. Singer (National Institutes of Health) and used between passages 5 and 45. Cells were grown in Dulbecco modified Eagle medium (DME) with 10% calf serum and $50 \mu\text{g}$ of gentamicin (Sigma Chemical Co.) per ml. At 12 to 24 h after the cell monolayer had become confluent, cells were infected at an input multiplicity of 2 PFU/cell. The cells were then maintained in DME with 2% calf serum.

SV40 strain 776, obtained from S. Weissmann (Yale University), was propagated at low input multiplicity (0.001 PFU/cell) in CV-1 cells obtained from M. L. DePamphilis (Harvard Medical School).

Labeling and extraction of nucleoprotein complexes. A protocol was devised to follow the effect of UV on the encapsidation of DNA synthesized several minutes before irradiation. At 33 h postinfection the cell cultures were labeled for 10 min with $80 \mu\text{Ci}$ of [^3H]deoxythymidine (Amersham Corp.; 40 to 60 Ci/mmol) per ml in DME containing 2% calf serum and 25 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (pH 7.0). Cells were then rinsed twice with TBS (150 mM NaCl, 5 mM KCl, 0.7 mM Na_2HPO_4 , 0.9 mM CaCl₂, 1 mM MgCl₂, 20 mM Tris [pH 7.4]) and covered with DME containing 2% serum, $100 \mu\text{g}$ of deoxythymidine, and $10 \mu\text{g}$ of deoxycytidine per ml (chase medium). After a 10-min chase, cells were rinsed with TBS, drained, and exposed to UV; the time of exposure is defined as $T = 0$ h. Cells were irradiated with a 15-W germicidal lamp at a fluence rate of 5.4 W m^{-2} , measured with an International Light IL770 germicidal radiometer. Under our conditions the average number of pyrimidine dimers per SV40 molecule was linear with fluence from 1.6 dimers at 10.8 J m^{-2} to 16 dimers at 108 J m^{-2} . Pyrimidine dimers were determined as sites susceptible to the UV endonuclease encoded by bacteriophage T4 (8, 21, 26). The lengths of the pulse and chase were chosen so

that at the time of irradiation most of the labeled SV40 DNA was covalently closed (data not shown). The nucleoprotein complexes were extracted by the method of Fernandez-Munos et al. (7) either immediately after irradiation or after various times of further incubation in the chase medium. Briefly, cells were scraped into cold hypotonic buffer (25 mM Tris [pH 7.9], 1 mM $MgCl_2$, 0.4 mM $CaCl_2$), and broken by 30 strokes in a tight-fitting Dounce homogenizer. The lysate was centrifuged at 2,000 rpm for 5 min in a centrifuge (International Equipment Co.; model PR-J). To separate NPC-I from NPC-II + V the supernatant was layered on 10 to 40% neutral sucrose gradients containing 2 mM Tris (pH 7.4). Gradients were centrifuged either for 70 min at 37,000 rpm in an SW41Ti rotor (Beckman Instruments, Inc.) or for 55 min at 45,000 rpm in an SW60Ti rotor (Beckman). Fractions were collected from the bottom of the centrifuge tubes, and the radioactivity in samples was determined after the addition of carrier DNA and precipitation with 1 M HCl. The fraction of [3H]DNA in the position of NPC-I or NPC-II and virions (NPC-II + V) was determined.

Inhibition of protein, RNA, and DNA syntheses. To determine the extent of inhibition of protein synthesis, cultures were labeled with either 1 μCi of ^{14}C -amino acid mixture (Amersham) per ml or 10 μCi of [3H]leucine (Amersham; 187 Ci/mmol) per ml starting at the time of irradiation or mock irradiation ($T = 0$ h). Incorporation into acid-precipitable material was determined on either the whole harvested cells, the NPC-containing supernatant prepared as described above, or the material in the neutral sucrose gradients. The results of all three types of measurements were the same.

Similar experiments were performed with 10 μCi of [3H]uridine (Amersham; 46 Ci/mmol) per ml to examine RNA synthesis.

To compare maturation after inhibition of either DNA or protein synthesis, hydroxyurea (2 or 10 mM) or cycloheximide (5×10^{-8} to 5×10^{-4} M) was added to cells at the time of irradiation or mock irradiation. Cells were harvested and processed as described above.

RESULTS

Kinetics of maturation of SV40 DNA in irradiated and unirradiated cells. SV40 DNA was pulse-labeled just before the infected cells were exposed to UV. NPCs were extracted either immediately after UV irradiation ($T = 0$ h) or after 6 h of incubation in chase medium ($T = 6$ h), and NPC-I was separated from NPC-II and virions (NPC-II + V) in neutral sucrose gradients. The kinetics of maturation of SV40 DNA was determined by measuring the amounts of pulse-labeled DNA in NPC-I and NPC-II + V in control and irradiated cells. Figure 1 shows typical patterns for unirradiated and irradiated cells. At $T = 0$ h the majority of [3H]DNA was in NPC-I whether or not the cells were irradiated (Fig. 1A). At $T = 6$ h 74% of the [3H]DNA in

unirradiated cells, but only 28% of the DNA in cells irradiated with $108 J m^{-2}$, had progressed to NPC-II + V (Fig. 1B).

The kinetics of maturation of SV40 DNA in unirradiated, lightly irradiated, and heavily irradiated cultures were determined over a 5-h period (Fig. 2). UV inhibition of maturation was not apparent in the first 20 to 40 min after irradiation; the difference between heavily irradiated and unirradiated cultures became apparent only at about 1 h. There was little or no further packaging of the heavily irradiated DNA beyond that which appeared by 1 to 2 h, whereas unirradiated DNA continued to be packaged beyond 5 h. In cells irradiated with $11 J m^{-2}$, conversion of [3H]DNA from NPC-I to NPC-II + V occurred to the same extent as in unirradiated cells.

To determine the dose response of maturation of SV40 DNA we compared the fraction of label in NPC-II + V at 5 h after various doses of irradiation. This time was chosen because progression of irradiated SV40 DNA seemed to reach a plateau by that time (Fig. 2), the level of which was dependent upon the UV exposure. The fraction of pulse-labeled SV40 DNA which progressed to NPC II + V by 5 h postirradiation declined exponentially with fluence (Fig. 3). The apparent D_{37} , based upon all of our UV experiments, was $117 J m^{-2}$; for the experiment shown in Fig. 3 the D_{37} was $95 J m^{-2}$.

Cause of the inhibition of maturation. The inhibition of packaging may be due to alterations in the DNA or to inhibition of the synthesis or modification of required proteins. To determine the cause we analyzed both the state of the DNA and the effects of UV on protein synthesis.

More than 90% of the [3H]DNA from both irradiated and unirradiated cells was covalently closed; thus, degradation of the pulse label cannot be the cause of the inhibition. Since a fraction of the prelabeled DNA is used as a template for further DNA synthesis (12, 24) and pyrimidine dimers appear to block replication forks (4, 5), we asked whether the [3H]DNA was accumulating as parental strands of replicative intermediates whose completion was inhibited. By $T = 2$ h, the fraction of label from the NPC-I pool which behaved as replicative intermediates on benzoylated-naphthoylated DEAE (BND)-cellulose columns (15) was 5 to 6% in unirradiated cultures and 13 to 19% in the cultures irradiated with $108 J m^{-2}$; irradiation of purified form I DNA with this fluence increased the fraction retarded on BND-cellulose from 5 to 9%. Thus, although there is some increase most of the still unpackaged [3H]DNA does not behave as repli-

FIG. 1. Distribution of ^3H -labeled SV40 DNA in nucleoprotein complexes extracted from irradiated and unirradiated cells. SV40-infected BSC-1 cells were labeled for 10 min with [^3H]thymidine and chased for 10 min. At this time ($T = 0$ h), some plates were exposed to 108 J m^{-2} ; others were left unirradiated. NPCs were extracted either immediately (A) or after a further 6 h of incubation in the chase medium (B), and the NPC-containing supernatants were sedimented on 10 to 40% neutral sucrose gradients. Sedimentation is from right to left. The slower-sedimenting material is NPC-I, the faster-sedimenting material is NPC-II + V (7, 24). Symbols: $\square$, unirradiated; $\circ$, 108 J m^{-2} . (Double tick marks for fraction number in panel B reflect scaling the gradients to the same distance on the X axis.)

cative intermediates; the inhibition of packaging seen at this fluence is not, therefore, merely due to accumulation of the DNA in replicative intermediates.

To test whether inhibition of replication itself caused the maturation block, the maturation of [^3H]DNA in the presence and absence of an inhibitor of DNA synthesis was determined. Hydroxyurea at 2 or 10 mM did not affect the fraction of prelabeled DNA packaged during a 5-h incubation in either irradiated or unirradiated cells (data not shown).

Since the DNA was in the form required for packaging (form I), and since inhibition of rep-

lication per se did not inhibit packaging, we examined the effects of UV on protein synthesis. UV lesions, presumably dimers, can act as blocks to transcription (11, 22), and C-C or C-U dimers introduced directly into RNA might act as blocks to translation (19). Irradiated and unirradiated cultures were covered with medium containing either [^3H]uridine, [^3H]leucine, or ^{14}C -amino acid mixtures to determine incorporation into acid-insoluble macromolecules. By 20 min after exposure to 108 J m^{-2} the level of both RNA synthesis and protein synthesis was less than 10% of that in control cells, and these processes remained inhibited for at least 5 h. UV

FIG. 2. Kinetics of maturation of ^3H -DNA in unirradiated, lightly irradiated, and heavily irradiated cultures. SV40-infected cells, labeled as in Fig. 1, were irradiated with either 11 J m^{-2} or 108 J m^{-2} or left unirradiated. NPCs were extracted either immediately or at various times after further incubation in chase media. The percentages of ^3H -DNA in the NPC-II + V pools were determined. The results of several experiments are presented. Symbols: $\circ$, $\bullet$, and $\triangle$, unirradiated; Δ and Δ , 11 J m^{-2} ; $\square$ and $\square$, 108 J m^{-2} .

FIG. 4. Correlation between inhibition of maturation and protein synthesis. The fractions of ^3H -DNA in NPC-II + V at $T = 5 \text{ h}$ are from the experiment shown in Fig. 3; the averages of the results from duplicate dishes are shown. In the same cultures, the fraction of protein synthesis was determined from the amount of ^{14}C -amino acid mixture incorporated into acid-insoluble material during these same 5 h, normalized to the incorporation in control cells (1.00).

FIG. 3. Fraction of ^3H -DNA in NPC-II + V at 5 h post-irradiation as a function of UV fluence. The fraction of ^3H -DNA in the NPC-II + V pool at $T = 5 \text{ h}$ was determined from gradients similar to those in Fig. 1 and plotted on a logarithmic scale. Solid and open symbols represent results from duplicate dishes.

inhibited protein synthesis in SV40-infected cells in a dose-dependent manner. The fraction of ^3H -labeled SV40 DNA that progressed to NPC-II + V within 5 h after irradiation was proportional to the extent of protein synthesis during that time (Fig. 4).

To determine whether inhibition of protein synthesis could account totally for the inhibition of maturation, the effect of cycloheximide on

maturation was determined. At $T = 0 \text{ h}$, unirradiated cultures were incubated with various doses of cycloheximide. Again, the fraction of label in NPC-II + V was proportional to the fraction of protein synthesis (Fig. 5). The extent of progression in cycloheximide-treated and UV-irradiated cells showed the same dependence upon residual protein synthesis. In an experiment comparing irradiated and cycloheximide-treated cells in which protein synthesis was inhibited to a similar extent, the kinetics of progression to NPC-II + V were parallel.

DISCUSSION

SV40 has been used as a model system in which to study the effects of UV irradiation on DNA replication and repair (21, 25, 26). This report demonstrates another effect of UV on the intracellular life cycle of SV40. UV inhibits the maturation of SV40 DNA from NPC-I, the nucleoprotein complex resembling cellular chromatin, to NPC-II. In uninhibited cells this progression occurs to a large extent within 4 to 6 h (Fig. 1 and 2) (1, 7, 9, 24) and correlates inversely with the ability of SV40 DNA to serve as a template for replication (24). UV inhibits maturation in a dose-dependent manner (Fig. 3). In heavily irradiated cells only one third of the labeled DNA progressed to NPC-II + V in the same 4 to 6 h, with most of the maturation

FIG. 5. Comparison of the effects of cycloheximide and UV fluence on maturation. After a 10-min pulse and a 10-min chase ($T = 0$ h) unirradiated cultures were incubated in chase medium containing cycloheximide. In parallel some cultures were irradiated and incubated in chase medium without cycloheximide. At $T = 5$ h the fraction of [3 H]DNA in NPC-II + V and the fraction of protein synthesis were determined as described in the legend to Fig. 4. The levels of cycloheximide shown (from least to most inhibitory) are 1×10^{-7} , 5×10^{-7} , 1×10^{-6} , 5×10^{-6} , and 5×10^{-4} M. UV fluences are 11, 32, 38, 54, 81, and 108 J m^{-2} . Symbols: $\square$, UV; $\bullet$, cycloheximide ($\square$ and $\bullet$ represent averages of two to five experiments); $\blacksquare$, average of 10 control experiments.

occurring early (Fig. 2 through 5). The inhibition of SV40 maturation does not correlate with inhibition of replication of the SV40 DNA (although replication is inhibited severely; data not shown); rather, it correlates with an inhibition of protein synthesis (Fig. 4 and 5).

The fraction of [3 H]DNA that can progress to NPC-II + V is proportional to the amount of protein synthesis remaining after treatment with either UV (Fig. 4 and 5) or cycloheximide (Fig. 5), two treatments which act by very different mechanisms. About one-third of the pulse-labeled SV40 DNA can be converted to NPC-II + V even at doses of UV or cycloheximide that severely inhibit protein synthesis (to 1 to 3% of control). This suggests that a pool of proteins required for progression of NPC-I to NPC-II exists, but is only sufficient for one-third of the newly replicated molecules at this time after infection (33 h). Since the inhibition of packaging is seen by 1 h after UV irradiation, the pool must be essentially exhausted within that time. Variations in the size of this pool of required proteins at different times after infection may well influence the fate of newly replicated molecules (24).

The maturation of SV40 DNA within cells must be considered in the design and analysis of experiments examining the effects of irradiation and other agents that disrupt the intracellular cycle of SV40. Since a large fraction of the intracellular SV40 DNA progresses to NPC-II + V within 5 h of its synthesis, the fate of SV40 DNA labeled for many hours before irradiation might not be representative of molecules undergoing replication or transcription at the time of irradiation or analysis. Also, maturation of a significant fraction of the DNA within several hours of synthesis may render this fraction inaccessible to cellular enzymes. These effects could explain the reports that there is little (26) or no (21) removal of pyrimidine dimers from prelabeled, UV-irradiated SV40 DNA.

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LITERATURE CITED

1. Baumgartner, I., C. Kuhn, and E. Fanning. 1979. Identification and characterization of fast sedimenting SV40 nucleoprotein complexes. *Virology* **96**:54-63.
2. Coca-Prados, M., and M.-T. Hsu. 1979. Intracellular forms of simian virus 40 nucleoprotein complexes. II. Biochemical and electron microscopic analysis of simian virus 40 virion assembly. *J. Virol.* **31**:199-208.
3. Coca-Prados, M., G. Vidali, and M.-T. Hsu. 1980. Intracellular forms of simian virus 40 nucleoprotein complexes. III. Study of histone modifications. *J. Virol.* **36**:353-360.
4. Edenberg, H. J. 1976. Inhibition of DNA replication by ultraviolet light. *Biophys. J.* **16**:849-860.
5. Edenberg, H. J. 1978. DNA replication in ultraviolet-irradiated mammalian cells, p. 489-492. In P. C. Hanawalt, E. C. Friedberg, and C. F. Fox (ed.), *DNA repair mechanisms*. Academic Press, Inc., New York.
6. Edenberg, H. J., S. Anderson, and M. L. DePamphilis. 1978. Involvement of DNA polymerase α in simian virus 40 DNA replication. *J. Biol. Chem.* **253**:3273-3280.
7. Fernandez-Munoz, R., M. Coca-Prados, and M.-T. Hsu. 1979. Intracellular forms of simian virus 40 nucleoprotein complexes. I. Methods of isolation and characterization in CV-1 cells. *J. Virol.* **29**:612-623.
8. Ganesan, A. K. 1973. A method for detecting pyrimidine dimers in the DNA of bacteria irradiated with low doses of ultraviolet light. *Proc. Natl. Acad. Sci. U.S.A.* **70**:2753-2758.
9. Garber, E. A., M. M. Seidman, and A. J. Levine. 1978. The detection and characterization of multiple forms of SV40 nucleoprotein complexes. *Virology* **90**:305-316.
10. Garber, E. A., M. M. Seidman, and A. J. Levine. 1980. Intracellular SV40 nucleoprotein complexes: synthesis to encapsidation. *Virology* **107**:389-401.
11. Giorno, R., and W. Sauerbier. 1976. A radiological analysis of the transcription units for heterogeneous nuclear RNA in cultured murine cells. *Cell* **9**:775-783.
12. Green, M. H., and T. L. Brooks. 1978. Recently replicated simian virus 40 DNA is a preferential template for transcription and replication. *J. Virol.* **26**:325-334.
13. La Bella, F., and C. Vescio. 1980. Late modifications of

- simian virus 40 chromatin occur during the lytic cycle in an immature form of virion. *J. Virol.* **33**:1138-1150.
14. **Lake, R. S., S. Barban, and N. P. Salzman.** 1973. Resolution and identification of the core deoxynucleoproteins of the simian virus 40. *Biochem. Biophys. Res. Commun.* **54**:640-647.
 15. **Levine, A. J., H. S. Kang, and F. E. Billheimer.** 1970. DNA replication in SV40 infected cells. I. Analysis of replicating SV40 DNA. *J. Mol. Biol.* **50**:549-568.
 16. **Mann, K., and T. Hunter.** 1979. Association of simian virus 40 T antigen with simian virus 40 nucleoprotein complexes. *J. Virol.* **29**:232-241.
 17. **Otto, B., and E. Fanning.** 1978. DNA polymerase α is associated with replicating SV40 nucleoprotein complexes. *Nucleic Acids Res.* **5**:1715-1728.
 18. **Pett, D. M., M. K. Estes, and J. S. Pagano.** 1975. Structural proteins of simian virus 40. I. Histone characteristics of low molecular weight polypeptides. *J. Virol.* **15**:379-385.
 19. **Ponta, H., M.-L. Pfennig-Yeh, E. F. Wagner, M. Schweiger, and P. Herrlich.** 1979. Radiation sensitivity of messenger RNA. *Mol. Gen. Genet.* **175**:13-17.
 20. **Reiser, J., J. Renart, L. V. Crawford, and G. R. Stark.** 1980. Specific association of simian virus 40 tumor antigen with simian virus 40 chromatin. *J. Virol.* **33**:78-87.
 21. **Sarasin, A. R., and P. C. Hanawalt.** 1980. Replication of ultraviolet-irradiated simian virus 40 in monkey kidney cells. *J. Mol. Biol.* **138**:299-319.
 22. **Sauerbier, W., R. L. Millette, and P. B. Hackett, Jr.** 1970. The effects of ultraviolet irradiation on the transcription of T4 DNA. *Biochim. Biophys. Acta* **209**:368-386.
 23. **Segawa, M., S. Sumio, and N. Yamaguchi.** 1980. Association of simian virus 40 T antigen with replicating nucleoprotein complexes of simian virus 40. *J. Virol.* **35**:320-330.
 24. **Wang, H.-T., and A. Roman.** 1981. Cessation of reentry of simian virus 40 DNA into replication and its simultaneous appearance in nucleoprotein complexes of the maturation pathway. *J. Virol.* **39**:255-262.
 25. **Williams, J. I., and J. E. Cleaver.** 1978. Perturbations in simian virus 40 DNA synthesis by ultraviolet light. *Mutat. Res.* **52**:301-311.
 26. **Williams, J. I., and J. E. Cleaver.** 1979. Removal of T4 endonuclease-sensitive sites from SV40 DNA after exposure to ultraviolet light. *Biochim. Biophys. Acta* **562**:429-437.