Translesion DNA Synthesis in the Dihydrofolate Reductase Domain of UV-Irradiated CHO Cells†

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ABSTRACT: The studies that document the coupling of strand-specific DNA repair to transcription of active genes exclude replicated DNA from the analysis. Yet cyclobutane pyrimidine dimers (CPD) induced by ultraviolet light (UV) persist in most of the genome in surviving Chinese hamster ovary (CHO) cells. The mechanisms that allow DNA replication to occur in the presence of damaged templates are poorly understood. We have investigated the distribution of CPD in the dihydrofolate reductase gene (*DHFR*) domain in replicated DNA. CHO B11 cells were incubated in the presence of BrdUrd after UV irradiation; the replicated DNA was separated from the unreplicated DNA by isopycnic sedimentation in CsCl, and then the parental and daughter strands were resolved in alkaline CsCl. We determined the fraction of a 14-kb *Kpn*I fragment of the *DHFR* gene that was resistant to digestion by T4 endonuclease V, a CPD-specific enzyme. In both parental and unreplicated DNA, ~80% of the CPD were removed from the transcribed strands while ~20% were removed from the nontranscribed strands of *DHFR* within 24 h. In a 15-kb *Kpn*I fragment that contains an origin of replication and is located ~15 kb downstream of *DHFR*, we found very low repair levels, whether it had been replicated or not. We detected no CPD in the daughter strands of either fragment analyzed. These results suggest that the replication forks can move through the damaged DNA in the absence of significant levels of repair or strand exchange and that the repair of CPD is not affected by replication in these cells.

The irradiation of cells with ultraviolet light (UV)[1] produces lesions in their DNA which have been shown to transiently inhibit replication. The primary lesions produced by moderate doses of UV (254 nm) are cyclobutane pyrimidine dimers (CPD); other, less frequent lesions include 6–4 pyrimidine–pyrimidone photoproducts [(6,4)PP] and oxidation products such as thymine glycols.

Research on the roles of prokaryotic DNA polymerases in DNA repair and in replication on damaged templates is abundant, and has been facilitated by the availability of mutants deficient in these polymerase activities. Studies with purified DNA polymerases in vitro have shown that lesions such as CPD pose blocks to strand elongation at the site of the lesion (Moore et al., 1980). On the other hand, Taylor and O'Day (1989) have shown that Pol I of *Escherichia coli* is able to bypass a CPD in a synthetic oligonucleotide when its concentration and that of dNTPs are high enough. Strand exchanges that result in damaged parental DNA becoming associated with intact daughter strands have been documented for *E. coli* by Ganesan (1974), but not for mammalian cells (Painter, 1974; Lehmann & Kirk-Bell, 1978).

The mechanisms by which mammalian DNA polymerases overcome lesions in vivo are unknown, although several models have been postulated. One of them is *translesion synthesis*, in which the polymerase inserts nucleotide(s) opposite non-informational or misinformational sites. Thus, the human DNA polymerase α_2 and the Pol I Klenow fragment of *E. coli* can bypass thymine glycol lesions in vitro (Clark & Beardsley, 1989).

A second mechanism is *discontinuous synthesis with gap formation*, in which replication is blocked when it encounters a lesion, but then it resumes a short distance downstream, leaving a gap opposite the lesion. In *E. coli*, such gaps are filled at a later time through a daughter strand gap repair mechanism. In mammalian cells, the appearance of gaps in the daughter strands can be due to other effects of the damage to the parental DNA, for instance, initiation of replication from a secondary origin beyond the lesion (Griffiths & Ling, 1987). Although the presence of gaps in daughter DNA strands has been documented in UV-irradiated mammalian cells, it is not known whether the gaps occur opposite lesions (Meneghini, 1981; Painter, 1985).

Most of the studies designed to elucidate damage-tolerance mechanisms in mammalian cells have been carried out in vitro, or have utilized viral DNA as the substrate. For example, Scaria and Edenberg (1988) showed that in CV-1 monkey cells infected with simian virus 40 (SV40) and irradiated with 75 J/m^2, CPD block elongation of viral daughter strands for at least 1 h. These studies must utilize large doses of UV, which might challenge the cellular repair capacity, in order to produce the desired frequency of CPD in the small viral DNA molecules. Indeed, when hamster or human cells were irradiated with only 5 J/m^2, van Zeeland and Filon (1982) observed that nascent DNA strands became larger than the inter-CPD distance within 15 min after irradiation, indicating that replication had paused only briefly at the CPD or that if gaps were left opposite CPD these gaps had been filled very rapidly.

Chinese hamster ovary (CHO) cells present an attractive system in which to study the replication of DNA-containing lesions caused by UV. These cells survive moderate doses of UV, replicating most of their DNA while exhibiting poor overall repair of CPD. In contrast, (6,4)PP are rapidly and efficiently repaired in these cells (Mitchell & Nairn, 1989). It has been shown that in CHO cells the repair of CPD in

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[1] Abbreviations: *DHFR*, dihydrofolate reductase (gene); CPD, cyclobutane pyrimidine dimer(s); (6,4)PP, 6–4 pyrimidine–pyrimidone photoproduct(s); ESS, endonuclease-sensitive site(s); LLUR, long-lived unreplicated region; UV, ultraviolet light; SV40, simian virus 40.

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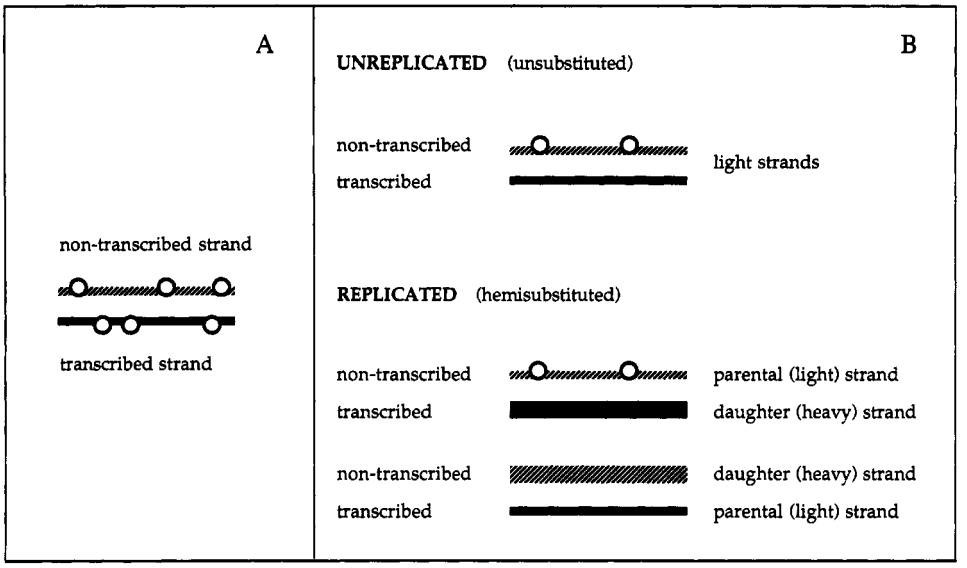

FIGURE 1: Diagram showing DNA strands and their nomenclature. (A) DNA immediately after UV irradiation; for clarity, the only photoproducts indicated are CPD (circles); (B) products found 24 h after irradiation and incubation with BrdUrd.

active DNA sequences is much more efficient than that in silent DNA sequences, and that for the genes so far examined this differential repair can be explained entirely by the preferential repair of the transcribed strand in the active sequences while the nontranscribed strand remains as poorly repaired as the bulk of the genome. Thus, a correlation between transcription and preferential repair has been established (Mellon et al., 1987).

The method usually utilized to study repair of CPD in specific sequences involves first the separation of the unreplicated DNA from the parental DNA that has replicated after UV irradiation by density-labeling with the thymidine analog bromodeoxyuridine (BrdUrd) during post-UV incubation and isolating unreplicated DNA in CsCl gradients. The DNA is restricted, and equal samples are treated or untreated with the CPD-specific endonuclease V of phage T4. The DNA is electrophoresed in alkaline agarose gels to separate full-length strands from strands nicked at sites of CPD. The genomic DNA is transferred to support membranes and detected by hybridization with radioactive sequence-specific probes. Exposure to X-ray film permits comparison of the amounts of radioactivity representing full-length fragments in samples treated or not treated with the endonuclease. The frequency of endonuclease-sensitive sites (ESS), which correspond to CPD, is derived from the fraction of molecules containing no ESS by using the Poisson expression (Bohr et al., 1985).

Previous investigations excluded replicated DNA to avoid overestimating repair, due to the presence of full-length DNA fragments from daughter strands. Figure 1 shows a schematic representation of the different classes of DNA strands that would be found 24 h after UV irradiation. For simplicity, we have not indicated leading or lagging strands in the diagrams. Only a small portion of the DNA remains unreplicated in CHO B11 cells 24 h after a low (10 J/m^2) dose of UV. The unreplicated DNA could represent a subclass of DNA that undergoes heterogeneous repair either as a cause or as a consequence of the fact that it has not replicated; if that were the case, the replicated DNA might exhibit a different pattern of CPD removal. Therefore, we analyzed the repair of CPD in the transcribed and nontranscribed DNA strands that had served as templates for replication, and compared the results to those for their unreplicated counterparts. In addition, we examined the daughter DNA strands for the presence of CPD, which would indicate the occurrence of strand exchanges. In our studies, we used CHO B11 cells, in which a stretch of DNA containing the dihydrofolate reductase (*DHFR*) gene has been amplified 50-fold. The *DHFR* "amplicon" has been extensively studied in several CHO cell lines by various groups of investigators. The map in Figure 2 is a composite of data published by Urlaub et al. (1983), Millbrandt et al. (1983), and Dijkwel and Hamlin (1988).

We found that the transcribed strand of the *DHFR* gene is preferentially repaired in replicated DNA as well as in unreplicated DNA. Thus, although most of the CPD remain in the nontranscribed strand, they do not seem to constitute significant blocks to replication. A region in the *DHFR* amplicon that contains an origin of replication was reported to exhibit extremely poor repair of CPD (Ho et al., 1989). We document here that this region is as poorly repaired in the replicated DNA as it is in the unreplicated DNA. We also document by two separate methods the lack of sensitivity of the BrdUrd-containing DNA strands to T4 endonuclease V, demonstrating that the daughter DNA is free of ESS in the fragments of the *DHFR* domain mentioned above and in the genome overall.

MATERIALS AND METHODS

Cell Culture and UV Irradiation. CHO B11 cells (obtained originally from R. T. Schimke, Department of Biological Sciences, Stanford University) were cultured and irradiated as previously described (Mellon et al., 1987). The cells were prelabeled with [^{14}C]dThd (0.01 μCi/mL) for 2–3 days, trypsinized, and replated at a density of 10^6 cells per 10-cm dish and cultured for 24 h in nonradioactive medium. After UV irradiation, the cells were incubated in fresh medium containing 10 μM BrdUrd, 1 μM FdUrd, 10 μM dCyt, and 0.1 mCi/mL [^{3}H]dThd. From the time of addition of BrdUrd-containing medium, all subsequent procedures were carried out under yellow lights to prevent photolysis of BrdUrd-substituted DNA (Hutchinson, 1973).

Repair Analysis in the DHFR Domain. High molecular weight DNA was isolated as previously described (Bohr et al., 1985). After separation of the parental and replicated DNA fragments by CsCl density centrifugation, the fractions containing DNA of normal and hybrid density were identified by their radioactivity, pooled, and dialyzed against 10 mM phosphate buffer, pH 6.8. In order to inhibit nicking of Br-

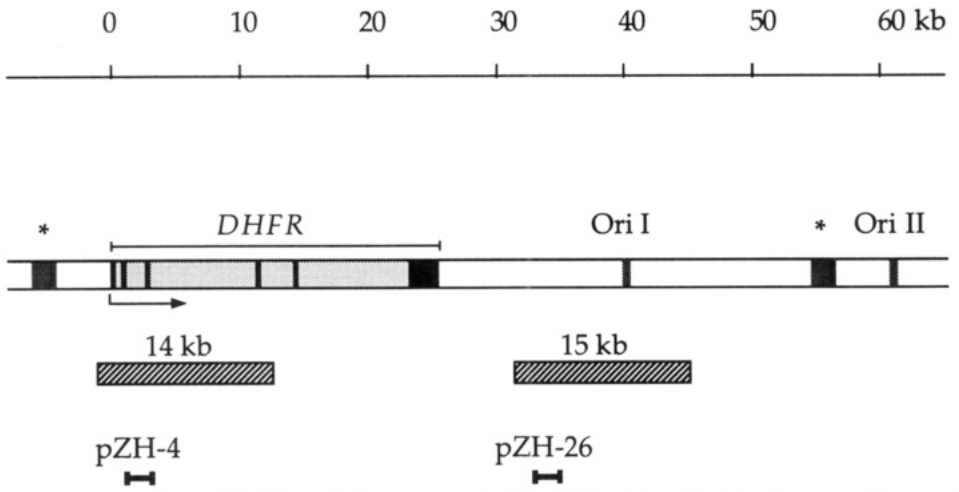

FIGURE 2: Map of the *DHFR* amplicon. Black bars indicate exons; Ori I and II, origins of replication; asterisks, matrix attachment sites; the arrow indicates the start site and direction of transcription for *DHFR*. The hatched bars indicate the sizes and locations of the genomic *Kpn*I fragments studied. The position and size of the DNA probes pZH-4 and pZH-26 are indicated.

dUrd-substituted DNA by alkali in subsequent steps (see text), the DNA samples were treated with 5 mM methoxyamine for 30 min at 37 °C (Liuzzi & Tarpaert-Borlé, 1988; K. Sweder, unpublished experiments). Light and heavy DNA strands were separated by alkaline CsCl density centrifugation (Smith et al., 1981).

The nicking of DNA at CPD by T4 endonuclease V is much less efficient in single-stranded DNA than in double-stranded DNA (Gordon & Haseltine, 1980). Thus, it was necessary to utilize larger amounts of enzyme. In order to minimize nonspecific nicking due to contaminating nucleases, we used T4 endonuclease V purified to apparent homogeneity, kindly supplied by Dr. R. S. Lloyd (Vanderbilt University). A control sample of unirradiated DNA was included in each experiment to allow correction for any nonspecific nicking. Treatment with T4 endonuclease V, alkaline agarose gel electrophoresis, Southern transfer, and hybridizations with ^{32}P-labeled probes were carried out as previously described (Mellon et al., 1987), except that we used phosphate buffer, pH 6.8, to dialyze and store the single-stranded DNA after preparation, and we eliminated the acid-depurination and alkali-nicking treatments of the DNA in agarose gels prior to blotting (K. Sweder, unpublished experiments). The DNA was transferred to Hybond N+ membranes (Amersham), which were then treated with alkali according to the manufacturer's instructions. Where indicated, membranes were deprobed by washes at 42 °C, first in 0.4 N NaOH for 15 min and then in 0.2 M Tris, 1% SDS, and 0.2 × SSPE for 30 min.

Probes. The probes utilized to detect the genomic fragments of interest are shown in Figure 2. Strand-specific, ^{32}P-labeled RNA probes were generated using the plasmid pZH-4 as a template (Mellon et al., 1987). Plasmid pZH-26 (Ho et al., 1989) was used to synthesize nick-translated DNA probes.

Analysis by Alkaline Sucrose Gradients. Cells were prelabeled with [^{14}C]dThd (0.04 μCi/mL) for 2 days. Fresh, nonradioactive medium was added to the cells 1 h before UV irradiation. After irradiation, the cells were incubated with medium containing [^{3}H]dThd (0.22 μCi/mL) with or without 10 μM BrdUrd and 1 μM FdUrd. Harvest, permeabilization, treatment with T4 endonuclease V in high-salt conditions, lysis, centrifugation in alkaline sucrose gradients, and determination of mean number average molecular weights (M_n) were as previously described (Ganesan et al., 1980).

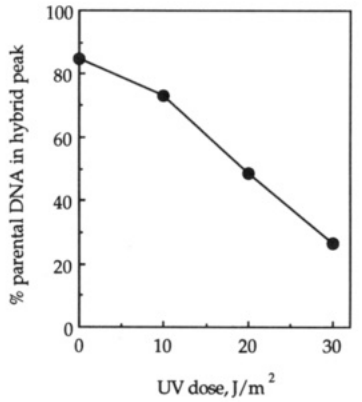

FIGURE 3: Effect of UV on DNA replication. The percent of the DNA that replicated at least once was calculated by adding the prelabel (^{14}C) counts of the fractions that were pooled for each peak of the radioactivity profile after centrifugation in a CsCl gradient. It is expressed as the ratio of ^{14}C counts in the hybrid peak to the sum of ^{14}C counts in the parental and the hybrid peaks.

RESULTS

UV irradiation causes inhibition of DNA replication. We measured the degree of inhibition of DNA synthesis in B11 cells by calculating the relative amount of DNA that had replicated in 24 h after treating the cells with various UV doses. We observed a decrease in replicated DNA proportional to the UV dose (Figure 3). We chose a UV dose of 10 J/m^2 for the experiments described below because it produces a convenient frequency of CPD for quantitation of repair in the DNA fragments chosen for this study. This dose produces a slight (8%) decrease in the amount of replicated DNA in 24 h.

The photographs in Figure 4 show autoradiograms from a repair experiment. Repair data were obtained by measuring the intensities of the hybridization signals in the autoradiograms. The ratios between the paired samples that had been treated or not with T4 endonuclease V were used to calculate the average number of ESS per fragment, using the Poisson expression. Comparison of ESS present in the repaired and the "0 hr" samples yielded the repair data. The initial frequency of lesions was similar for the transcribed and the nontranscribed strands in the 14-kb *Kpn*I *DHFR* fragment and both strands of the 15-kb *Kpn*I downstream fragment (Table I legend). Unirradiated samples, treated or not with T4 endonuclease V, were analyzed in parallel in each

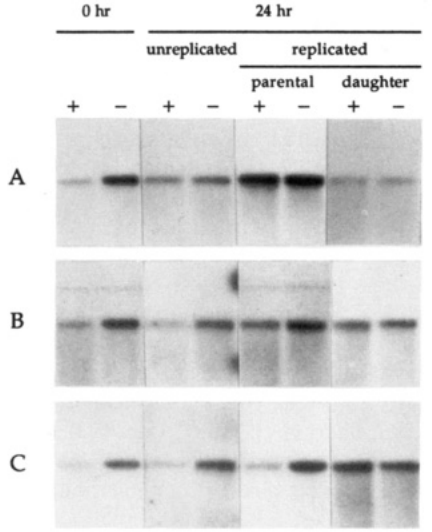

FIGURE 4: Autoradiogram illustrating repair in the *DHFR* domain. DNA was harvested immediately after UV irradiation (0 hr) or after 24 h of repair (24 hr). Single-stranded DNA samples were treated (+) or not (–) with T4 endonuclease V prior to electrophoresis. The *DHFR* and downstream *Kpn*I fragments were detected by hybridization to strand-specific RNA probes made from pZH-4 or to nick-translated DNA probes made form pZH-26, respectively. For the unsubstituted DNA (first six lanes), one membrane was hybridized, and several autoradiograms were obtained after exposures for different lengths of time to optimize the quantification of DNA for each pair (enzyme-treated or not) of bands; the best exposures are shown in the figure, thus, the mosaic-like appearance of the photograph. Data for the BrdUrd-substituted daughter DNA (two rightmost lanes) were obtained from three separate membranes. (A) Transcribed strand of *DHFR*; (B) nontranscribed strand of *DHFR*; (C) downstream fragment.

Table I: Percent ESS Removed from Specific Sequences in the *DHFR* Amplicon[a]

	DHFR gene		downstream fragment
	TS	NTS	BS
unreplicated DNA	82 ± 8	16 ± 12	7 ± 9
template DNA	78 ± 16	28 ± 19	3 ± 3

[a] Data shown represent the mean and standard deviation from five experiments for the *DHFR* gene and three experiments for the downstream fragment. The initial frequency of CPD was 1.1 ± 0.3 for the transcribed strand (TS) and 1.0 ± 0.2 for the nontranscribed strand (NTS) of the *DHFR* gene fragment, and 1.1 ± 0.3 for both strands (BS) of the downstream fragment.

experiment as controls for nonspecific nicking. The values, which ranged between 0 and 0.07 nick/fragment, were subtracted from those obtained from irradiated samples (data not shown).

The repair of CPD in the unreplicated fraction of the DNA in the *DHFR* gene confirms our previous observation (Mellon et al., 1987): the transcribed strand exhibits very efficient repair while the nontranscribed strand is poorly repaired. We show here that the strands that served as templates for replication are repaired to the same extent and with the same selectivity for the transcribed strand as are the unreplicated DNA strands (Figure 4, panels A and B; Table I). In the case of the nontranscribed strands of the *DHFR* gene fragment, we do not consider that the numerical differences between repair values in the template and the unreplicated DNA are significant; due to the logarithmic relationship between the CPD-free fraction (zero class) and the frequency of CPD in a restriction fragment, calculations of low levels of repair are subject to more error in the measurement than when the repair is proficient. This problem is discussed in Bohr and Okumoto (1988).

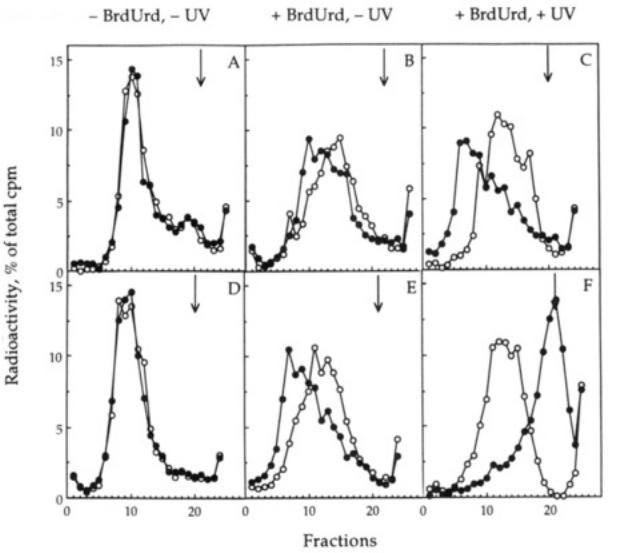

FIGURE 5: Radioactivity profiles of alkaline sucrose gradients, showing the effect of BrdUrd incorporation on DNA strand size and the insensitivity of daughter strands to T4 endonuclease V. B11 cells were prelabeled with [^{14}C]dThd, irradiated or not as indicated, and incubated for 24 h in medium containing [^{3}H]dThd (left panels) and BrdUrd and FdUrd (center and right panels). Top panels, not treated with T4 endonuclease V; bottom panels, treated with T4 endonuclease V. Closed symbols, ^{14}C-labeled parental and unreplicated DNA; open symbols, ^{3}H-labeled daughter DNA. The position of λ DNA (24.25 kb) is indicated by an arrow in each panel. The direction of sedimentation is from right to left.

Initially, we experienced difficulties when we attempted to apply the methodology described above for detection of ESS in daughter strands. Visualization of ethidium bromide-stained alkaline agarose gels indicated that equivalent amounts of unreplicated and BrdUrd-substituted DNA were loaded onto the gels and that the average size (~5 kb) of the respective *Kpn*I digests was similar; however, when the DNA was transferred to membranes and hybridized to a radioactive probe, we were unable to obtain a signal from the substituted DNA. Zwanenburg et al. (1984) reported that BrdUrd-substituted DNA contained alkali-labile sites. 5-Halo substitution weakens the glycosidic bond of pyrimidines, rendering them sensitive to hydrolytic and thermolytic cleavage (Shapiro & Kang, 1969). The resulting apyrimidinic sites are in turn sensitive to alkaline lysis and perhaps to the AP-endonucleolytic activity of T4 endonuclease V. To study the effect of BrdUrd incorporation on the sensitivity of the DNA to alkali and to T4 endonuclease V, we utilized alkaline sucrose gradients to compare the sizes of parental and daughter bulk DNA under various conditions. The entire population of cells was examined in these experiments, without separating the replicated and unreplicated DNAs; we assume that most of the ^{3}H label was associated with daughter DNA, while the ^{14}C-labeled DNA included 10–20% unreplicated and 80–90% template DNA.

The results (Figure 5) show that the size of the daughter strands containing BrdUrd is reduced with respect to the parental strands (panel B) while both strands are of the same size in the absence of BrdUrd (panel A). This reduction in size indicates that an average of 4.3 interruptions (single-strand breaks or gaps) per 10^4 kb appeared in each BrdUrd-substituted daughter strand. These breaks probably originated during the alkaline lysis of the cells or the centrifugation in alkaline sucrose gradients.

When cells were grown with BrdUrd, the size distribution of the unsubstituted DNA strands showed an increase of

smaller-sized fragments, perhaps due to small alkali-labile BrdUrd-containing patches introduced during normal DNA turnover.

We tested the sensitivity of these various DNAs to incision by T4 endonuclease V. It can be seen that only the unsubstituted DNA from irradiated cells exhibits a reduction in size upon treatment with this CPD-specific enzyme (panel F). The size of this DNA, when compared to the initial inter-CPD distance (data not shown), reflects the removal of ~20% of the CPD from the genome overall.

Thus, we conclude that (a) the incorporation of BrdUrd does not create sites sensitive to T4 endonuclease V, although it produces breaks or alkali-sensitive sites, and (b) the daughter, BrdUrd-containing DNA strands show no ESS. This insensitivity to T4 endonuclease V was also observed in daughter DNA synthesized from irradiated templates in the absence of BrdUrd (data not shown).

To allow the examination of repair in the *DHFR* fragment in daughter DNA, several steps were taken to minimize the appearance of breaks in BrdUrd-containing sequences. First, we addressed the sensitivity of BrdUrd-substituted DNA to light of wavelengths shorter than 400 nm (Hutchinson, 1973) by shielding the cells and the DNA from white light sources or by working under yellow lights. Second, we treated the DNA with methoxyamine, a compound which binds to abasic sites, protecting them from nicking by alkali (Liuzzi & Tarpaert-Borlé, 1988). Third, we eliminated the alkaline and acid treatments of the agarose gels prior to Southern transfer (K. Sweder, unpublished experiments), and lastly, we utilized a phosphate-based buffer that maintained its neutral pH at different molarities, to dialyze and store the purified DNA.

These technical modifications allowed the detection of full-length *Kpn*I fragments of daughter DNA, but separate membranes had to be used to hybridize to each probe, since most of the genomic DNA was lost from the membranes when they were deprobed. We determined that the daughter, BrdUrd-containing DNA strands are insensitive to T4 endonuclease V, indicating that no ESS were present in the daughter strands within the fragments analyzed (Figure 4, rightmost lanes).

Initiation of replication requires the synthesis of oligoribonucleotide primers, which is carried out by a primase activity associated with DNA polymerase α in mammalian cells. This small amount of RNA synthesis, although quite different from transcription, might serve as a signal for selective repair. A 15-kb *Kpn*I fragment located downstream of *DHFR* in CHO cells has been shown by Ho et al. (1989) to exhibit very poor repair of CPD. This fragment contains an origin of replication, and it appears not to be transcribed (Foreman & Hamlin, 1989). We wanted to determine whether this fragment would show an enhancement of repair when it is replicated. To test this idea, we reprobed the membranes used to obtain the data shown in Figure 4 (panels A and B) with nick-translated pZH-26. The results show that replication of this region does not affect the very low level of repair of CPD observed in the unreplicated DNA (Figure 4, panel C; Table I).

DISCUSSION

We have demonstrated that the selective removal of CPD from the transcribed strand of the expressed *DHFR* gene in CHO cells is not affected by the replication of this region and that the poor repair of CPD in the nontranscribed strand or in a transcriptionally silent region that contains an origin is not enhanced by its use as a template for replication. Thus, there is no better or more homogeneous repair in the replicated fraction of the DNA than that in those DNA fragments that have not yet replicated, and, conversely, there is no significant bias in the replication of DNA that favors CPD-free templates.

The importance of the role of active transcription or of the activities associated with transcription in the preferential repair of CPD in active genes has been demonstrated in mammalian cells (Mellon et al., 1987), in bacteria (Mellon & Hanawalt, 1989), and in a yeast minichromosome (Smerdon & Thoma, 1990). These studies suggest a strong correlation between the ability of cells to remove CPD from active genes and their resistance to UV, and provide an explanation for the ability of many rodent cell lines to survive UV irradiation while removing few CPD from their genomes. Moreover, it was shown recently that a human mutant cell line, XP129, exhibits the CPD repair characteristics of rodent cells but resistance to UV irradiation similar to that of normal human cells (Lommel and Hanawalt, personal communication).

In order to complete a replication cycle, the entire genome must be used as template for the synthesis of daughter DNA strands. Will the replication machinery discriminate between active and silent sequences on the basis of their CPD content? Our results suggest that at low UV doses the DNA polymerases in B11 cells are able to synthesize DNA across CPD efficiently. Sweder and Hanawalt (personal communication) have demonstrated that CPD-containing DNA can be replicated in a UV-sensitive CHO mutant cell line, in the complete absence of repair. The trans-CPD synthesis model is also supported by the study of van Zeeland and Filon (1982) in which it was shown that the delay in DNA chain elongation in hamster and human cells irradiated with low doses of UV was only 15–30 min. Therefore, it appears that inhibition of elongation is not a major component of the profound inhibitory effect of UV on DNA synthesis. According to Painter (1985), the main effect of irradiation on replication is due to inhibition of replicon initiation. At UV doses higher than 10 J/m^2, we observe a significant reduction in the amount of replication in B11 cells. This could be due to damage in molecules other than DNA, to an increase in photoproducts that are more efficient at blocking replication than CPD, or to a saturation of the tolerance mechanisms that allow replication of damaged templates.

A predictable consequence of the persistence of CPD in the nontranscribed strand of an active gene is that the mutations found in the gene would be mostly due to lesions in that strand. Indeed, Vrieling et al. (1991) have determined than in UV-irradiated V79 hamster cells, most of the mutations can be attributed to CPD in the nontranscribed strand of the *HPRT* gene. Analysis of DNA repair in the same gene revealed a strong bias for CPD removal from the transcribed strand. A similar correlation between strand-specific repair and mutation frequency was found for the *HPRT* gene in human cells treated with benzo[*a*]pyrene diol epoxide (Chen et al., 1992).

Vrieling et al. (1989) found that in V-H1 cells, a repair-deficient, UV-sensitive mutant of V79 hamster cells, the transcribed strand of the *HPRT* gene, which is the lagging strand, contained most of the mutations induced by UV irradiation; this mutational bias is the reverse of that shown for wild-type cells mentioned in the previous paragraph. The authors hypothesize that their findings are due to differences in the fidelity of DNA replication of the leading and the lagging strand. The *DHFR* gene is replicated starting from an origin-containing region ~15 kb downstream of the gene (Ori I in Figure 2; Hamlin & Ma, 1990). This means that transcription and replication occur in opposite directions and that the leading strand is the nontranscribed strand while the lagging strand

is the transcribed strand. Replication of mammalian nuclear DNA requires the sequential action of several DNA polymerases: after primers have been synthesized on both strands by polymerase α, polymerase δ extends the leading strand and polymerase ξ synthesizes the lagging strand (Linn, 1991). It is possible that polymerases δ and ξ exhibit different tolerance levels for lesions or that the nature of their mode of action results in a differential treatment of lesions in the opposite strands. Studies of replication of SV40 in UV-irradiated monkey cells by Sarasin and Hanawalt (1980) indicated that replication was delayed by lesions but that it eventually resumed, without removal of the CPD from the parental DNA strands. These results suggested that the synthesis of the daughter strands was continuous, bypassing the lesions in the template strands. A model was proposed by these authors, on the basis of the model of Meneghini and Hanawalt (1976) in which synthesis on the leading strand is blocked by a lesion, while synthesis on the lagging strand continues at the next Okasaki fragment leaving a gap; a polymerase activity, perhaps damage-induced, then fills in the gaps opposite lesions on the lagging strand and continues synthesis, bypassing lesions on the leading strand.

Another mechanism by which gaps would be generated when cells replicate damaged DNA was suggested by Griffiths and Ling (1987). These authors have presented evidence for the activation of secondary origins of replication as a response to DNA-damaging agents, to allow the replication of regions between the sites at which DNA polymerases are stalled at lesions. This mechanism would also leave gaps opposite the lesions, which would be filled in later. The presence of gaps in DNA strands synthesized after UV irradiation has been inferred from the work of Lehmann (1974) and Meneghini (1976). In the model illustrated in Figure 6, based on Painter (1985), blocks of leading strands remain until lagging strands from adjacent origins approach and replicate past the lesions, leaving gaps; if two or more lesions block leading strands between origins, long-lived unreplicated regions (LLUR) are generated (Park & Cleaver, 1979) until silent origins are activated allowing replication to be complete. Alternative ways to resolve an LLUR between blocked forks include deletion of the affected sequence and strand exchange with homologous, undamaged sister chromatids (not shown). Any of the above-mentioned mechanisms (activation of silent origins, deletions, strand exchanges, gap filling) could be induced by the presence of DNA lesions.

Our experiments were not designed to detect gap formation during DNA synthesis with damaged templates. However, we did not detect differences between the average size of the daughter DNA strands from cells that had been irradiated or not irradiated; thus, if any gaps had occurred, they were not observed 24 h after UV irradiation.

We were not able to detect CPD in the daughter strands of the *DHFR* gene or the downstream fragment. Strand exchanges between parental oligonucleotides containing lesions and homologous daughter sequences have been proposed as a mechanism by which damaged DNA could be replicated in mammalian cells (Meneghini & Hanawalt, 1976). Clark and Hanawalt (1984) found evidence for ESS in daughter DNA strands of late-replicating, but not in early-replicating, simian virus 40 in infected monkey kidney cells irradiated with 100 J/m^2. Thus far, no evidence for strand exchange within the mammalian genome has been reported. We conclude that if transfer of CPD-containing sequences from parental to daughter strands occurs in B11 cells, it must be at a level below our ability to resolve it (i.e., <0.1 CPD per fragment).

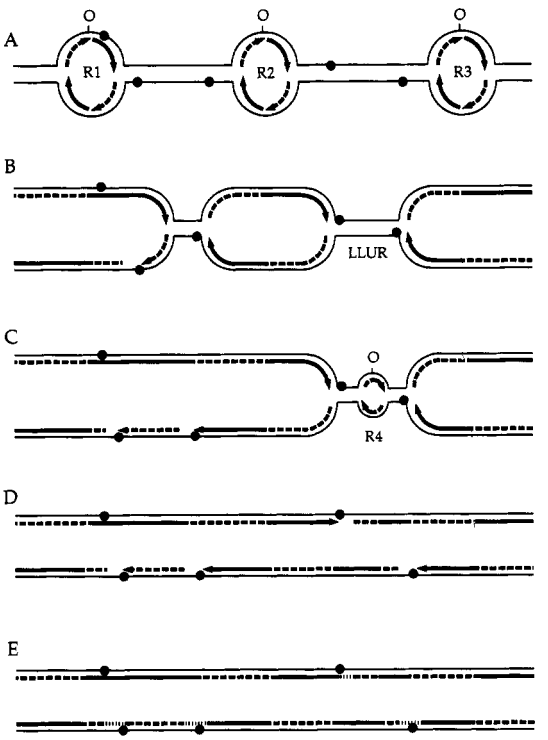

FIGURE 6: Model for replication of CPD-containing DNA. (A) The region depicted contains three replicons (R1, R2, and R3) that have just initiated replication from their respective origins (O); UV irradiation has produced randomly located CPD, represented by dots. Thin lines, parental strands; thick solid lines, leading strands; thick dashed lines, lagging strands. (B) Replication continues, but unwinding is blocked when the leading strands reach a CPD in R2 and R3; this situation creates a long-lived unreplicated region (LLUR) between these two replicons. Synthesis of the lagging strand reinitiates past the CPD in R1, leaving a gap opposite the CPD. (C) The progression of replication from R1 continues until R1 and R2 are joined, leaving a gap opposite the CPD at the joining point. A new replicon (R4) is activated within the LLUR. (D) Synthesis initiated in R4 allows the LLUR to be replicated so that all four replicons are joined, still leaving gaps opposite CPD. (E) The gaps opposite CPD are eventually filled. Replication has been completed, generating continuous daughter strands copied from lesion-containing parental strands. Note: The order of the events described may not reflect their sequence in vivo.

Our current hypothesis is that there are at least two modes for repair of CPD. One of them occurs on the transcribed strand of expressed genes during active transcription, is fast and efficient, and is essential for survival. A second mode operates on the genome overall, is slower and/or less efficient than the first one, and is not essential for survival. In all likelihood, these two mechanisms share the main components of the repair machinery; the first one requires some additional factor that couples it to transcription. Selby and Sancar (1991) have reported such a factor that is required for transcription-dependent repair of CPD in *E. coli*.

There may be regions of the genome whose conformation allows the same incidence of CPD as that for the genome overall but that are more difficult to repair. We find that an origin region is poorly repaired in B11 cells. Smerdon and Thoma (1990) showed that an ARS1 region of a yeast minichromosome was poorly repaired, compared to sequences that were transcribed; the latter sequences exhibited strand-specific repair that correlated with their transcriptional activity. Origins of replication are thought to be localized in genomic regions with complex secondary structures that may hinder the access of repair enzymes. A detailed analysis of repair of CPD in the origin region of the *DHFR* amplicon in B11 cells is under way.

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