Repair of Individual DNA Strands in the Hamster Dihydrofolate Reductase Gene after Treatment with Ultraviolet Light, Alkylating Agents, and Cisplatin*

(Received for publication, July 16, 1992)

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We have analyzed gene-specific and strand-specific DNA damage and repair in the dihydrofolate reductase gene in hamster cells. Cells were UV-irradiated or treated with two types of chemotherapeutics, alkylating agents or cisplatin. UV-induced pyrimidine dimers were detected using a previously published technique in which the T4 endonuclease V enzyme is used to create nicks at the lesion sites. 6-4 photoproducts were detected in a similar assay using ABC excinuclease after prior reversal of the pyrimidine dimers with photolyase. Adducts formed by the alkylating agents nitrogen mustard and dimethyl sulfate were quantitated by generating strand breaks at abasic sites after neutral depurination. Cisplatin-induced intrastrand adducts were detected with ABC excinuclease, and cisplatin interstrand cross-links were detected using a denaturation-reannealing reaction before electrophoresis. In accord with previous reports by other investigators, we find distinct strand specificity of the repair of pyrimidine dimers after UV; the transcribed strand was much more efficiently repaired than the nontranscribed strand. In contrast, there was little or no strand bias in the repair of the 6-4 photoproducts. For alkylating agents, a slight bias toward repair in the transcribed strand was found after treatment with nitrogen mustard, but there appeared to be no bias in the repair after treatment with dimethyl sulfate. Cisplatin interstrand cross-links are repaired with equal efficiency from the two strands, but the more common cisplatin-induced lesion, the intrastrand adduct, is preferentially repaired from the transcribed strand. In conclusion, there is strand bias in the repair of pyrimidine dimers and cisplatin intrastrand adducts, but the strand specificity of repair may not be a general feature for all DNA lesions, as we found little or no strand bias in the repair of other lesions studied.

Recently, there has been accumulating interest in the heterogeneity of DNA repair processes in mammalian cells. Over the past few years, methods have been available to measure gene-specific repair, (1), and it has now been reported in several studies that transcriptionally active genes are preferentially repaired in mammalian cells (2). The results support the general conclusion that there is more efficient repair of pyrimidine dimers (PD) in transcriptionally active genes than in inactive genomic regions or in the genome overall. It was initially discovered that the active, essential DHFR gene was efficiently repaired in hamster cells (1), where there is little repair in the overall genome. This phenomenon has been termed preferential DNA repair and has been found in a number of active genes in mammalian cells, in lower eukaryotes, and in bacteria (2–4). Using the same general technique, but probing the membranes with single-stranded RNA probes (riboprobes), it was shown that the preferential DNA repair in the mammalian DHFR gene of UV-induced PD was largely due to repair in the transcribed strand, i.e. there was little or no repair in the nontranscribed strand (5). Other investigators have since found strand specificity of repair of UV-induced pyrimidine dimers in other genes in yeast (6) and human cells (7). Since strand selectivity of repair of pyrimidine dimers has also been reported in Escherichia coli (8), this process might be a general feature of the fine structure of DNA repair of pyrimidine dimers.

It has been observed for several mammalian genes that there is a strand bias for mutation fixation that may be due to a strand selectivity of DNA repair. For example, in the human HPRT gene, strand bias for mutation has been shown to be due to strand selective repair resulting from the persistence of unrepaired premutagenic lesions in the nontranscribed DNA strand (9–11). In the hamster DHFR gene, mutations induced by (±)-3,4-dihydroxy-1,2-epoxy-1,2,3,4-tetrahydrobenzo[c]phenanthrene are also preferentially located in the nontranscribed strand (12). There is a reasonable correlation between the strand bias of the DNA repair and the strand bias of the mutations as examined collectively in the HPRT gene after UV damage to the cells (13). One limitation in such studies is that whereas gene-specific repair can now be measured separately for the two major UV photoproducts, the type of photoproduct that caused the mutation in repair-proficient cells cannot easily be distinguished. In a study by McGregor et al. (10), a predicted strand bias for mutation fixation was found in repair-proficient cells but not in repair-deficient Xeroderma pigmentosum complementation group A cells. This supports the notion that the strand-selective repair process is responsible for the strand bias of the mutations in repair-proficient cells. It is important, however, to consider

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¶ The abbreviations used are: PD, pyrimidine dimers; DHFR, dihydrofolate reductase; HPRT, hypoxanthine guanine phosphoribosyltransferase; 6-4 PP, 6-4 photoproducts; IA, cisplatin intrastrand adduct; ICL, cisplatin interstrand cross-link; T4 endo, T4 endonuclease V; CHO, Chinese hamster ovary; kb, kilobase.
that since these mutations are fixed by replication, the strand bias could also arise from a bias in replication after damage.

UV (254 nm) irradiation of cells causes the formation of two major photoproducts in DNA. The predominant lesion (65–85% of total) is the cyclobutane PD, while the less common (10–30%) is the 6–4 photoproduct (6–4 PP). Since 6–4 PPs are cytotoxic and mutagenic in mammalian cells (14–16), we recently developed an assay for the measurement of 6–4 PP repair in specific genes. This approach involved the reversal of UV-induced PDs with photolyase followed by cutting of the remaining UV photoproducts (almost exclusively 6–4 PPs) with *E. coli* ABC exinuclease (17).

We have also developed techniques to detect lesions caused by alkylating agents and cisplatin in specific genes. Cisplatin is the prototypical heavy metal compound with anti-tumor activity. The most frequent cisplatin DNA lesion is the intrastrand adduct (IA) between adjacent purines (GG or AG) (18), whereas the interstrand cross-links (ICL) account for only about 1% of the total DNA lesions (19). We detect the cisplatin IA by cleavage with the ABC exinuclease as described above, and the cross-links are detected by a denaturation-renaturing method.

Alkylating agents include simple monofunctional methylating agents such as dimethyl sulfate and bifunctional agents such as nitrogen mustard (HN2), which forms both monoadducts and interstrand DNA cross-links. The predominant lesion is the alkylation of the N7 position of guanine, but many other base modifications are possible, and the spectrum varies for the individual agents (20). In our assay, the N7 methylations are detected by cleavage in a two-step reaction, neutral depurination followed by alkaline hydrolysis.

We have examined the strand selectivity of DNA repair for lesions other than pyrimidine dimers for two main reasons: 1) to determine whether the enzymology involved in the gene-specific repair of pyrimidine dimers differs from that involved in the repair of other types of damage, which addresses the question of whether strand specificity is restricted to the repair of pyrimidine dimers or is a general feature of mammalian DNA repair; 2) if the strand specificity of DNA repair correlates with strand specificity of mutations after several types of damage, it would support the general hypothesis that the mutational bias is caused by the strand selectivity of the repair process. We have thus examined the strand specificity of the DNA repair in our model gene, DHFR, after treatment of hamster cells with UV (PD and 6–4 PP), alkylating agents (HN2 and dimethyl sulfate), and cisplatin.

**MATERIALS AND METHODS**

**Cell Lines, Culture Conditions, and Probes**—The Chinese hamster ovary (CHO) cell line CHO-B11, which contains an amplified DHFR gene (21), was grown in Ham's F-12 medium without glycine, hypoxanthine, or thymidine (GIBCO), supplemented with 10% dialyzed fetal calf serum, and maintained in 500 nM methotrexate. The cells were subcultured to ensure exponential growth 1 day before irradiation. ABC excinuclease and DNA photolyase were purified as described elsewhere (22, 23). The T4 endonuclease V (T4 endo) was produced from an overproducing strain provided by Dr. DeRiel, Temple University. The DNA probes were as previously described elsewhere (22, 23). The T4 endo was used to produce plasmid pZ3d8. Large scale cultures were grown to produce plasmid DNA as described by Humphreys et al. (28).

**Analysis of PD and 6–4 PP Repair in the DHFR Gene**—The initial steps of the assay were as described by Bohr and Okumoto (29). The CHO cells, grown in monolayer, were irradiated with UV doses (254 nm) ranging from 20 to 60 J/m². Cells at 0-h repair were lysed immediately, whereas those for repair time points were incubated in bromodeoxyuridine and fluorodeoxyuridine to density label the DNA replicated after damage. After UV irradiation of the cells and DNA isolation, samples were treated with restriction endonuclease, and the parental DNA was separated on CsCl gradients (29). The DNA was divided into two portions. The first was treated with T4 endo to measure PD as described (29). The second portion was treated with DNA photolyase and light, which specifically and quantitatively reverses PD (30). The remaining, non-dimer photoproducts are almost exclusively 6–4 PP (31). Samples treated with photolyase were preincubated at room temperature in the dark in a buffer containing 50 mM Tris, pH 7.5, 100 mM NaCl, 1 mM EDTA, 10 mM β-mercaptoethanol, and 0.25 μg of photolyase/μg of DNA. The samples were then placed in a small reaction tube and irradiated in a monochromator at 405 nm for 30 min at room temperature. The DNA was extracted to transfer competent DH5α cells to ampicillin resistance using X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) screening to identify white recombinant clones (26). A lysozyme-boiling minilysis technique (27) was used to prepare DNA from small cultures and led to identification of plasmid pZ3d8. Large scale cultures were grown to produce plasmid DNA as described by Humphreys et al. (28).
with phenol and phenol-chloroform followed by ether, precipitated with ethanol, and resuspended in TE (10 mM Tris, 1 mM EDTA, pH 8.0) to a concentration of approximately 0.2 μg/μl. The photoactivatable DNA was then divided into three aliquots. One aliquot was treated with T4 endo to demonstrate that virtually all of the PD were repaired by the photoreactivation treatment. Another was treated with T4 endo to excise the remaining DNA adducts. A third was used as a control. The three samples were run in parallel on an alkaline-agarose gel, transferred to a support membrane, and treated with T4 endo to demonstrate that virtually all of the PD were converted to apurinic sites. Then the samples were treated with ABC excinuclease to excise the DNA at the remaining adducts. The three samples were run in parallel with AHC excinuclease to excise the DNA at the remaining DNA adducts. The three samples were treated with T4 endo to demonstrate that virtually all of the PD were converted to apurinic sites. Then the samples were treated with ABC excinuclease to excise the DNA at the remaining adducts.

RESULTS

Strand Repair of Pyrimidine Dimers—Fig. 2 shows the Southern blot analysis of the formation and repair of PD in the transcribed and nontranscribed strand of the CHO DHFR gene using riboprobes from pZ3d8. Each blot was first probed for the transcribed strand, deprobed, and then probed for the nontranscribed strand. Lanes are run in parallel with or without treatment with the T4 endo. The control, 0-h, and no-UV doses show that there is no nonspecific cutting by T4 endo. There is increased cutting by T4 endo in the treated lanes of the 20–60 J/m2 samples. The important lanes to note are the 40 J/m2 24-h time points (+) treated with T4 endo. Whereas a band is visible indicating repair in the transcribed strand, there is no repair in the nontranscribed strand. The quantitation of this analysis is shown in Table I. The data are based on two biological experiments and several gels from each. The data for PD repair using a double-stranded probe overnight at 48 °C in fresh buffer after adding riboprobe heated at 65 °C for 15 min in 1 ml of hybridization buffer. After hybridization, membranes were washed in 2 × SSPE for 5 min at room temperature, then twice in PSE (0.25 M sodium phosphate, 0.001 M EDTA, pH 7.2, containing 2% SDS) at 65 °C for 15 min, followed by 1–2 washes in PES (0.04 M sodium phosphate, 0.001 M EDTA, pH 7.2, containing 1% SDS) at 65 °C for 20 min each wash. Heat-inactivated RNase (Boehringer Mannheim 1119915) was then added to 2 × SSPE (19 units/250 ml), and the membrane was washed in this solution for 2 min at room temperature to remove nonspecifically bound probe. After autoradiography, the resulting bands were analyzed as described for double-stranded probing.

Table I

| UV dose (J/m²) | Repair | Pyrimidine dimers in DHFR strands | Percent repair | J/m² | h |
|---------------|--------|----------------------------------|---------------|------|---|
|               |        | Both | T² | N² | Both | T | N |
| 0             | 0      | 0    | 0  | 0  | 0    | 0  | 0 |
| 20            | 1.67   | 2.19 | 1.82 | 0 | 0 | 0 |
| 40            | 2.55   | 3.03 | 2.60 | 0 | 0 | 0 |
| 60            | 3.15   | 3.91 | 3.42 | 0 | 0 | 0 |
| 80            | 2.65   | 2.93 | 2.77 | 0 | 0 | 0 |
| 24            | 1.54   | 1.41 | 2.30 | 0 | 0 | 0 |

* T, transcribed strand.  
* N, nontranscribed strand.
were published previously (17). The new results presented here clearly show that there is strand selectivity of the repair of PD in the DHFR gene. The repair is confined to the coding strand with almost no repair in the noncoding strand. When the filter was reprobed with a double stranded DNA probe generated by multiprimer labeling, the repair measurements comprised an average between the two strands.

Strand Repair of 6–4 Photoproducts—Fig. 3 shows the Southern blot analysis of 6–4 PP repair in the two strands of the DHFR gene, and the quantitation is shown in Table II. For each time point, we have included the photolyased sample treated with T4 endo as a control for the efficiency of the photolyase treatment. It is evident from the autoradiogram that the photolyase reaction was complete, and this is confirmed by the quantitation that shows it went to >90% completion. The density of the photolyased, ABC excinuclease-treated band where the repair is monitored is seen to increase with time, and this occurs both in the transcribed and nontranscribed strands. As see in Table II, the initial level of 6–4 PP is very similar in the two strands, and the repair efficiency is similar in the transcribed and nontranscribed strands. When the filter was probed with the regular double-stranded DNA probe, the repair efficiency was calculated as an average between the two strands. The table shows the results of two separate biological experiments.

In Fig. 4, we have plotted the repair kinetics for the removal of the two main photoproducts in the individual strands of the DHFR gene. The repair of 6–4 PPs was faster than that of PD in the DHFR gene, and whereas there was distinct strand selectivity for PD repair (Fig. 4B), strand selective repair of 6–4 PPs was not evident (Fig. 4A). It is difficult to determine whether the small difference observed between the two strands in Fig. 4A, particularly at 4 h, represents strand bias or whether it is due to variability in the assay.

Strand Repair of Nitrogen Mustard and Dimethyl Sulfate—Fig. 5 shows blots of strand repair of HN2 adducts in the hamster DHFR gene after treatment of the cells with 200 μM HN2 for 30 min. Parallel lanes 1 and 2 are identical samples, thus providing a control for loading. The control lanes (C) have not been depurinated, whereas all other samples have undergone neutral depurination and alkaline hydrolysis. The reappearance of band (repair) is readily visible with time after the 0-h time point. Below the blot, we have plotted the repair efficiency. It is seen that at all time points there is more efficient repair in the transcribed than in the nontranscribed strand, although the difference is minimal. When probing with the double-stranded probe, the results fall between the data for the individual strands. The results (see Table III) with HN2 are based on two separate biological experiments and several individual gels. For dimethyl sulfate (Fig. 6), the composition of the blot is similar to that for HN2, and the repair is also similar in the two strands, although there is again more repair in the transcribed than in the nontranscribed strand. Cells were treated with dimethyl sulfate at 150 μM for 30 min. The data (Table III) are based on several independent determinations from one biological experiment.

Strand Repair of Cisplatin Intrastrand Adducts and Interstrand Cross-links—Fig. 7 shows the damage and repair in the individual DNA strands of cisplatin IA. Nontreated DNA is not shown, but the nonspecific cutting with ABC excinuclease.

![Fig. 3. Strand repair of 6–4 photoproducts in the CHO DHFR gene after UV irradiation.](image)

![Fig. 4. Strand repair of UV photoproducts in the CHO DHFR gene.](image)

**TABLE II**

| H after | Incisions in DHFR strands | Percent repair |
|---------|---------------------------|----------------|
| UV dose | Both T N | Both T N | Both T N | Both T N |
| 40 J/m² | | | | |
| 0 0 0 | 0 0 0 | 0 0 0 | 0 0 0 | 0 0 0 |
| 4 0.91 0.90 0.94 | 0.91 0.90 0.94 | 0.91 0.90 0.94 | 0.91 0.90 0.94 | 0.91 0.90 0.94 |
| 8 0.43 0.50 0.56 | 0.43 0.50 0.56 | 0.43 0.50 0.56 | 0.43 0.50 0.56 | 0.43 0.50 0.56 |

* T, transcribed strand.

* N, nontranscribed strand.

* (1) and (2) indicate separate biological experiments.
FIG. 5. Strand repair of nitrogen mustard adducts in the CHO DHFR gene. Cells were treated with 200 μM HN2 for 30 min. The DNA was extracted, restricted, and the parental DNA separated. Samples were subjected to a neutral depurination followed by an alkaline hydrolysis to detect alkaline labile sites, mainly due to N7 methylation. C, untreated (HN2) DNA. Lanes 1 and 2 are identical samples run in parallel. The percent repair is plotted. Coding, transcribed; noncoding, nontranscribed.

**TABLE III**

| Nitrogen mustard | Incisions in DHFR | N° |
|------------------|-------------------|----|
| h after 200 μM   |                   |    |
| 0                | 1.73 ± 0.36       | 1.85 ± 0.57 |
| 2                | 1.11 ± 0.27       | 1.27 ± 0.07 |
| 4                | 0.96 ± 0.34       | 1.46 ± 0.17 |
| 8                | 0.85 ± 0.16       | 1.12 ± 0.12 |
| 24               | 0.40 ± 0.25       | 0.65 ± 0.33 |

| Dimethyl sulfate | Incisions in DHFR | N° |
|------------------|-------------------|----|
| h after 150 μM   |                   |    |
| 0                | 1.54              | 1.57 |
| 2                | 0.93              | 0.96 |
| 4                | 0.79              | 0.69 |
| 8                | 0.73              | 0.69 |
| 24               | 0.37              | 0.28 |

* T, transcribed strand.  
* N, nontranscribed strand.

did not exceed 10%. The repair is seen as the reappearance of bands in the ABC exonuclease-treated lanes from 0 to 24 h. The data are shown in Table IV. There is less repair in the nontranscribed strand, although since the nontranscribed blot was exposed longer than the transcribed blot, this is not readily visible. The plot shows about 2-fold more repair in the transcribed strand than in the nontranscribed strand. The difference is not as dramatic as for pyrimidine dimers, but we consistently found this strand bias in several gels from two different biological experiments. Fig. 8 shows the results from the cisplatin ICL assay. Samples were run before and after denaturation. In the denatured samples, the repair can be seen as the removal of the double-stranded (D) DNA and a corresponding increase in single-stranded (S) native DNA. As seen on the plot, Fig. 8, and from the data in Table IV, there is no difference between the two DNA strands with regard to the repair of cisplatin ICL.

**DISCUSSION**

In this study, we have analyzed the strand bias of DNA repair after different types of DNA damage. We find that for some lesions, pyrimidine dimers, and cisplatin intrastrand adducts there is a distinct bias of the repair with a preferential removal of lesions in the transcribed strand. For a number of other lesions, the difference between the strands is minimal or not present. Based on this data we conclude that there are differences in the DNA repair pathways of these different types of damage.

In this study, we demonstrate that 6–4 PP repair in the CHO DHFR gene shows little or no strand selectivity, in contrast to the pronounced strand-selective repair of PDs. Our results may have important implications for both DNA repair and mutagenesis. The lack of distinct strand specificity of the gene-specific repair of 6–4 PP as contrasted with the PD supports other information to suggest that there are different pathways of repair for these two UV-induced photoproducts. The mode of repair may in some manner be dependent upon the precise distortion that these agents inflict upon the chromosome structure.

In addition, we have studied the strand-specific repair of
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FIG. 7. Strand repair of cisplatin intrastrand adducts in the CHO DHFR gene. Cells were treated with 300 μM cisplatin for 1 h. After purification, restriction, and CsCl gradient centrifugation, the samples were treated with ABC excinuclease (ABC EN), which cleaves at sites of the intrastrand adducts. The damage and repair was quantitated in both strands, and the strand bias of repair is shown in the plot. Coding (○), transcribed; noncoding (■), nontranscribed.

TABLE IV
Strand-specific removal of cisplatin lesions from the CHO DHFR gene

| Intrastrand adducts | Incisions in DHFR | h after 300 μM | T | N* |
|---------------------|-------------------|---------------|---|----|
|                     |                   | 0             | 2.12 ± 0.60 | 2.36 ± 0.64 |
|                     |                   | 8             | 1.15 ± 0.43 | 1.74 ± 0.54 |
|                     |                   | 24            | 0.93 ± 0.40 | 1.55 ± 0.49 |

| Interstrand cross-links | Frequency of cross-linking in DHFR | h after 300 μM | T | N |
|-------------------------|-------------------------------|---------------|---|---|
|                         |                               | 0             | 2.60 | 2.67 |
|                         |                               | 8             | 1.24 | 1.20 |
|                         |                               | 24            | 0.87 | 0.80 |

*a T, transcribed strand.

Fig. 8. Strand repair of cisplatin interstrand cross-links in the CHO DHFR gene. Cells were treated with 300 μM cisplatin for 5 h. Purified, restricted DNA was denatured and renatured before separation by neutral agarose gel electrophoresis. The nondenatured samples are shown as controls. D, double-stranded (cross-linked) DNA; S, single-stranded (native) DNA. The plot shows the repair in the transcribed, nontranscribed, and in both strands. Coding (○), transcribed; noncoding (■), nontranscribed; ▽, both coding and non-coding.

The difference between the strands in a repair-proficient background would thus be expected to be smaller than we find in the repair-deficient background using UV. Given this difference in overall genome repair, the relative strand bias observed here for the repair of cisplatin IA may be of the same magnitude as that of pyrimidine dimers.
For most of the lesions examined, the frequency of initial adducts formed in the transcribed and in the nontranscribed strand is very similar, but for HN2 there are slightly more lesions formed in the nontranscribed strand. This is in accord with our previous observations of differences in the gene-specific frequency of induced nitrogen mustard lesions (2) (but not of lesions induced by several other agents) and with data obtained by Barlett et al. (36) that also report a small heterogeneity in the formation of these lesions within the genome.

These experiments were done at doses currently used for studies of DNA damage and repair in general as well as for studies of this type. It would be preferable, but rather unfeasible, to perform a dose-response repair analysis using a wide concentration spectrum for all the compounds we have used. Our techniques are, unfortunately, limited to these relatively high doses for these experiments, since we need to be able to detect in the range of 0.5–4 lesions in each fragment that we are analyzing. Although it is a limitation that we cannot do our studies at significantly lower levels of compounds than used, we consider it an advantage of this work that we are comparing the repair in a situation where the initial level of lesions introduced by the different compounds used is fairly similar.

Our findings, taken together with those previously reported, suggest that strand specificity of DNA repair is not a universal aspect of the DNA repair mechanisms but rather it is limited to the repair of certain adducts. However, it is noteworthy that for the lesions where we find no significant strand bias, we consistently find more efficient repair in the transcribed than in the nontranscribed strand. For most lesions, there is good correlation between the present results and our previous findings of preferential repair in the hamster DHFR gene as compared with a nontranscribed, downstream region. For example, for cisplatin IA, we previously reported that there is preferential DNA repair (35), and we now find that there is also strand-selective repair. Preferential repair of cisplatin IA was not as distinct as for pyrimidine dimers, and the strand selectivity observed is also not as distinct. Dimethyl sulfate was not preferentially repaired (37) and is also not strand selectively repaired. Cisplatin ICLs were not preferentially repaired (35) and are also not strand selectively repaired. Pyrimidine dimers were preferentially repaired (1), and they are also strand selectively repaired. In contrast, we reported that there was preferential repair of 6–4 PP (17) and of HN2 (37) in this hamster DHFR locus. For both of these lesions, we now find that there is little or no strand selectivity of the repair. This may indicate that there are several repair mechanisms and that the nontranscribed strand is repaired in a different mode than the noncoding DNA.

Several experients have shown that the efficiency of repair in a gene can correlate with its transcriptional activity. Gene-specific DNA repair efficiency of pyrimidine dimers can be modulated by transcriptional induction of structural genes such as in the case of the hamster of human metallothionein genes (38, 39), and inhibitors of transcription can block the gene-specific repair (40). As with UV-induced pyrimidine dimers, gene-specific repair of alkylation damage can also correlate with transcriptional activity; in rat cells, there is substantially more repair of N-methylpurines in the active insulin gene than when it is not transcribed (41).

The strand specificity of pyrimidine dimer repair discussed above further documents the association between DNA repair processes and the transcription complex. This association appears to apply better to the strand selectivity of the repair than to the preferential gene-specific repair. An E. coli cell-free system has recently been described (42) in which gene- and strand-specific repairs can be studied. It was found that the strand-specific repair was coupled much stronger with transcription than was the gene-specific repair. Whereas the strand specificity of the repair of pyrimidine dimers appears to be closely associated with transcription, there are data suggesting that the gene-specific repair efficiency may not always correlate with transcriptional activity (43). In addition, in differentiating mouse cells, it has been demonstrated that the repair of some genes correlates with the stage of differentiation rather than with gene expression (44).

It is possible that the strand bias of repair is related to the degree of transcription blockade caused by a lesion. UV-induced pyrimidine dimers are known to block transcription, whereas there is much less information about the blockade caused by the other lesions we have studied. Another possibility is that strand bias correlates with the degree of chromatin distortion invoked by the lesion.

Recently, findings on the mutational spectrum found in specific genes after DNA damage have been related to the strand specificity of DNA repair. McGregor et al. (10) showed that UV-induced mutations in the HPRT gene of human cells occurred with a strand bias consistent with preferential repair of the transcribed strand. These results support the previous findings of Vrieling et al. (45) demonstrating strand bias for UV-induced mutations in the HPRT gene of hamster V79 cells. McGregor et al. (10) interpreted their results as reflecting preferential repair of the transcribed strand of HPRT, because repair-proficient cells that were not allowed time for repair after UV irradiation (i.e., cells irradiated in the S phase as opposed to the G1 phase of the cell cycle) and repair-deficient cells (XP group A) did not reveal the strand bias for UV mutations observed in G1-irradiated repair-proficient cells. Experiments of Vrieling et al. (45) used asynchronous hamster cell cultures, but their results are also generally consistent with these conclusions. In a very recent report, Chen et al. (46) find a good correlation between the strand specificity of the repair of benzo[a]pyrene diol epoxide adducts and the distribution of mutations in the human HPRT gene.

Our results showing that in the CHO B-11 hamster DHFR gene there is no strand bias for repair of 6–4 PPs, as opposed to a distinct transcribed strand selectivity for repair of PDs (Fig. 4), suggest that repair of 6–4 PPs should not affect the strand distribution of UV-induced base substitution mutations in mammalian cells. However, it is important to note that the studies on UV-induced mutation spectra were done at a 10-fold lower dose than our studies and that it is likely that this difference in dose makes it problematic to directly compare the results from these different approaches. At this time, it is not possible to perform direct gene-specific repair studies of 6–4 PP at lower doses than those used here because of the need to induce a sufficient number of lesions into the restriction fragment assayed. It would obviously be desirable to develop methodology that would allow such repair experiments to be conducted at lower doses. Results of such studies should aid in further understanding of the roles of PDs and 6–4 PPs in UV toxicity and mutagenesis in mammalian cells.

Acknowledgment.—We thank Scott K. Coleman for technical assistance.

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