# ENHANCEMENT OF POSTREPLICATION REPAIR IN ULTRAVIOLET-LIGHT-IRRADIATED CHINESE HAMSTER CELLS BY IRRADIATION IN $G_2$ OR S-PHASE

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ABSTRACT Postreplication repair in synchronous Chinese hamster cells was determined after split doses of ultraviolet (UV) radiation. Repair was enhanced by irradiation of cells in $G_2$ or S-phase with a small dose of UV radiation at least 1.5 h before a three-fold larger dose of UV. There was significantly greater enhancement when the first dose was given in $G_2$ than when it was given in the S-phase 0.5–1.5 h before the test dose. These data indicate that enhancement of postreplication repair does not require active DNA replication and qualitatively is independent of when in the cell cycle the cells are irradiated.

## INTRODUCTION

Chinese hamster and many other mammalian cells are able to cope with damage in their DNA produced by ultraviolet (UV) radiation by DNA repair processes (1). Although Chinese hamster cells are defective in excision repair of pyrimidine dimers (2,3), it is thought that they can survive a dose of UV radiation that produces approximately $10^6$ dimers per cell by relying upon postreplication repair (4). Postreplication repair, in its broadest definition, can be considered as the processes by which a cell can replicate DNA, using a damaged template. The molecular weight of the newly synthesized DNA observed immediately after UV irradiation is smaller than that from unirradiated cells and is eventually chased into higher molecular weight DNA resembling parental DNA in size (4).

In Chinese hamster cells the presumptive gaps (4) left in daughter DNA after replication upon a template containing dimers are eventually filled in and joined together. However, other data (5,6) suggest that the replication machinery bypasses the lesion in the parental DNA without leaving a gap in the daughter strand.

The process of chasing small molecular-weight DNA into larger DNA can be enhanced in Chinese hamsters (7) and xeroderma pigmentosum cells (8,9) by prior

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irradiation of cells with a small dose of UV radiation or treatment with *N*-acetoxy-acetylaminofluorene. This enhancement of repair was not observed when cells were incubated with cycloheximide (7) between the split doses of UV.

Recovery from UV irradiation is primarily associated with the S-phase of cell cycle and it has been suggested that this recovery is due to postreplication repair (4,10). Recently, Meyn et al. (11) showed that Chinese hamster ovary cells irradiated with UV early in the first S-phase recovered normal DNA replication kinetics in the second S-phase, even though dimers were still present in the DNA. Buhl et al. (12) observed that high molecular weight DNA was synthesized 12 h after UV irradiation in human cells. Most other studies (4) on postreplication repair of UV-damaged DNA have been with asynchronous populations and have not analyzed the postreplication repair of DNA in S-phase from cells irradiated in other segments of the cell cycle. In this paper, we investigated the postreplication repair capacity of synchronous cells given split doses of UV radiation and asked whether prior DNA replication was required for enhancement of postreplication repair.

We observed that the repair capacity of cells irradiated with UV in the S-phase is enhanced by prior UV irradiation in either $G_2$ or in S-phase. Thus it appears that enhancement of postreplication repair in Chinese hamster cells does not require active DNA replication.

## METHODS

### *Cell Culture and Synchrony*

Chinese hamster V79 cells were grown in Dulbecco's modified Earle's salts medium supplemented with 400 $\mu$g/ml glutamine and 140 U/ml of penicillin and 140 $\mu$g/ml of streptomycin. Approximately $1.8 \times 10^6$ cells were plated in 10 ml of medium in 75 $cm^2$ Corning tissue culture bottles (Corning Glass Works, Science Products Div., Corning, N.Y.). After 3 h, 0.03 $\mu$Ci/ml of [$^{14}$C]thymidine (dT) (50 Ci/mol [New England Nuclear, Boston, Mass.]) was added to label parental DNA and cells were incubated for 40 h at 37°C in a humid atmosphere containing 10% $Co_2$.

Cells in exponential growth were collected by shaking off mitotic and early $G_1$ cells (13), plated into 60-mm Falcon dishes (Falcon Plastics, Division of BioQuest, Oxnard, Calif.), and synchronized by incubation for 8 h in hydroxyurea (14). Hydroxyurea was removed by washing cells twice with prewarmed medium as as to allow cells to go through S. To collect cells at the beginning of the second S-phase, cells were incubated 8 h later in 1 mM hydroxyurea for 4 h and allowed to progress through $G_2$, mitosis, and $G_1$ (see Fig. 1).

Progression through the cell cycle was monitored by pulse-labeling cells with 2.5 $\mu$Ci/ml [$^3$H]dT (60 Ci/mmol) (New England Nuclear) for 0.25 h at the various times shown in Fig. 1. Visual observation of cells showed mitotic cells at 7 and 8 h after washing off hydroxyurea. The cells used in our experiments appeared to progress through S-phase approximately 1 h later than those observed in earlier experiments (15), although the degree of synchrony was comparable.

### *UV Irradiation and Alkaline Sucrose Sedimentation*

The medium was removed from the plates and approximately 90,000 cells were irradiated with 254-nm radiation at a dose rate of 0.28 $Wm^{-2}$ at various times after shake-off or removal of

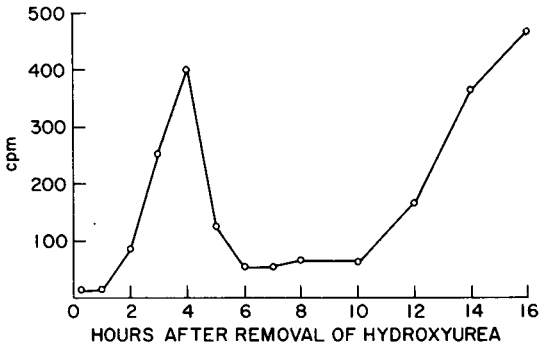


FIGURE 1 DNA synthesis after cell synchronization by mitotic shake-off and hydroxyurea. Cell synchrony was monitored by incubating cells with [$^3$H]dT for 15 min and determining the amount of [$^3$H]dT incorporated into newly synthesized DNA. Cells were incubated with 1 mM hydroxyurea between 8 and 12 h to block cells from entering the second S-phase.

hydroxyurea. At the times indicated in the figures, cells were pulse-labeled with 10 $\mu$Ci/ml [$^3$H]dT (60 Ci/mmol) for 30 min and then chased in unlabeled dT (4 $\mu$g/ml) for 1 h. After the chase, the cells were washed with an EDTA-containing solution (16) and exposed to 2,000 R X-rays (17); 45,000 cells in 50 $\mu$l were lysed in 0.2 ml of a 1 M NaOH, 0.01 M EDTA solution layer on top of a 5.2 ml of 5–20% alkaline sucrose gradient, as previously described (7). Samples were centrifuged in a SW 50.1 rotor of a Beckman model L2-65 centrifuge (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.), at 30,000 rpm for 130 min and collected onto paper strips. The radioactivity of each fraction was determined as previously described (7).

## RESULTS

### *Postreplication Repair of Newly Synthesized DNA in Cells Given Split Doses of UV*

Cells were synchronized by incubating mitotic and $G_1$ cells with 1 mM hydroxyurea. After removal of hydroxyurea, as described under Methods, they were allowed to progress through S-phase of the cell cycle, which took approximately 5 h (Fig. 1). At 6 h some cells were irradiated with 2.5 $Jm^{-2}$ UV. 2 h later, hydroxyurea was added and the cells were incubated for 4 h to collect cells at the beginning of the second S-phase ($S_2$-phase) (12 h, shown on Fig. 1). Immediately after removal of hydroxyurea, some cells were irradiated with 2.5 $Jm^{-2}$. After 1.5 h, cells given UV were given 7.5 $Jm^{-2}$, while cells not given UV were irradiated only with 10 $JM^{-2}$ UV. Irradiation of cells with split or single doses of UV inhibited DNA synthesis 82 and 75%, respectively. As shown in Fig. 2, the newly synthesized DNA from cells given 10 $Jm^{-2}$ was smaller in size than the DNA from cells not irradiated with UV. However, the newly synthesized DNA from cells given $UV_1$ (2.5 $Jm^{-2}$) in $G_2$ (6 h on Fig. 1) or in S (12 h on Fig. 1) and $UV_2$ (7.5 $Jm^{-2}$) in S (13.5 h on Fig. 1) sedimented further down the gradient than cells given only 10 $Jm^{-2}$ UV radiation 1.5 h into $S_2$ (Fig. 2). These data showing enhancement of postreplication repair in synchronized cell populations are consistent with previous data (7) showing enhancement in asynchronous cell populations of Chinese

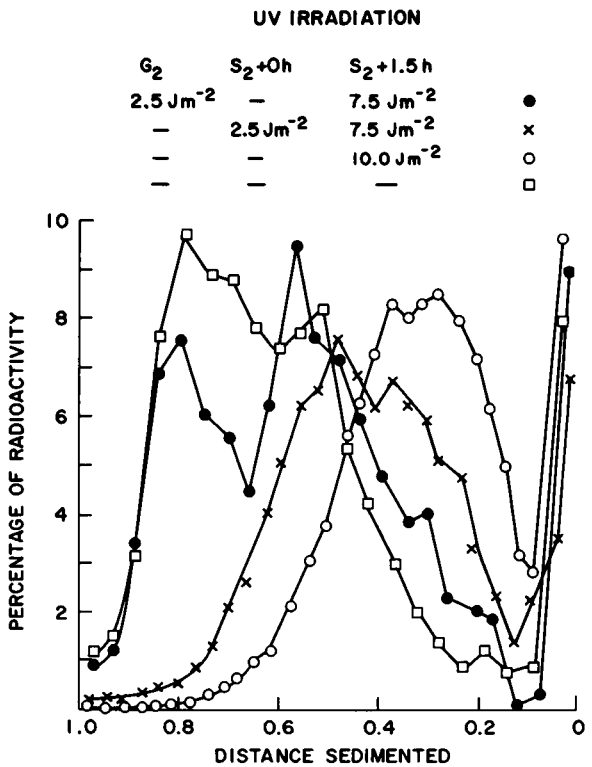


FIGURE 2 Sedimentation profiles of pulse-chased DNA from cells given split doses of UV at different times in the cell cycle. Cells were irradiated with 2.5. $Jm^{-2}$ UV in $G_2$ phase (•) or immediately after removal of the second hydroxyurea block ($S_2$ + 0 h) (×) and irradiated again 1.5 h into the second S-phase ($S_2$ + 1.5 h). Cells were irradiated (○) or not irradiated (□) with 10.0 $Jm^{-2}$ UV irradiation at $S_2$ + 1.5 h. 0.25 h after the last irradiation, cells were pulse-labeled with [$^3$H]dT for 0.5 h and chased in unlabeled medium for 1 h before sedimentation in alkaline sucrose, as described under Methods.

hmaster cells. The qualitatively greater enhancement observed when cells were given $UV_1$ in $G_2$ 7.5 h before $UV_2$ than $UV_1$ in S 1.5 h before $UV_2$ irradiation suggests that irradiation of cells in S-phase is not a prerequisite for enhancement of postreplication repair.

### *Effect of Irradiation in $G_2$ on Enhancement of Postreplication Repair*

To determine whether the enhancement observed in Fig. 2 was due to the presence of photoproducts during the 1.5 h of DNA synthesis in the S-phase before $UV_2$ irradiation, $UV_2$ was given as soon as possible after removal of the second hydroxyurea block. Ideally one should study repair as soon as cells enter S-phase. However, at centrifugation times that did not pellet parental DNA, we observed that the size of newly synthesized DNA at pulse-chase times earlier than 0.75 h into S-phase was too small to detect significant differences between sizes of irradiated and nonirradiated DNA. For example, the size of newly synthesized DNA from nonirradiated cells pulse-labeled at

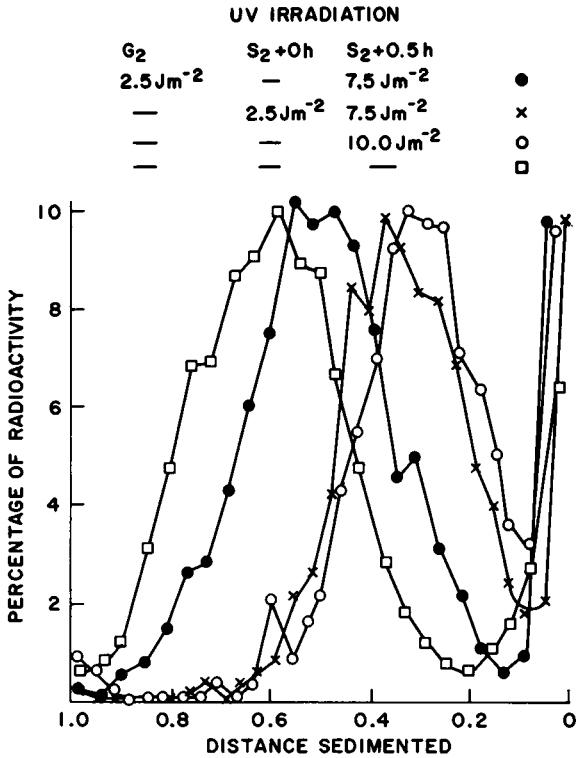


FIGURE 3 Alkaline sucrose gradient profiles of DNA from cells given split doses of UV in $G_2$ or S-phase. Cells were irradiated with 2.5 $Jm^{-2}$ UV in $G_2$-mitosis (•) or at $S_2$ + 0 (×) and then with 7.5 $Jm^{-2}$ at $S_2$ + 0.5 h; or irradiated with 10.0 $Jm^{-2}$ (○) at $S_2$ + 0.5 h. Remainder of the procedures are described in Fig. 2.

0.75 h after removal of hydroxyurea was smaller than pulse-chased DNA from cells labeled 1.5 h into the $S_2$-phase (compare Fig. 3 with 2). Also as shown in Fig. 3, the newly synthesized DNA from cells irradiated with 2.5 $Jm^{-2}$ UV in $G_2$ (6 h on Fig. 1) and given 7.5 $Jm^{-2}$ at 0.5 h after removal of the second hydroxyurea block (12.5 h on Fig. 1) was appreciably larger in size, i.e., further down the gradient, then pulse-chased DNA from cells given 10 $Jm^{-2}$ UV 0.5 h into $S_2$-phase.

The following data argue that the enhancement as observed above was not due to the 0.5 hr cells were in S-phase. As shown in Fig. 3, the size of newly synthesized DNA from cells given 2.5 $Jm^{-2}$ at 0 h and 7.5 $Jm^{-2}$ at 0.5 h after removal of the second hydroxyurea block was only slightly larger than the DNA from cells given 10 $Jm^{-2}$ at 0.5 h into the second S-phase. DNA synthesis was inhibited 71 and 77% in cells given 2.5 $Jm^{-2}$ UV in $G_2$ or at the beginning of the second S-phase, respectively (6 and 12 h on Fig. 1), and 7.5 $Jm^{-2}$ UV 0.5 h into the $S_2$-phase. Similar patterns of enhancement have been observed with cells given 2.5 $Jm^{-2}$ 4 and 7 h after release from the first hydroxyurea block. Three trivial explanations to account for these observations were considered and ruled out. (*a*) $UV_1$ could alter the size of the cell; that would reduce

the amount of $UV_2$ penetrating the cell. Previous data (7) showed that the number of thymine-containing dimers was greater in cells given split doses of UV than in cells given a single dose of UV. (*b*) $UV_1$ could change the distribution of the length of growing replicons or inhibit the initiation of new replicons. Other data in Chinese hamster (7,18) and in human cells (9) show that $UV_1$ primarily inhibits the rate of chain elongation and not initiation. (*c*) The photoproducts induced by $UV_1$ irradiation could be removed by excision repair before $UV_2$ irradiation. Using UV-endonuclease from *Micrococcus luteus*, we have observed that the number of UV-endonuclease-sensitive sites in the DNA from Chinese hamster cells irradiated with 3 $Jm^{-2}$ UV decreases from 8.1 per $10^8$ daltons 0 h after UV irradiation to 6.7 per $10^8$ daltons 4 h after UV irradiation. This decrease (approximately 16%) in endonuclease-sensitive sites is too small to account for the enhancement observed.

## DISCUSSION

Postreplication repair is greater when UV is given in fractionated doses than when the sum of doses is given at one time. The data presented in this paper indicate that the enhancement of postreplication repair observed in cells given a small dose of UV during $G_2$ cannot be due to the presence of photoproducts at the beginning of the $S_2$-phase but must reflect events that also take place before the cells enter S-phase. These data indicate that enhancement depends upon the time between the split doses and not upon the cell cycle phase when the first UV is given.

The enhanced ability to convert small molecular-weight DNA into larger DNA after UV irradiation cannot be explained simply by the excision of dimers or to alterations in cell size or replicons produced by $UV_1$ (7,9,18). Also, cell death or mitotic delay produced by the first UV dose are unlikely to cause a confused interpretation of these data, since cell survival at 2.5 $J/m^2$ is over 95% (19) and progression through the cell cycle is not significantly altered.

A reasonable explanation for our data is that the first dose increases the rate of postreplication repair of DNA in cells given the second dose. Such an increase has been observed in Chinese hamster (7), and xeroderma pigmentosum (8,9) cells given UV or treated with *N*-acetoxy-acetylaminofluorene. Although the exact mechanisms of enhancement of repair are as yet unknown, the process could involve: (*a*) increases in the rate of filling in of gaps left in daughter DNA as a result of replication upon a damaged template; or (*b*) modification of the replication machinery so that it can bypass the lesion in parental DNA (5,6); or (*c*) modification of photoproducts so that they are no longer recognized as blocks to DNA replication (12); or (*d*) increases in recombinational exchanges of dimers. Cells in the S-phase are more sensitive to killing (18) by UV irradiation than at any other phase. These effects appear to be due to an interference in the replication of new DNA by the presence of photoproduct in parental DNA. Interestingly, fractionation of UV doses leads to an increase in survival (20). Also the inhibition of DNA synthesis in the first S-phase by UV is not exhibited in the second S-phase (11). Since very few dimers are removed and little or no recombinational ex-

change takes place (21), these data suggest that recovery from the effects of UV irradiation is due either to a modification of the UV-induced damages or the DNA replication machinery such that replication proceeds normally on the damaged template.

Recent research (21) using alkaline sucrose sedimentation and fiber autoradiography showing that the rate and extent of daughter DNA fork progression was the same in irradiated and nonirradiated cells favors the idea that the replication machinery is modified by the first UV irradiation, so that cells can enhance the postreplication repair of the second UV irradiation.

The research carried out at Brookhaven National Laboratory was under auspices of the U.S. Department of Energy.

*Received for publication 17 October 1977 and in revised form 20 March 1978.*

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