THE EARLY AND LATE MODES OF DNA REPLICATION IN ULTRAVIOLET IRRADIATED SYRIAN HAMSTER EMBRYO CELLS

J. DONIGER AND JOSEPH A. DIPAOLO, *Laboratory of Biology, Division of Cancer Cause and Prevention, National Cancer Institute, Bethesda, Maryland 20205 U.S.A.*

ABSTRACT The nature of DNA replication in UV irradiated Syrian hamster embryo cells (HEC) was investigated by measuring the size distribution of nascent daughter strand DNA. During the early mode nascent strands are made in smaller pieces than in nonirradiated cells. The late mode begins when nascent strands recover to normal size. This was observed in HEC 5 h post-UV. When the late mode is operational, nascent strands elongate to parental size in <2 h, whereas >3 h are required during early mode function. Evidence from split dose experiments demonstrates that the recovery of the size of nascent strands is not due to enhanced gap filling. Furthermore, pyrimidine dimers are probably recognized differently by the replication complex during early and late mode DNA synthesis. The late mode of replication could account for the ability of HEC to survive UV irradiation even though they are inefficient in both excision and postreplication repair.

INTRODUCTION

The interrelationships of DNA repair to cell survival, DNA replication, mutagenesis, and neoplastic transformation are being studied using Syrian hamster embryo cells (HEC)[1] as part of a program to understand the modulation of neoplastic transformation caused by treating cells with two or more agents. When HEC are damaged by UV light (254 nm) irradiation, the cells replicate their DNA, and have a high rate of survival even though most of the pyrimidine dimers (the major UV-induced photoproduct in DNA) remain in the DNA. Moreover, although the rates of excision repair and postreplication repair are 4–5 and 2–3 times slower, respectively, than those observed in human cells, the survival to UV irradiation damage is similar (1). Therefore, the existence of other repair mechanisms has been postulated to account for the survival of HEC.

The recovery of the size of nascent daughter DNA strands several hours post-UV has been observed in cells of several mammalian species (2, 3). By definition, the "early mode" of replication in UV irradiated cells occurs before recovery of nascent strand size and is followed by the "late mode." These experiments show that HEC exhibit early and late modes of replication; the time required to elongate nascent strands to parental size is less for the late mode than the early mode; the late mode of replication is not solely due to enhanced postreplication repair; and most of DNA replication occurs by the late mode.

[1] *Abbreviations used in this paper:* CM, Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum; HEC, Syrian hamster embryo cell; NTE buffer, 100 mM NaCl, 50 mm Tris-HCl pH 7.5, 10 mM EDTA; UV, ultraviolet light.

MATERIALS AND METHODS

For each experiment, fresh HEC from fetuses 13–14 d in gestation (16-to-19-mm crown-rump length) were used. Primary and subsequent passage cells were grown as monolayers in plastic Petri dishes in CM at 37°C in an 11% CO_2 humidified atmosphere. In all experiments, 2-day-old secondary or tertiary hamster cultures obtained by seeding 2.5×10^6 cells/100-mm dish were used.

Cells were UV irradiated 24 cm from a single 15 W General Electric germicidal lamp (G15T8) (General Electric Co., West Lynn, Mass.) at a fluence of 0.6 J/m^{2}s. The fluence rate was measured by an International photometer (International Scientific Instruments Inc., Santa Clara, Calif.) calibrated by the potassium ferri-oxalate procedure. Before UV irradiation, the medium was removed. CM (2 ml) was replaced after irradiation.

For postreplication repair experiments, HEC, plated at 5×10^4 (to maintain log phase growth) in a 60-mm dish were labeled overnight in CM containing 0.1 μCi/ml [^{14}C]thymidine (50 mCi/mmol). The medium with radioactivity was removed 1 h before UV irradiation, the cells were washed, and 2 ml of fresh CM were added. After irradiation, the cells were incubated according to the protocol used (see text). The cells were pulse labeled for 15 min with [^{3}H]thymidine (20 Ci/mmol, 50 μCi/ml). Where indicated, the pulse label was chased for 1, 2, or 3 h with CM containing 20 μM unlabeled thymidine. Incubation was terminated by washing the cells with saline/EDTA (0.8% NaCl, 0.115% Na_2HPO_4, and 0.02% each of KH_2HPO_4, Na_2EDTA, and KCl). The cells were X-irradiated (1,100 R, 50 KvP, Picker portable industrial apparatus, Model T55-433; Picker Corp., Cleveland, Ohio) to facilitate the unraveling of the DNA. After a 2-ml sample of saline/EDTA was added to the dishes, the cells were detached with the aid of a rubber policeman, washed once by centrifugation ($1,000 \times g$ for 4 min) and resuspended in 0.5 ml saline/EDTA. Between 5×10^4 and 10^5 cells were lysed on a 0.3 ml pad of 1 N NaOH, 10 mM EDTA, layered on top of a 5–20% linear sucrose gradient (2 M NaCl, 0.33 N NaOH, 10 mM EDTA), seated on 0.1 ml of 60% (wt/vol) sucrose, 0.5 N NaOH. The gradients were centrifuged in a SW50.1 rotor (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.) for ~130′ at 30,000 rpm and terminated when $\omega^2 t = 7.7 \times 10^{10}$ rad^2/s. ~20 fractions were collected from each gradient by pumping through a 20-gauge canula inserted to the bottom of the tube. The DNA in each fraction was precipitated with 1 N HCl and collected on Whatman GF/C glass fiber filters (Whatman Inc., Clifton, N.J.). The filters were washed once with 1 N HCl and twice with 95% ethanol. After drying, the radioactivity on the filters was counted in a toluene based scintillation solution. The gradients were precalibrated using the following phage DNA markers: (*a*) T_2; (*b*) T_4; (*c*) T_7; (*d*) nicked ϕX174; (*e*) lambda. The data were analyzed and plotted by computer.

To determine the amount of the genome replicated after UV irradiation, cells were plated and prelabeled as for postreplication repair experiments. Some of the cells were labeled overnight with [^{3}H]thymidine (0.25 μCi/ml, 0.25 μg/ml) instead of [^{14}C]thymidine. After irradiation (10 J/m^2), the cells were incubated for 6 h in fresh CM containing BrdUrd (5 μg/ml). Subsequently, the medium was removed and the plates were frozen at −40°C. After thawing, the cells were lysed by the addition of 1% Sarkosyl in NTE buffer. RNAse (50 μg/ml) was added, the dishes were incubated for 30 min at 37°C, followed by 1 h further incubation in the presence of proteinase K (50 μg/ml). The extracted DNA was sheared by passage through a 20-gauge needle three times. ^{3}H-labeled DNA (from cells incubated without BrdUrd), ^{14}C-labeled DNA (from cells incubated with BrdUrd), and CsCl (60.8%, wt/wt of NTE) were mixed in a 1:4:42 ratio by volume. Density equilibrium was established by centrifugation for 48 h in a SW50.1 rotor at 33,000 rpm. The gradients were fractionated into ~50 fractions and the radioactivity was determined. The density of fractions 10, 20, and 30 was determined by measuring the refractive index of 2-μl samples. The data were analyzed and plotted by computer.

RESULTS

The size distribution of nascent DNA daughter strands, pulse labeled for 15 min at various times post-UV (10 J/m^2), was determined by alkaline sucrose sedimentation (Fig. 1). During the first 3 h, most of the pulse label was in DNA strands smaller than observed in

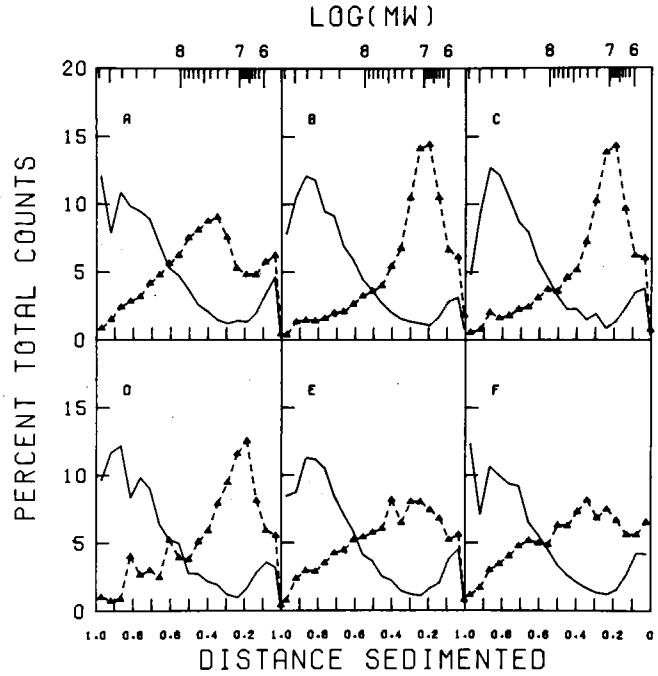

FIGURE 1 Alkaline sucrose sedimentation analysis of nascent daughter strands pulse labeled at various times after HEC were UV irradiated. HEC were labeled overnight with [^{14}C]thymidine (—), UV irradiated (10 J/m^2) and pulse labeled for 15 min with [^{3}H]thymidine (▲—▲) at different times post-UV. At the end of the pulse, the cells were lysed and the DNA sedimented in alkaline sucrose. Sedimentation is from right to left. The calculated molecular weight for a given distance sedimented is shown at the top. Between 2,000 and 5,000 cpm of each label was applied to the gradients and the fractions were counted for a minimum of 2 min. (*A*) nonirradiated cells; (*B*) irradiated cells, pulse label at 0.5 h, (*C*) 1.5 h, (*D*) 3 h, (*E*) 5 h, or (*F*) 7 h, post-UV.

nonirradiated control cells. The molecular weight of the mode is ~9 × 10^6, not very different from the distance between pyrimidine dimers (5 × 10^6, data not shown). By 5 h post-UV, the size of the nascent strands has started to recover and approaches that of nonirradiated cells. Therefore, the early mode of replication in HEC is operational during the first 3 h post-UV and the late mode after 5 h post-UV. The rate of chase of nascent strands, pulse labeled at 0.5 and at 5 h post-UV, respectively, was compared (Fig. 2). The nascent strands replicated at 0.5 h post-UV require in excess of 3 h to elongate to parental size while those replicated at 5 h post-UV reach parental size in ~2 h.

D'Ambrosio and Setlow (4) have shown that the postreplication repair rate, i.e., the rate of chase of pulse labeled nascent strands into parental DNA in Chinese hamster cells, can be enhanced by pretreating the cells with a low dose of UV or *N*-acetoxy-acetylaminofluorene 2–12 h before a second dose. The following experiments were designed to determine whether the late mode of replication is the result of enhanced postreplication repair. HEC were pulse labeled with [^{3}H]thymidine 0.5 h after UV irradiation with either a single dose of 10 J/m^2 or a double dose of 2.5 J/m^2 plus 7.5 J/m^2 separated by 2.5 h. The size distributions of nascent strands from cells irradiated with 10 J/m^2 in two doses was slightly larger than that of cells

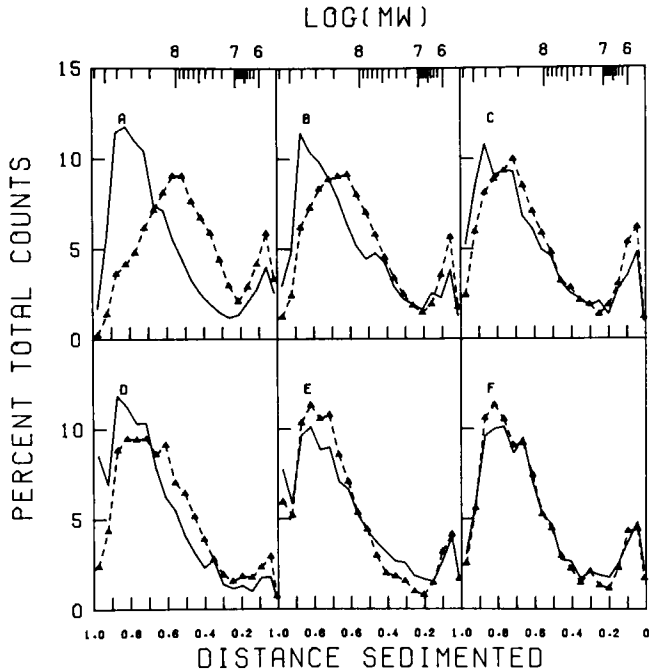

FIGURE 2 Chase of nascent strands, pulse labeled during the early or late modes of replication, into parental size DNA. HEC were labeled overnight with [^{14}C]thymidine (—), UV irradiated (10 J/m^2), and pulse labeled with [^{3}H]thymidine (▲—▲) for 15 min at 0.5 h (*A*, *B*, and *C*) or 5 h (*D*, *E*, and *F*) post-UV. The pulse label was chased by incubating the cells with excess cold thymidine (20 mM) for 1 h (*A* and *D*), 2 h (*B* and *E*), or 3 h (*C* and *F*). Other details as in Fig. 1 and Materials and Methods.

irradiated once (Fig. 3 *A*). There was more pulse label in DNA strands >2 × 10^8 daltons (the size of parental DNA) in the cells receiving the split dose due to enhancement. When the interval between the two dose irradiations was increased from 2.5 to 5.5 h no significant difference in the distribution of nascent strands was observed (Fig. 3 *B*). Therefore, the amount of enhancement was not time dependent within the interval studied. Moreover, when HEC were pulse labeled 6 h after receiving UV irradiation in a single dose of 10 J/m^2, i.e., during the late mode, the size distribution of nascent strands was clearly larger than that obtained when cells were irradiated with two doses totaling 10 J/m^2 separated by 5.5 h and pulse labeled 0.5 h later (Fig. 3 *C*) The mode molecular weight was 33 × 10^6 when the cells were irradiated with the single dose as compared to 11 × 10^6 when two irradiations were used. In both cases, the initial irradiation was 6 h before pulse labeling, and the template strands contained the same number of UV-induced photoproducts because the cells received the same total dose. Excision repair during this period is negligible (1). If the late mode of replication were due to enhanced postreplication repair, the size distributions in the above two cases should have been the same. Therefore, the difference in the size distributions of nascent strands observed in Fig. 3 *C* suggests that enhanced postreplication repair cannot account for the recovery of the size of nascent strands during the late mode of replication. It could be argued, however, that 10 J/m^2 elicits more enhancement of postreplication repair than does 2.5 J/m^2. This would also explain the data in Fig. 3 *C*. If this were the case, the recovery of the

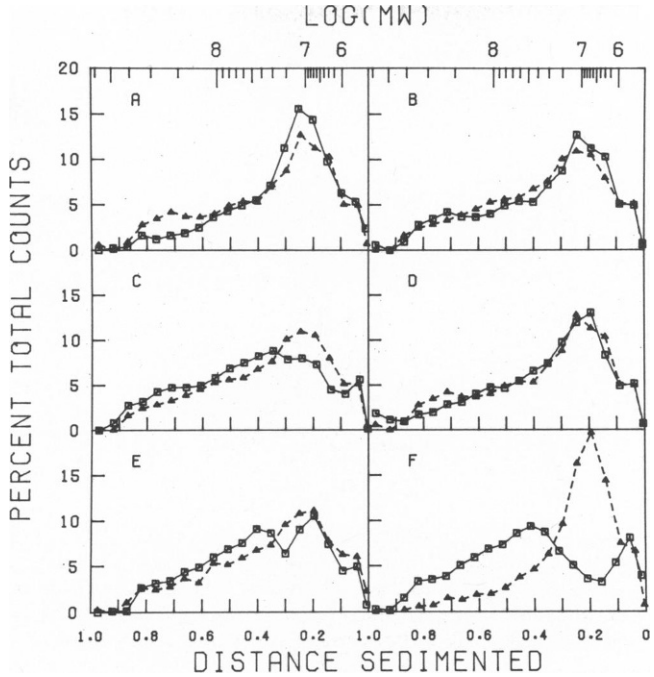

FIGURE 3 A comparison of the effects of double UV doses to single ones on the size distribution of pulse labeled nascent strands in HEC. HEC were labeled overnight with [^{14}C]thymidine (profiles not shown) UV irradiated, and pulse labeled with [^{3}H]thymidine for 15 min. Other details as in Fig. 1 and Materials and Methods. Whenever the cells were given two UV doses, the pulse label was added 0.5 h after the second dose. (*A*) Total UV irradiation was 10 J/m^2; single dose 0.5 h before pulse label, □; two doses, 2.5 and 7.5 J/m^2 separated by 2.5 h, ▲. (*B*) Two UV doses totaling 10 J/m^2; 2.5 h interval, □; 5.5 h interval, ▲. (*C*) Total UV irradiation was 10 J/m^2; single dose 6 h before pulse label, □; two doses, 2.5 and 7.5 J/m^2 separated by 5.5 h, ▲. (*D*) Total UV irradiation was 10 J/m^2; single dose 3 h before pulse label, □; two doses 2.5 and 7.5 J/m^2 separated by 2.5 h, ▲. (*E*) Total UV irradiation was 20 J/m^2; single dose 6 h before pulse label, □; two doses, 10 and 10 J/m^2, separated by 5.5 h ▲. (*F*) Unirradiated control □; single dose of 20 J/m^2 0.5 h before pulse label, ▲.

size of nascent strands should also be observed at 3 h post-UV because enhancement is the same at 3 and 6 h post-UV (Fig. 3 *B*). This was not observed (Fig. 1). Furthermore, when the size distribution of nascent strands from cells pulse labeled 3 h post-UV with a single dose of 10 J/m^2 was compared to that from cells receiving two doses, totaling 10 J/m^2, separated by 2.5 h and pulse labeled 0.5 h later, the two distributions were not significantly different (Fig. 3 *D*). This shows that enhancement is not dose dependent in the range of 2.5–10 J/m^2. These experiments show that recovery of nascent strands is not solely the result of enhanced postreplication repair. This conclusion is confirmed by the fact that results similar to those in Fig. 3 *C* were obtained when 20 J/m^2 irradiation 6 h before pulse labeling was compared to 10 J/m^2 followed 5.5 h later by 10 J/m^2 followed 0.5 h later by pulse labeling (Fig. 3 *E*). For comparison purposes, the size distributions of nascent strands in nonirradiated controls (Fig. 3 *F*, □) and in cells pulse labeled 0.5 h after 20 J/m^2 UV (Fig. 3 *F*, ▲), are presented.

The amount of DNA replicated after HEC were UV irradiated 10 J/m^2 was determined by incubating the cells with BrdUrd and determining the percent of the DNA which has a

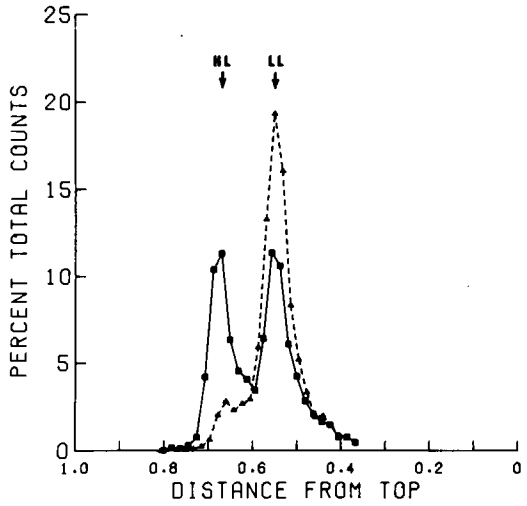

FIGURE 4 The fraction of the HEC genome replicated within 6 h post-UV. HEC-labeled overnight with [^{14}C]thymidine were UV irradiated (10 J/m^2) and incubated in CM containing BrdUrd (5 μg/ml) for 6 h. The DNA was then extracted from the cells and subjected to CsCl density gradient sedimentation. *HL* and *LL* designate the positions in the gradients of heavy light and light light DNA, respectively. Other details as in the Materials and Methods. DNA from nonirradiated control cells (□—□) or from UV irradiated cells (▲—▲).

hybrid density when banded in CsCl density equilibrium gradients. Only 10% of the genome replicated in the first 6 h post-UV when the cells were irradiated with 10 J/m^2 (Fig. 4). Evidence that the entire genome of irradiated HEC (10 J/m^2) replicated was obtained when the cells were incubated with BrdUrd for 36 h (data not shown). Similar results have been demonstrated for CHO (5). Therefore, at least 90% of the genome replicates by the late mode.

DISCUSSION

HEC exhibit the same recovery in the size distribution of nascent strands replicated in irradiated cells by 5 h post-UV (Fig. 1) as occurs in human (3) and Chinese hamster cells (2). Less time is required for the nascent strands replicated by the late mode to elongate to parental size than is required with respect to the early mode (Fig. 2).

The following model is proposed to explain the nature of early and late mode DNA replication. The small nascent strands observed during the early mode are due to gaps in the daughter strand which occur because of damages in the template strand. The filling in of the gaps (probably by de novo synthesis) results in full sized daughter strand DNA. During the late mode, the damaged bases in the template or their environment are altered such that gaps are no longer made in the daughter strands. No further repair is necessary to make the daughter strands full size. Three reports are consistent with the hypothesis that gaps in the daughter strand are responsible for the short nascent strands observed during the early mode. Doniger (6) reported that the short nascent strands were observed in Chinese hamster cells (V-79) irradiated with 5 J/m^2 when there was no radiation effect on the rate of replication

fork elongation or the size distribution of growing replicons. Meneghini (7) showed the presence of gaps by the sensitivity of newly replicated DNA in UV irradiated human cells to a single strand specific endonuclease. Such sensitivity could only occur if the template strands opposite the nascent daughter strands had single strand regions in them. Finally, Setlow and D'Ambrosio (8) have measured the gap size by the BrdUrd photolysis technique. After pulse labeling nascent strands in UV irradiated cells, the cells were incubated in the presence of BrdUrd until the nascent strands elongated to parental size. Single strand breaks were introduced with 313-nm light in a dose-dependent manner into DNA containing BrdUrd. The BrdUrd-substituted stretches adjacent to nascent strands replicated in UV irradiated V79 or XP variant cells were only 200–300 bases long.

Since the short nascent strands synthesized during the early mode can be chased into parental size DNA, the gaps must be filled. This gap filling, traditionally referred to as postreplication repair, apparently occurs by de novo synthesis because the pyrimidine dimers present in the template strands are not transferred (or recombined) into the daughter strands during the gap filling period (9, 10) as is the case in bacteria (11).

The conversion from the early mode to the late mode could occur for one of three reasons: (*a*) The damage in the template strand responsible for the gaps is excised. (*b*) The rate of gap filling becomes enhanced to such a rate so that time of gap filling is shorter than the time for the replication fork to travel the distance between two lesions. If this occurs, small nascent strands cannot be observed. (*c*) Gaps are no longer made during late mode replication.

Data suggest that lesions leading to gaps in the nascent strands are not excised by the time the late mode occurs. It is assumed that the gaps are due to the presence of pyrimidine dimers. ~10% of pyrimidine dimers are excised in HEC within the first 6 h post-UV (1). However, another photoproduct could cause the gaps. Two lines of evidence argue against this explanation. First, the small size of nascent strands in UV irradiated chicken and rat kangaroo cells can be reversed by photoreactivation (12, 13) suggesting that pyrimidine dimers are responsible for the gaps. Second, if caffeine was added to HEC 4 h post-UV, when the late mode was becoming operational, the size distribution of nascent strands slowly decreased between 1 and 3 h post-caffeine. By 3 h post-caffeine (7 h post-UV), the size distribution was similar to that in cells pulse labeled 0.5 h post-UV only. Thus, while caffeine can block gap filling almost immediately during the early mode of replication (6), when applied during the late mode, it was responsible for slowly reestablishing the early mode (details to be presented elsewhere). This shows that damages in the template strand persist when the late mode functions.

An enhanced rate of gap filling cannot be solely responsible for the late mode of replication (Fig. 3). Therefore, many gaps are not formed during the late mode. Either the nature of DNA replication changes several hours post-UV or some alteration of the DNA damage or its nuclear protein environment occurs which affects the recognition of the damaged template by the replication complex. The latter probably occurs because the size distribution of nascent strands replicated after the split dose of 10 J/m^2 plus 10 J/m^2 separated by a 5.5-h interval (Fig. 3*E*, △) appears to be the average of the size distributions observed when the cells are irradiated with 10 J/m^2 0.5 and 6 h, respectively, before pulse labeling (Fig. 3 *A* and *C*, □). In this case, the pyrimidine dimers produced 0.5 h before pulse labeling led to gaps in the daughter strand but the dimers produced 6 h before the pulse did not lead to gaps.

When the late mode of replication becomes operational, only a small fraction of the DNA has been replicated (Fig. 4). Therefore, most of the daughter strands are replicated with only a few gaps. Whether the daughter strands replicating during the late mode contain base changes opposite pyrimidine dimers in the template strands is unknown. However, the ability of UV irradiated cells to make parental size daughter strands without gaps is the first requirement for cell survival. Excision and postreplication repair are much less efficient in HEC than in human cells, while the survival after UV irradiation is about the same (1). The ability of HEC to replicate most of the DNA with few daughter strand gaps appears to account for the ability of these cells to survive UV irradiation without relying heavily on postreplication (i.e., gap filling) or excision repair.

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REFERENCES

1. DONIGER, J., and J. A. DIPAOLO. 1980. Excision and post-replication DNA repair capacities, enhanced transformation, and survival of Syrian hamster embryo cells irradiated by ultraviolet light. *Cancer Res.* **40**:582–587.
2. MEYN, R. E., and R. M. HUMPHREY. 1971. Deoxyribonucleic acid synthesis in ultraviolet-light-irradiated Chinese hamster cells. *Biophys. J.* **11**:295–301.
3. BUHL, S. N., R. B. SETLOW, and J. D. REGAN. 1973. Recovery of the ability to synthesize DNA in segments of normal size at long times after ultraviolet irradiation of human cells. *Biophys. J.* **13**:1265–1275.
4. D'AMBROSIO, S. M., and R. B. SETLOW. 1976. Enhancement of post-replication repair in Chinese hamster cells. *Proc. Natl. Acad. Sci. U.S.A.* **73**:2396–2400.
5. MEYN, R. E., R. R. HEWITT, L. F. THOMSON, and R. M. HUMPHREY. 1976. Effects of ultraviolet irradiation on the rate and sequence of DNA replication in synchronized Chinese hamster ovary cells. *Biophys. J.* **16**:517–525.
6. DONIGER, J. 1978. DNA replication in ultraviolet light irradiated Chinese hamster cells: The nature of replicon inhibition and post-replication repair. *J. Mol. Biol.* **120**:433–446.
7. MENEGHINI, R. 1976. Gaps in DNA synthesized by ultraviolet light-irradiated WI38 human cells. *Biochim. Biophys. Acta.* **425**:419–427.
8. SETLOW, R. B., and S. M. D'AMBROSIO. 1978. Size of putative gaps in post-replication repair. *Biophys. J.* **21**:94 *a*. (Abstr.).
9. LEHMANN, A. R., and S. KIRK-BELL. 1978. Pyrimidine dimer sites associated with daughter strands in UV-irradiated human fibroblasts. *Photochem. Photobiol.* **27**:297–307.
10. D'AMBROSIO, S. M., and R. B. SETLOW. 1978. On the presence of uv-endonuclease sensitive sites in daughter DNA of uv-irradiated mammalian cells. *In* DNA Repair Mechanisms. P. C. Hanawalt, E. C. Friedberg, and C. F. Fox, editors. ICN-UCLA Symposia on Molecular and Cellular Biology. **9**:499–503.
11. GANESAN, A. K. 1974. Persistence of pyrimidine dimers during post-replication repair of ultraviolet light-irradiated *Escherichia coli* K12. *J Mol. Biol.* **87**:103–119.
12. LEHMANN, A. R., and S. STEVENS. 1975. Postreplication repair of DNA in chick cells: studies using photoreactivation. *Biochim. Biophys. Acta.* **402**:179–187.
13. BUHL, S. N., R. B. SETLOW, and J. D. REGAN. 1974. DNA repair in *Potorous tridactylus*. *Biophys. J.* **14**:791–803.