Increased UV Resistance of a Xeroderma Pigmentosum Revertant Cell Line Is Correlated with Selective Repair of the Transcribed Strand of an Expressed Gene

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Received 27 July 1992/Returned for modification 14 October 1992/Accepted 17 November 1992

A UV-resistant revertant (XP129) of a xeroderma pigmentosum group A cell line has been reported to be totally deficient in repair of cyclobutane pyrimidine dimers (CPDs) but proficient in repair of 6-4 photoproducts. This finding has been interpreted to mean that CPDs play no role in cell killing by UV. We have analyzed the fine structure of repair of CPDs in the dihydrofolate reductase gene in the revertant. In this essential, active gene, we observe that repair of the transcribed strand is as efficient as that in normal, repair-proficient human cells, but repair of the nontranscribed strand is not. Within 4 h after UV at 7.5 J/m², over 50% of the CPDs were removed, and by 8 h, 80% of the CPDs were removed. In contrast, there was essentially no removal from the nontranscribed strand even by 24 h. Our results demonstrate that overall repair measurements can be misleading, and they support the hypothesis that removal of CPDs from the transcribed strands of expressed genes is essential for UV resistance.

DNA repair is a complex process essential for normal cellular growth and viability. This is well demonstrated by the human autosomal recessive disease xeroderma pigmentosum (XP). Afflicted individuals are sun sensitive and suffer from an elevated incidence of cancer, particularly skin cancers on sunlight-exposed regions of the body. Cultured cells from XP individuals are substantially more sensitive to the lethal and mutagenic effects of UV irradiation than are cells from unaffected individuals. (For brevity, we will refer to cultured repair-proficient human cells derived from unaffected individuals as normal human cells.) The molecular defect responsible for the classical XP phenotype is in an early step of the nucleotide excision repair process, the major mechanism by which UV photoproducts are removed from DNA (for a review of XP, see reference 8). The complexity of excision repair in human cells is indicated by the existence of seven complementation groups of XP (3, 30).

The two predominant DNA photoproducts formed by irradiation with 254-nm UV light are the cyclobutane pyrimidine dimer (CPD) and the pyrimidine (6-4) pyrimidone photoproduct (6-4 PP). It is estimated that the induction of CPDs is threefold higher than that of 6-4 PPs (19). Cells in the classical XP complementation groups and the majority of cellular mutants that are defective in repair of UV-induced DNA damage are defective in repair of both lesions. However, a few rodent and human cell mutants that have altered repair capacities for only one of these photoproducts have been identified (6, 13, 20, 27). These mutants should be useful for evaluating the respective biological roles of the photoproducts and may also provide insight into the mechanism of excision repair in mammalian cells.

The initial attempts to use these mutants to assess the biological roles of the CPD and the 6-4 PP compared repair in the overall genome with biological end points such as lethality and mutagenesis. These studies resulted in conflicting conclusions. For example, the Chinese hamster ovary

(CHO) cell mutant UV61 efficiently removes 6-4 PPs but not CPDs when repair is assayed as an average over the entire genome (27). The UV resistance of UV61 cells is intermediate between that of the parental CHO cells and that of mutants that are deficient in repair of both photoproducts (21, 27). These results suggest that repair of CPDs is essential for full UV resistance and that 6-4 PP removal may also be important. However, a different conclusion might be reached on the basis of the phenotype of another hamster cell mutant, VH-1. These cells are devoid of CPD repair but carry out 6-4 PP repair at a reduced rate (20), yet they are as UV sensitive as mutants that are deficient in repair of both photoproducts (35). These results suggest that repair of CPDs is essential for resistance to UV but that repair of 6-4 PPs may not be. A third conclusion has been drawn on the basis of the overall repair characteristics of a human cell line derived from XP cells. The revertant, designated XP129, was created by mutagenizing XP12RO, a simian virus 40 (SV40)-immortalized cell line belonging to XP complementation group A (XP-A) and selecting for increased UV resistance. XP129 cells are as resistant to UV as are normal human cells. However, while the repair of 6-4 PPs has returned to normal, the repair of CPDs in the genome overall appears to remain deficient (6). On the basis of these results, Cleaver et al. (5) concluded that 6-4 PPs make a major contribution to the lethal effects of UV irradiation and that CPDs may not be relevant to most of the biological effects of UV.

The studies described above measured repair as an average over the entire genome. However, the mammalian genome consists of diverse functional classes of DNA and is organized into a complex chromatin structure. An accurate assessment of the biological role of a particular DNA lesion must consider possible differences in the effects of persisting lesions in different classes of DNA in the genome. Preferential repair of CPDs in transcriptionally active DNA has been demonstrated in *Escherichia coli*, *Saccharomyces cerevisiae*, and rodent and human cells in culture (2, 16, 17, 23, 26). These and other studies suggest that selective removal of CPDs from expressed genes may be crucial for cellular

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resistance to UV (for a review, see reference 24). Cultured rodent cells exhibit poor removal of CPDs from the genome overall, yet their UV resistance is similar to that of normal human cells. Bohr et al. (2) demonstrated that CHO cells efficiently remove CPDs from the active, essential dihydrofolate reductase (DHFR) gene. They suggested that all essential DNA sequences might be preferentially repaired in rodent cells and that UV resistance might depend upon repair of these essential sequences rather than upon repair of the genome overall. In a recent study, we demonstrated a correlation between the level of CPD repair in the transcribed strand of an active gene and the degree of UV sensitivity of CHO cell mutants (14). We found a moderate level of selective repair of the transcribed strand of the DHFR gene in UV61 cells, which have intermediate UV sensitivity and repair 6-4 PPs but not CPDs in the genome overall. We detected no repair of the gene in the UV-sensitive UV5 cells. The correlation is strengthened by the results of Zdzienicka et al. (36). They detected no removal of CPDs from the hypoxanthine phosphoribosyl-transferase gene of the UV-sensitive hamster cell mutant VH-1, which has no overall repair of CPDs but moderate repair of 6-4 PPs.

The results of these studies of repair in genes in hamster cell mutants that have altered repair capacities for only one of the two principal photoproducts support the hypothesis that removal of CPDs from transcriptionally active DNA is crucial for cellular resistance to UV. However, the most dramatic example of a cell line with differential repair of these two photoproducts is the XP-A revertant XP129. This cell line seemed ideal for a definitive study of the contribution of selective repair of CPDs in active genes to UV resistance. The UV resistance of XP129 cells is indistinguishable from that of normal human cells and is substantially higher than that of the extremely sensitive parental cell line, yet there is little repair of CPDs in the genome overall in XP129 cells (6). The critical question is whether the UV resistance is due solely to the repair of 6-4 PPs or whether the preferential repair of CPDs in active genes is also essential. In this report, we describe our in-depth characterization of CPD repair in XP129 cells. We examined repair in the genome as a whole and carried out a detailed analysis of repair in the DHFR gene. We assayed repair in three different restriction fragments within the gene, analyzed repair of the transcribed and the nontranscribed DNA strands, and determined how the UV fluence affects the efficiency of removal of CPDs from the respective strands. We compared repair in the DHFR gene in the XP129 cell line with that in the parental XP12RO cell line and with that in a normal human cell line.

MATERIALS AND METHODS

Cell lines and culture conditions. The SV40-immortalized human cell line GM00637E (GM637) was obtained from the Human Genetic Mutant Cell Repository. The SV40-immortalized XP-A cell line XP12RO and its revertant XP129 were generously provided by James Cleaver (University of California, San Francisco). Cells were grown as monolayers in minimum essential medium (GIBCO) supplemented with 10% fetal bovine serum, 2 mM glutamine, penicillin, and streptomycin in humidified 5% CO₂-95% air at 37°C. All cell lines tested free of mycoplasma, using in-solution hybridization with a probe homologous to mycoplasma rRNA (Gene-Probe, San Diego, Calif.), both before the study and after 2

months of continuous culturing in medium without antibiotics. Actively growing cells were used in all experiments.

UV irradiation and repair analysis in total cellular DNA. The analysis of repair in total cellular DNA was carried out essentially as described by van Zeeland et al. (28). Briefly, cells were labeled with [³H]thymidine and then irradiated at an incident dose rate of 0.33 J/m²/s with a Westinghouse IL782-30 germicidal lamp (predominantly 254 nm). The cells were either processed immediately after irradiation or incubated in growth medium for 24 h at 37°C. The cells were then trypsinized, resuspended, permeabilized, and treated with 2 M NaCl to render all of the CPDs accessible to T4 endonuclease V. After incubation with T4 endonuclease V, the cells were lysed directly on top of alkaline sucrose gradients and centrifuged in a Beckman SW50.1 rotor for 100 min at 45,000 rpm (T4 endonuclease V treated) or for 110 min at 35,000 rpm (mock treated). The frequency of CPDs in the DNA was determined from the number-average molecular weights, calculated from the radioactivity profiles of the gradients.

UV irradiation and repair analysis in the DHFR gene and in each strand of the gene. The UV irradiation of the cells and the analysis of repair in specific restriction fragments were carried out essentially as described previously (1, 14). Briefly, radioactively labeled cells were irradiated as described above, either lysed immediately after irradiation or incubated for the specified repair times in growth medium containing bromodeoxyuridine and fluorodeoxyuridine to density label the newly replicated DNA, and then lysed. The lysates were treated with proteinase K, and the DNA was further purified by phenol-chloroform extractions and treatment with RNase. The DNA was digested to completion with *Hind*III, *Kpn*I, or *Pst*I and centrifuged in CsCl gradients to separate the unreplicated DNA from the replicated DNA. The gradients were fractionated and assayed for radioactivity. The fractions containing the unreplicated DNA were pooled and dialyzed to remove the CsCl. Equal amounts of DNA were either treated or mock treated with the enzyme T4 endonuclease V, which produces a single-strand break specifically at each CPD (11, 28). The DNA was denatured, electrophoresed through 0.5% alkaline agarose gels to separate full-length restriction fragments from the digestion products of T4 endonuclease V, transferred to Hybond N⁺ membranes (Amersham), and hybridized with either ³²P-labeled DNA probes or strand-specific ³²P-labeled RNA probes. After a stringent wash, the membranes were exposed to X-ray film with an intensifying screen, and the resulting autoradiograms were scanned with a Quick Scan R and D densitometer (Helena Laboratories). The Poisson expression was applied to calculate the number of CPDs per fragment from the ratio of intensities of bands from enzyme-treated and mock-treated DNA samples.

Probes for specific DNA sequences in the DHFR region. Plasmid pBH31R1.8 is a 1.8-kbp *Eco*RI human genomic fragment cloned into pBR325 (34). pZH6 is a 935 bp *Eco*RI-*Pst*I fragment from the 5' end of pBH31R1.8 subcloned into pGEM3Z (17). pZH15 is a 788-bp *Pst*I-*Sst*I fragment from the 3' end of the pBH31R1.8 fragment subcloned into pSP72 (Promega). pGEM0.69EH is a 690-bp *Eco*RI-*Hind*III fragment from intron V of the human DHFR gene cloned into pGEM2 (33). ³²P-labeled DNA probes were produced by nick translation of the plasmid; strand-specific RNA probes were generated by in vitro transcription of linearized plasmid in the presence of [³²P]CTP, using the procedure recommended by Promega (Madison, Wis.).

FIG. 1. Map of the human DHFR gene locus. Exons are represented as gray boxes. The direction of transcription of the DHFR gene is indicated as a solid arrow, and that of DUT is indicated as a dashed arrow. The locations of the *Hind*III, *Kpn*I, and *Pst*I restriction fragments used in the repair analysis are shown as black bands. The positions of the human genomic inserts in plasmids pBH31R1.8, pZH6, pZH15, and pGEM0.69EH used to generate probes are indicated.

RESULTS

The removal of CPDs from the DHFR gene and from each strand of the gene was determined in three SV40-immortalized human cell lines: the normal GM637 line, the XP-A line XP12RO, and its UV resistant revertant XP129. A map of the DHFR region is shown in Fig. 1. Repair of CPDs in XP129 cells was examined in greater detail, including in the genome as a whole, in three different restriction fragments within the gene, and in the transcribed and nontranscribed strands of the DHFR gene after UV at 7.5 and 20 J/m².

Repair in the overall genome in the revertant XP129. XP129 cells were previously characterized as being defective in overall removal of CPDs. Cleaver et al. (6) found that only 6% of the CPDs were removed from the genome overall in 24 h after UV at 13 J/m². To ensure that the cells that we obtained had retained this characteristic, we examined repair in the overall genome. Radioactively labeled cells were UV irradiated with 7.5 J/m² and either processed immediately or reincubated for 24 h. The cells were then permeabilized, treated or mock treated with the CPD-specific enzyme T4 endonuclease V, and subjected to lysis on top of alkaline sucrose gradients and centrifugation (Fig. 2). The radioactivity profiles show only a slight increase in the average size of the T4 endonuclease V-treated DNA from cells that were allowed to repair for 24 h after UV, indicating that few CPDs were removed in that time. We calculate that less than 20% of the CPDs were removed from the genome overall in 24 h after UV at 7.5 J/m².

Repair in the *Hind*III restriction fragment of the DHFR gene in three human cell lines. To assay removal of CPDs from the DHFR gene, we used a method (first described by Bohr et al. [2]) to quantitate repair in specific restriction fragments. Purified DNA from UV-irradiated cells was restricted with *Hind*III and divided into two equal samples, one of which was treated with T4 endonuclease V. The DNA was electrophoresed in a denaturing alkaline agarose gel to separate the full-length restriction fragments from the digestion products of T4 endonuclease V, transferred to a nylon membrane, and hybridized with ³²P-labeled pBH31R1.8. The frequency of CPDs in the restriction fragment was calculated from the band intensities by using the Poisson

FIG. 2. Radioactivity profiles of total cellular DNA from XP129 cells UV irradiated with 7.5 J/m². (A) Mock-treated cells lysed immediately (○) and 24 h (△) after UV irradiation; (B) T4 endonuclease V-treated cells lysed immediately (□) and 24 h (▲) after UV irradiation. The position of the ¹⁴C-labeled phage lambda DNA marker (48.5 kb) is indicated by the arrow. Sedimentation is to the left.

expression. Although UV irradiation inhibits DNA replication in human cells, in 24 h after UV at 7.5 J/m², we observed some newly replicated DNA (data not shown). The newly synthesized strand is free of CPDs and if included in the repair analysis would result in an artificially elevated estimate of the amount of repair. Therefore, the procedure that we used includes steps to remove newly replicated DNA from the analysis.

We measured repair in a 22-kb *Hind*III restriction fragment located primarily within the DHFR gene (Fig. 1). The results are shown in Fig. 3. The presence of CPDs immediately after irradiation is indicated by the relatively light band intensities in the lanes from the T4 endonuclease V-treated DNA. Repair is observed as a reappearance of the band relative to the band in the lane from the mock-treated DNA. We found that 67% of the CPDs were removed in 24 h after UV at 7.5 J/m² in GM637 cells (Fig. 3A). In contrast, we detected no removal of CPDs in the parental XP12RO cells (Fig. 3B). However, we did detect 57% repair in XP129 cells (Fig. 3). Our results demonstrate efficient repair of CPDs in the DHFR gene in the UV-resistant revertant XP129, but the extent of repair detected was less than that in the normal GM637 cells.

Repair in the transcribed and nontranscribed strands of the *Hind*III restriction fragment of the DHFR gene in three human cell lines. Although CPDs are removed from both strands of the DHFR gene in normal human cells, it has been

FIG. 3. Autoradiograms illustrating removal of CPDs from the *Hind*III fragment of the DHFR gene in 24 h after UV. The lanes containing DNA that was mock treated (–) or T4 endonuclease V treated (+) are indicated. All lanes contain DNA from cells that were irradiated with 7.5 J/m² except those that are designated as unirradiated (unirrad). (A) Normal human cells (GM637) and XP-A revertant cells (XP129); (B) XP-A cells (XP12RO) and XP-A revertant cells (XP129). The lanes containing DNA from unirradiated cells demonstrate that there is essentially no nonspecific nicking activity in the T4 endonuclease V preparation.

shown that the rate of repair of the transcribed strand is more rapid than that of the nontranscribed strand. In CHO cells and in XP complementation group C cells, there is rapid, efficient repair of the transcribed strand but essentially no repair of the nontranscribed strand (17, 29). Therefore, we assayed repair in each strand of the DHFR gene. The membranes that we used to generate the autoradiograms depicted in Fig. 3 were deprobed and rehybridized with strand-specific probes derived from pZH6 (Fig. 1). We first hybridized with a probe that detects the transcribed strand and then, after deprobing, hybridized with a probe for the nontranscribed strand (Table 1). In 24 h after 7.5 J/m², we observed very little repair in either strand in the parental XP12RO cells, as expected from the results shown in Fig. 3. In GM637 cells, there was essentially complete repair (97%) in the transcribed strand and 55% repair in the nontranscribed strand. In XP129 cells, 71% of the CPDs were removed from the transcribed strand and 34% were removed from the nontranscribed strand.

These results suggest that the revertant XP129 repairs each strand of the DHFR gene but that the level of repair in

TABLE 1. Removal of CPDs from the *Hind*III fragment of the DHFR gene^a

Strand probed	% CPD removal		
	XP129	XP12RO	GM637
Both	58	0	67
Transcribed	71	6	97
Nontranscribed	34	1	55

^a Repair was assayed 24 h after UV at 7.5 J/m². XP129 is an XP-A revertant, XP12RO is the parental XP-A line, and GM637 is a repair-proficient human cell line.

FIG. 4. Removal of CPDs from each strand of the *Kpn*I fragment of the DHFR gene after UV at 7.5 J/m² in XP129 cells. Symbols: ●, transcribed strand; ○, nontranscribed strand. Each point represents the average of at least two determinations from two separate experiments.

each strand is reduced from that of normal human cells. It is important to note, however, that the *Hind*III fragment that we analyzed includes a segment outside the gene (Fig. 1). Two-thirds of the fragment is within the gene, but one-third of the fragment extends into the 5' flanking region. This region contains a transcription unit (divergent upstream transcript [DUT]) whose transcript originates in the promoter region of the DHFR gene but is transcribed in the other direction (10). Thus, the transcribed strand of the DHFR gene is the nontranscribed strand of DUT and vice versa. Mellon et al. (17) showed that selective repair changes strands in that region; i.e., in each gene, it is the transcribed strand that is preferentially repaired. Thus, the repair that we observed in the nontranscribed strand of the DHFR gene might actually represent repair of the transcribed strand of DUT.

Repair in the transcribed and nontranscribed strands of the *Kpn*I fragment of the DHFR gene in the revertant XP129. To eliminate the difficulty in interpreting strand-specific repair results in the *Hind*III fragment, we also analyzed repair in XP129 cells in a fragment that is located entirely inside the DHFR gene. Membranes were sequentially probed, with an intervening deprobing step, for each of the strands of the 20-kb *Kpn*I fragment, using probes derived from pGEM0.69EH (Fig. 1). Repair of the transcribed strand was very rapid (Fig. 4). Within 4 h after UV at 7.5 J/m², over half of the CPDs were removed, and by 8 h, repair was nearly complete. In stark contrast, there was essentially no repair of the nontranscribed strand even 24 h after irradiation. From these results, it clear that repair of the transcribed strand of the DHFR gene in XP129 cells resembles the rapid and complete repair of the transcribed strand seen in normal human cells (17, 29). Unlike normal human cells, however, which also repair the nontranscribed strand, albeit more slowly than the transcribed strand, XP129 cells carry out essentially no repair of the nontranscribed strand. The strong strand bias of repair that we observed in the *Kpn*I fragment provides evidence that the repair that we observed in each strand of the *Hind*III fragment probably occurred exclusively in the region of each strand that is transcribed and that the region of each strand that is not transcribed was probably poorly repaired.

Effect of UV fluence on the removal of CPDs from each strand of the DHFR gene in the revertant XP129. We also

FIG. 5. Removal of CPDs from each strand of the *Pst*I fragment of the DHFR gene after UV at 20 J/m² in XP129 cells. Symbols: ■, transcribed strand; □, nontranscribed strand. Each point represents the average of three determinations from two separate experiments.

measured the rates of repair in each strand of the DHFR gene after UV at 20 J/m². Irradiation with this higher fluence makes it difficult to determine accurately the initial CPD frequency in a 20-kb fragment because the fraction of fragments free of CPDs is extremely low. Therefore, we assayed repair in the 8.3-kb *Pst*I fragment by using strand-specific probes derived from pZH15 (Fig. 1). As we saw with the lower fluence, there was essentially no repair of CPDs in the nontranscribed strand (Fig. 5). In the transcribed strand, about 20% of the CPDs were removed in 4 h after irradiation, and within 24 h, more than half of the CPDs had been removed. These results demonstrate that there is significant repair of the transcribed strand in XP129 cells even after UV at 20 J/m².

DISCUSSION

The biological role of CPDs must be evaluated in the context of preferential repair of transcriptionally active DNA. We detected no removal of CPDs from either strand of the DHFR gene in the UV-sensitive XP-A cell line XP12RO. We observed that all of the CPDs were removed from the transcribed strand and that over 50% were removed from the nontranscribed strand within 24 h in the UV-resistant human cell line GM637. When we examined repair in a fragment entirely within the gene, we detected essentially no removal of CPDs from the nontranscribed strand in the revertant XP129, but we observed that removal from the transcribed strand was essentially the same as the rapid and complete repair observed for the transcribed strand of normal human cells. The earlier characterization of XP129 cells indicated that they are unable to remove CPDs but that they have regained the ability to repair 6-4 PPs (6). Because the cells are as UV resistant as normal human cells, this led the authors to conclude that cell killing by UV light is due to 6-4 PPs and not CPDs (5). However, in our fine-structure analysis, we observed efficient repair of CPDs in the transcribed strand of the DHFR gene. We demonstrate a correlation between the proficiency of CPD removal from the transcribed strand of an active gene and the degree of UV sensitivity of three human cell lines which differ markedly from each other in their overall genomic repair of the two principal UV-induced photoproducts. Thus, in contrast to the conclusion reached after the initial characterization of the XP-A revertant (5, 6), we conclude from our results that

repair of CPDs is probably essential. The cellular lethality of UV irradiation appears to be due at least in part to the presence of CPDs in the transcribed strands of active, essential genes. 6-4 PPs presumably also contribute, but it is easy to overestimate the importance of 6-4 PPs when repair of CPDs in transcriptionally active DNA is not considered.

An expected consequence of the altered DNA repair capacity in the XP129 revertant is a corresponding change for mutation induction. Preliminary analysis of mutations to 6-thioguanine resistance in genomic DNA suggests that the mutation induction by UV in XP129 cells is substantially higher than that in XP12RO cells but only slightly higher than that in GM637 cells (6). Our findings, in XP129 cells, of significant repair of CPDs in the transcribed strand and poor repair in the nontranscribed strand are consistent with these data. Recent strand-specific analysis of mutations in the hypoxanthine phosphoribosyltransferase gene in repair-deficient hamster cells and in XP-A cells suggests that, at least in some sequences, the mutational load due to lesions in the nontranscribed strand is dramatically less than expected (9, 15, 18, 31). Whatever the origin of this unexpected bias, it could explain why the lack of CPD repair in the nontranscribed strand in XP129 cells does not greatly increase the frequency of 6-thioguanine resistance. In contrast to the results for repair-deficient mutants, strand-specific mutational analysis in repair-proficient hamster and human cells reveals a strong bias for mutations resulting from lesions in the nontranscribed strand (4, 15, 18, 32). These results suggest that preferential repair of the transcribed strand exerts a substantial influence on UV-induced mutagenesis.

The molecular nature of the XP mutants. The complete lack of repair of both CPDs and 6-4 PPs in the parental XP-A cell line XP12RO results from a nonsense mutation in the fifth of six exons in the XP-A complementing (XPAC) gene (22). XP12RO cells are homozygous for a C→T transition at nucleotide 619 which changes the Arg-207 codon (CGA) to a nonsense codon (TGA). The lack of repair in XP12RO cells could result simply from the production of a nonfunctional truncated protein lacking 67 C-terminal amino acids. However, the mutation also results in a significant reduction in the level of XPAC mRNA, probably due to mRNA instability (22). Indeed, no XPAC protein was detected in partially purified XP12RO cell extracts immunoblotted with an antibody raised against the deduced polypeptide sequence of the XPAC protein (12). Therefore, it is quite likely that the lack of repair in XP12RO cells is the consequence of an absence of the XPAC protein.

The XP129 revertant remains deficient in repair of CPDs in the genome overall but has acquired the ability to repair 6-4 PPs in the genome overall and CPDs in transcriptionally active DNA. The reversion may result from a secondary mutation in the XPAC gene. Preliminary sequence information (12) indicates that in XP129 cells there is a T→G transversion at nucleotide 619, the location of the premature stop codon in the parental XP12RO cells. This transversion would result in a full-length XPAC protein in which the arginine present at codon 207 in the normal human XPAC protein is substituted by glycine in the XP129 XPAC protein. This amino acid substitution might alter the structure of the XPAC protein in a way that decreases either its affinity for or ability to function on CPDs in the bulk of the genome. The altered XPAC protein may maintain the ability to function on CPDs associated with blocked RNA transcripts and 6-4 PPs because these lesions may cause a greater distortion of the DNA structure than do CPDs in the bulk of the genome or

because the affinity of the XPAC protein is greater for these lesions.

Alternatively, the primary effect of the amino acid substitution in the XP129 XPAC protein may be in the level of the protein available for repair. A reduced level of the XPAC protein would result if the mutation in XP129 cells decreases the stability of the protein or mRNA relative to that in normal human cells. A reduced level of the protein could also result if only one allele of the XP129 XPAC gene has the secondary mutation while the other allele maintains the original XP-A mutation. When partially purified extracts of XP129 cells were immunoblotted for the XPAC protein, the level detected was about 30% of that seen in normal cells (12). If CPDs associated with blocked transcripts and 6-4 PPs cause more distortion of the DNA than do CPDs in the bulk of the genome or if the XPAC protein has a higher affinity for or greater ability to function on these lesions, then they may be preferentially repaired when the level of XPAC protein is limited.

Is the revertant XP129 the human analog of Chinese hamster cells? At first glance, the ability of XP129 cells to repair 6-4 PPs and CPDs in the transcribed strand very efficiently while remaining deficient in repair of the majority of CPDs might seem unique, but this is precisely the UV repair phenotype of cultured Chinese hamster cells. Just like the XP129 revertant, wild-type Chinese hamster cells are as UV resistant as normal human cells, and they repair 6-4 PPs efficiently but are relatively deficient in repair of CPDs in the genome overall (19, 21). They perform rapid, efficient repair of CPDs in the transcribed strand of active genes but essentially no repair of CPDs in the nontranscribed strand (17, 32). These similarities between XP129 cells and Chinese hamster cells suggest that the rodent XPAC protein may be more like the XPAC protein of the XP129 revertant than like the normal human protein. Although the sequence of the Chinese hamster XPAC gene is not available, a comparison of the published sequences for the mouse and normal human XPAC genes shows that at the site of the mutation in XP12RO cells and the site of the secondary mutation in XP129 cells, the mouse sequence codes for arginine just as the normal human XPAC sequence does (25). This finding does not rule out the possibility that the rodent XPAC protein is more like that of the revertant than like that of normal human cells. But if so, it is a structural or functional similarity that results from something more complex than the rodent and the revertant XPAC proteins simply containing the same amino acid at the site of the XP12RO mutation.

The level of the XPAC protein in XP129 cells appears to be reduced relative to that in normal human cells (12). Perhaps the level of the XPAC protein in Chinese hamster cells is also low. It may be that in rodent cells, just as we suggested for the XP129 revertant, a limited amount of XPAC protein results in preferential repair of certain lesions, either because the XPAC protein has a higher affinity for those lesions or because those lesions cause more distortion to the DNA, as may be the case with CPDs associated with blocked transcripts and 6-4 PPs. Perhaps CPDs not associated with transcription are repaired only when the level of XPAC protein is not limited, such as in normal human cells.

Effect of UV fluence on the removal of CPDs. When repair of the transcribed strand of the DHFR gene in the revertant XP129 is expressed as the percentage of CPDs removed per fragment, repair is approximately 80% complete within 8 h after 7.5 J/m² and approximately 20% complete within 8 h after 20 J/m². However, when repair is expressed as the actual number of CPDs removed per kilobase of DNA, there

is no significant difference between the two fluences up to 8 h after UV. The number of CPDs removed per kilobase is roughly linear with time up to 8 h after 7.5 J/m², after which repair is essentially complete, and up to at least 24 h after 20 J/m². Therefore, although the percentage of CPDs removed is less after the higher fluence, the actual number of CPDs removed per kilobase of DNA is basically the same after either fluence.

In a recent study, Cleaver et al. (7) detected very little removal of CPDs from the DHFR gene after UV at 26 J/m² in XP129 cells. They examined the same 22-kb *Hind*III restriction fragment in which we observed substantial repair after a lower fluence of UV. In addition, we detected efficient repair after 20 J/m² in the transcribed strand of the *Pst*I fragment that lies entirely inside the *Hind*III fragment. The repair that we observed may not be easily detected under the conditions used by Cleaver et al. First, since there is no removal of CPDs from the nontranscribed strand, the amount of repair detected in the fragment will be roughly half that which would be detected with use of a transcribed strand-specific probe. Second, when the initial frequency of CPDs per fragment is too high, it is difficult to detect low to moderate levels of repair. This is why we matched our fragment sizes and UV fluences to keep our initial frequency of CPDs between one and two per fragment. Cleaver et al. examined a 22-kb fragment after 26 J/m²; using Poisson analysis, we estimate that this should have produced approximately four CPDs per fragment. This is a zero class of less than 0.02 so that even if 25% of the CPDs were to be removed from the fragment (this would be 50% from the transcribed strand), the zero class would rise to only 0.05. When the zero classes are this low, it is very difficult to quantitate repair accurately because the bands in the lanes of the T4 endonuclease V-treated DNA are difficult to detect relative to the bands in the lanes of the mock-treated DNA. With more complete repair (as would be the case in normal human cells which repair both strands), the bands become easy to detect. For example, if 60% of the CPDs are removed from the fragment, the zero class will rise to 0.20. In XP129 cells, which repair only the transcribed strand, it is possible that there is still significant repair after 26 J/m² which is not easily detected in large fragments. Alternatively, the repair might decline dramatically after UV at 20 J/m².

ACKNOWLEDGMENTS

We thank J. Cleaver for providing the XP129 and XP12RO cells. We thank G. Attardi for providing plasmid pBH31R1.8, I. Mellon and G. Spivak for pZH6, G. Spivak and C. Kane for pZH15, and B. Dolnick for pGEM0.69EH. We are very grateful to C. A. Smith and A. K. Ganesan for many helpful discussions and critical reading of the manuscript.

This work was supported by Outstanding Investigator Award CA44349. L.L. was supported as a predoctoral trainee by Public Health Service grant CA09302.

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