Transcription-Coupled Repair of Psoralen Cross-Links but Not Monoadducts in Chinese Hamster Ovary Cells†

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ABSTRACT: We have examined the rate and extent of removal of 4′-(hydroxymethyl)-4,5′,8-trimethylpsoralen (HMT) cross-linkable monoadducts and interstrand cross-links from restriction fragments within the amplicon containing the dihydrofolate reductase (DHFR) gene in the Chinese hamster ovary (CHO) cell line B_{11}. The rate and extent of removal of HMT cross-links was significantly greater in an actively transcribed fragment than in a nontranscribed extragenic fragment of similar size. For the 5′ half of the DHFR gene, approximately 80% of the HMT cross-links were removed in 8 h, in agreement with results reported by Vos and Wauthier [Vos, J. M., & Wauthier, E. L. (1991) *Mol. Cell Biol. 11*, 2245–2252, 1991]. However, few cross-links were removed in that period from the nontranscribed fragment, whose 5′ end is approximately 7 kb downstream from the DHFR transcription unit and which includes a putative replication initiation site. Even after 24 h, only about 50% of the cross-links had been removed from this fragment. In contrast, both the rate and the extent of removal of cross-linkable HMT monoadducts were similar in the two fragments with 50% of the cross-linkable monoadducts removed in 24 h. Moreover, monoadducts formed in the bulk of the genome were removed in 24 h. Moreover, monoadducts formed in the bulk of the genome were removed at a slightly slower rate and to a lesser extent (30% in 24 hours) than those from either of these specific sequences. These results suggest that HMT cross-links are more efficiently removed from actively transcribed sequences than from nontranscribed sequences, but that the transcriptional state does not strongly influence the removal of cross-linkable monoadducts.

A number of studies have demonstrated that ultraviolet (UV)[1] light induced cyclobutane pyrimidine dimers (CPDs) are preferentially removed from actively transcribed sequences in *Escherichia coli*, yeast, and cultured mammalian cells. In most cases this repair is due to preferential repair in the transcribed strands of the genes. This suggests that transcription, or some structural or functional component associated with transcription, is intimately involved with repair of transcribed genes (Hanawalt & Mellon, 1993; Smith & Mellon, 1989). In fact, a transcription repair coupling factor has been isolated from *E. coli* and shown to be the product of the *mfd* gene (Selby & Sancar, 1993).

Although it is clear that transcription plays some role in the removal of CPDs from active genes, the situation for chemical adducts is less clear. Tang and co-workers reported that removal of *N*-(deoxyguanos-8-yl)-2-aminofluorene (dG-C8-AF) adducts in Chinese hamster ovary (CHO) cells was equally efficient in a fragment within the DHFR gene and in a non-transcribed fragment 3′ to the gene (Tang et al., 1989). Furthermore, both fragments were repaired at the same rate as the bulk of the genome, with 70% of the adducts removed within 24 h. However, Chen and associates recently reported preferential removal of (±)-7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (BPDE) adducts in diploid human fibroblasts. About 87% of the BPDE adducts were removed from the transcribed strand of the HPRT gene within 24 h compared to 58% removal from the nontranscribed strand, 12% removal from both strands of the inactive 754 locus, and 38% removal from the genome overall (Chen et al., 1992).

Interstrand cross-linking agents comprise another class of bulky chemical adducts that have been used extensively in DNA repair studies. By precluding strand separation for the essential processes of transcription and replication, cross-links are lethal at very low frequencies compared to those agents that do not form cross-links. The effect of transcription and/or chromatin structure on the repair of cross-linking has been investigated, initially using psoralens as model adducts. Psoralens are a class of bifunctional linear tricyclic compounds that intercalate principally at 5′-TpA-3′ sequences in DNA and when photoactivated with long-wavelength UV (340–380 nm) light (UVA) can covalently react with the 5′–6′ double bond of the pyrimidine base to form a monoadduct. Upon absorption of a second photon, a psoralen monoadduct linked to the DNA through the furan ring may react further with the 5′–6′ double bond of a pyrimidine base on the opposite strand to form an interstrand cross-link (Cimino et al., 1985; Hearst, 1981; Hearst et al., 1984). Zolan et al. (Zolan et al., 1982) showed that psoralen adducts in general were poorly removed from the highly repetitive α DNA sequences in primate cells as compared to those in the bulk of the genome. Vos and Hanawalt showed that cross-links by 4′-(hydroxymethyl)-4,5′,8-trimethylpsoralen (HMT) adducts in the human DHFR gene were removed more rapidly than were HMT monoadducts from the same sequence (Vos & Hanawalt, 1987). Furthermore, Islas et al. showed that the removal of HMT cross-linking was more proficient in the

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[1] Abbreviations: UV, ultraviolet; CHO, Chinese hamster ovary; DHFR, dihydrofolate reductase; HMT, 4′-(hydroxymethyl)-4,5′,8-trimethylpsoralen; CPD, cyclobutane pyrimidine dimer; dG-C8-AF, *N*-(deoxyguanos-8-yl)-2-aminofluorene; BPDE, (±)-7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene; UVA, long-wavelength UV (360 nm); BrdUrd, bromodeoxyuridine; FdUrd, fluorodeoxyuridine.

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actively transcribed DHFR gene than in the transcriptionally silent *c-fms* protooncogene, despite a higher initial level of cross-linking in the DHFR gene (Islas et al., 1991). Within 24 h, approximately 90% of the psoralen cross-links had been removed from the DHFR gene, while approximately 40% of cross-links were removed from the bulk of the genome, and hardly any cross-links were removed from the *c-fms* gene. Some preferential repair of cross-linkable monoadducts in the DHFR gene was observed, although the effect was not as dramatic as that seen for cross-links. Approximately 60% of the cross-linkable monoadducts were removed from the DHFR gene as compared to only about 35% from the bulk of the genome. These studies suggested that transcription plays some role in the processing of psoralen cross-links. Furthermore, nitrogen mustard induced interstrand cross-links were removed more rapidly from the actively expressed *c-myc* gene than from the rest of the genome in human Colo320HSR cells, suggesting that transcription may play a role in the removal of this type of cross-link as well (Futscher et al., 1992). However, Jones et al. reported no difference in the removal of *cis*-diamminedichloroplatinum II (cisplatin) induced cross-links from a *Kpn*I restriction fragment within the CHO DHFR gene and a nontranscribed *Kpn*I restriction fragment downstream (Jones et al., 1991). Approximately 90% of the cross-links induced by a 1-h exposure to 300 μM cisplatin were removed from both fragments in 24 h. Interestingly, there was a general trend toward preferential repair of total cisplatin adducts (mostly *intra*strand cross-links) in the actively transcribed genes.

These studies demonstrate that the evident repair characteristics of bulky chemical lesions may vary considerably, possibly depending on the cell lines chosen, the assays used, or the particular chemical damaging agents, even though the same nucleotide excision repair mechanism is implicated in their removal. We have used the psoralen derivative HMT to measure the processing of both psoralen monoadducts and interstrand cross-links in a restriction fragment within the DHFR gene and in a nontranscribed extragenic fragment downstream from the gene in CHO B_{11} cells. We used the denaturing/renaturing agarose gel electrophoresis technique developed by Vos and Hanawalt (Vos & Hanawalt, 1987) to measure very low cross-link and monoadduct frequencies. We found that HMT-induced cross-links are processed much more efficiently in the DHFR gene than in the nontranscribed fragment. While both fragments had nearly identical initial cross-linking frequencies, within 8 h approximately 85% of the cross-linking in the DHFR fragment was removed, but only about 5% of the cross-linking was removed from the extragenic fragment. In contrast, the repair characteristics for HMT monoadducts in the two fragments were identical; approximately 65% of cross-linkable monoadducts were removed within 8 h. These results demonstrate that, for a single psoralen species that generates two qualitatively different types of bulky chemical lesions, the relationship between repair and transcription for the respective lesions is fundamentally different.

MATERIALS AND METHODS

Materials. Both ^{3}H-labeled (16 Ci/mmol) and unlabeled HMT were obtained from HRI Associates (Concord, CA). The probes used have been described previously (Mellon et al., 1987). The pZH-4 probe hybridizes to the 14-kb *Kpn*I fragment at the 5′ end of the DHFR gene, and pZH-26 hybridizes to a 14-kb *Kpn*I fragment approximately 7 kb downstream from the DHFR transcription termination site (Figure 1). Probes were prepared by nick translation using

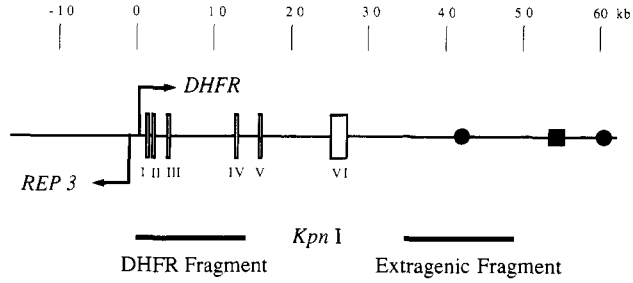

FIGURE 1: Genomic map of the DHFR domain in CHO cells. Roman numerals show the six exons of the DHFR gene. Arrows indicate the start and direction of transcription for the DHFR gene as well as a divergent upstream transcript designated REP 3 (Linton et al., 1989). The closed circles and closed box indicate sites of initiation of DNA replication and a matrix attachment region, respectively (Dijkwel & Hamlin, 1988). The thick bars indicate the location of the two 14-kb *Kpn*I fragments used in this study. The DHFR fragment is completely contained within the transcriptionally active DHFR gene. The extragenic fragment is downstream of the DHFR transcription unit and therefore not transcribed.

[α-^{32}P]dCTP (3000 Ci/mmol; Amersham, Arlington Heights, IL).

Cell Culture. Chinese hamster ovary (CHO) B_{11} cells (Kaufman & Schimke, 1981), amplified about 50-fold for the DHFR gene, were grown in minimal essential medium (MEM) supplemented with 10% dialyzed fetal bovine serum, glutamine (10 mM), $NaHCO_3$ (7% stock) (GIBCO, Grand Island, NY), and 0.5 μM methotrexate (Sigma, St. Louis, MO) in 10-cm dishes (Falcon).

Psoralen Treatment and DNA Preparation. Cells were prelabeled for two doublings with 0.1 μCi/mL [^{3}H]thymidine (80 Ci/mmol; NEN-DuPont, Boston MA) in the presence of 10 μM unlabeled thymidine. The medium was removed prior to psoralen treatment and the cells were washed twice with phosphate-buffered saline (PBS). Two milliliters of a 1.0 μg/mL (3.8 μM) solution of HMT in PBS was added to the cells. To allow time for intercalation of the psoralen into DNA, cells were incubated for 10 min at 4 °C in the absence of light. Cells were then irradiated through the bottom of the plastic tissue culture dishes with two 15-W General Electric F15T8-BLB bulbs at a fluence of 0.6 kJ/(m^2 min) for 5 min at 4 °C. The cells were then washed and incubated with PBS for 10 min at 37 °C to remove any unbound HMT, and either lysed immediately (10 mM Tris-HCl, pH 8.0, 1 mM EDTA, 0.5% SDS, and 200 μg/mL proteinase K) or incubated in the presence of 10 μM BrdUrd and 1 μM FdUrd (to density label replicated DNA) to allow for processing of the psoralen photoadducts. The cell lysates were extracted with phenol, phenol:chloroform and chloroform; the DNA was precipitated with ethanol and dissolved in TE (10 mM Tris-HCl, pH 8.0, and 1 mM EDTA). The dissolved DNA was restricted with *Kpn*I (5 units/μg DNA; BRL, Bethesda, MD) and centrifuged to equilibrium in isopycnic CsCl gradients to separate replicated from unreplicated DNA. The gradients were fractionated, the fractions were assayed for radioactivity, and the unreplicated DNA was pooled and dialyzed extensively against TE.

Quantitation of Psoralen Adducts in Specific Sequences. The assay we have used to measure HMT cross-links and cross-linkable monoadducts in defined genomic restriction fragments has been described in detail elsewhere (Vos, 1988; Vos & Hanawalt, 1987). Briefly it involves dividing each DNA sample into three equal aliquots. One aliquot is run on a neutral agarose gel in its native state and represents the sum of cross-linked and non-cross-linked DNA (i.e., total DNA). This aliquot is also used as an internal control to correct for sample loading variation between different time points. The

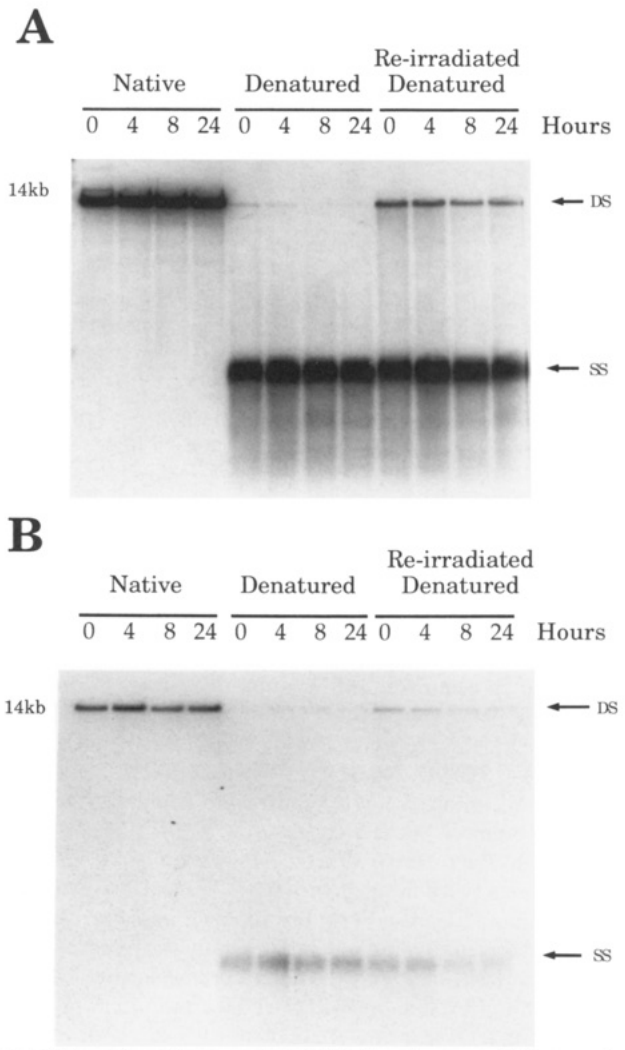

FIGURE 2: Autoradiograms of the DHFR and extragenic fragments. Each sample was divided into three equal parts. The denatured aliquots show the amount of cross-linking in the DNA fragment. cross-linked DNA will reanneal and migrate at the position of double-stranded DNA in the gel (DS). Samples that are re-irradiated before they are denatured show the amount of cross-links and cross-linkable monoadducts. Subtracting the frequency of cross-linking derived from the denatured bands from that from the re-irradiated and denatured bands yields a measure of cross-linkable monoadducts. Panel A is a representative autoradiogram from the DHFR fragment. Panel B is a representative autoradiogram from the extragenic fragment.

second aliquot is denatured and then loaded onto the neutral agarose gel. The cross-linked DNA strands rapidly renature, while the non-cross-linked DNA remains single-stranded. After hybridization with a ^{32}P-labeled probe for the sequence of interest, two bands appear, one representing the double-stranded cross-linked DNA and the other, migrating further down the gel, representing the unmodified single-stranded DNA (e.g., Figure 2A, denatured T_0 lane). The ratio of the intensities of the band of cross-linked DNA and the band of undenatured total DNA yields the fraction of cross-linked DNA. One could also represent the cross-link fraction as the ratio of cross-linked DNA to the sum of cross-linked DNA and single-stranded DNA within each lane; however, we find obtaining an accurate measure for single-stranded DNA to be problematic because of the spreading seen in the single-stranded DNA band (see SS bands in Figure 2). The third aliquot is re-irradiated extensively with UVA *in vitro* to convert all cross-linkable monoadducts into cross-links. The fraction of DNA renatured will then be the sum of cross-linked and cross-linkable monoadducted DNA. By subtracting the fraction of cross-linked DNA derived in the denatured sample from the fraction of cross-linked DNA from the re-irradiated/denatured sample, we obtain the fraction of DNA containing cross-linkable monoadducts. Once the cross-link fraction is determined, the Poisson equation is used to calculate the cross-linking frequency (Vos, 1988). Densitometry of autoradiographs is performed on a Helena Laboratories Quick Scan densitometer. Because of the range of intensities seen in the autoradiograms, multiple exposures were performed for each filter to ensure that we were within the linear range of the film for the bands to be analyzed. Furthermore, internal standards were used to control for different exposure times. It should also be noted that at very low cross-linking levels (such as those seen for the cross-links in this study) the cross-linking frequency approaches the value for the cross-linked fraction and becomes linear (Vos, 1988). Hence one can calculate the relative repair values directly from the intensities of cross-linked bands (after correcting for any loading error derived from the native DNA lanes). Although this approach can only provide relative repair values and does not establish absolute cross-linking frequencies, by obviating the need for multiple exposures, it serves as an important check for the calculation of cross-link frequencies. The repair values derived from the calculation of cross-linking frequencies and from the relative repair values agreed to within 5% of each other.

Repair of Psoralen Monoadducts in the Total Genome. Repair of HMT monoadducts was determined by a slight modification of an assay described previously (Islas et al., 1991). Briefly, cells were prelabeled for two doublings with [^{32}P]orthophosphate (NEN-DuPont, Boston, MA) and treated as above except that [^{3}H]HMT was used. The cells were lysed, and the DNA was sheared by two passages through an 18-gauge needle, purified by centrifugation in neutral CsCl gradients, and dialyzed extensively against TE. The samples were then alkali denatured (0.1 M NaOH for 10 min at 55 °C), neutralized with Tris-HCl on ice, and centrifuged in isopycnic CsCl gradients at pH 10.8. These gradients allow the separation of cross-linked DNA (renatured and therefore double-stranded) from monoadducted (single-stranded) or nonadducted DNA (also single-stranded). The ratio of ^{3}H (in HMT) to ^{32}P (in DNA) yields the relative abundance of psoralen adducts in both the monoadducted and the cross-linked peaks. Since, under the psoralen treatment described here, less than 5% of all photoadducts were cross-links (data not shown), accurate measurements were obtained only for monoadducted DNA.

RESULTS

We used the renaturing agarose gel electrophoresis technique to measure the processing of psoralen cross-links and cross-linkable monoadducts in specific genomic sequences (Vos, 1988; Vos & Hanawalt, 1987). This technique allowed us to measure the removal of HMT photoadducts from a transcribed *Kpn*I fragment in the DHFR gene (DHFR fragment) and from a nontranscribed *Kpn*I fragment downstream of the DHFR gene (extragenic fragment) in the same experiment. We also measured the repair of [^{3}H]HMT monoadducts in the overall genome by the technique previously developed to resolve monoadducted and nonadducted DNA from cross-linked DNA (Islas et al., 1991).

The DHFR domain in CHO B_{11} cells (Figure 1) is amplified approximately 50-fold. The 14-kb DHFR fragment is entirely contained within the DHFR transcription unit. The 14-kb extragenic fragment begins approximately 7 kb downstream from the DHFR transcription termination site. This fragment contains a site for the initiation of DNA synthesis in another CHO line, CHOC400, and therefore B_{11} may also contain an

Table 1: Cross-Link and Monoadduct Frequencies as a Function of Time[a]

	HMT cross-links			HMT Cross-linkable monoadducts		
time (h)	cross-link freq	SD	repair (%)	monoadduct freq	SD	repair (%)
			DHFR fragment			
0	0.046	±0.015	0	0.185	±0.119	0
4	0.019	±0.008	58.9	0.131	*b*	29.5
8	0.009	±0.009	80.9	0.041	±0.018	77.6
24	0.005	±0.002	89.0	0.039	±0.004	78.9
			Extragenic Fragment			
0	0.038	±0.013	0	0.252	±0.061	0
4	0.039	±0.017	0	0.153	*b*	39.2
8	0.031	±0.015	18.4	0.086	±0.026	65.8
24	0.016	±0.009	57.9	0.071	±0.018	71.6

time (h)	Total Genome—Monoadducts[b] $^3H/^{32}P$ ratio	repair (%)
0	1.80	0
4	1.58	11.8
8	1.32	26.3
24	1.23	31.3

[a] Cross-linking and cross-linkable monoadduct frequencies for the DHFR and extragenic fragment as a function of repair time are given. All values are expressed as the number of adducts per 14-kb fragment ± 1 SD. All values are averaged from three independent biological experiments except where noted. The values for the removal of monoadducts from the total genome are expressed as the ratio of ^3H-labeled HMT to ^{32}P-prelabeled DNA. Repair is measured as the relative decrease in the [^3H]HMT counts as a function of time. [b] Averaged from two biological experiments only.

origin of replication in this fragment (Anachkova & Hamlin, 1989; Hamlin & Ma, 1990). The extragenic fragment is not transcribed.

Processing of HMT Cross-Links. CHO B_{11} cells were exposed to 1.0 μg/mL HMT and 3.0 kJ/m^2 UVA light to induce a detectable number of psoralen photoadducts. Under these conditions, the initial fraction of DNA migrating in the position of double-stranded (DS) DNA is very small (see Figure 2, T_0 denatured lanes) for both fragments, suggesting that the initial cross-linking frequencies for both fragments were identical and very low, on the order of 0.04 cross-link per 14-kb fragment (0.3 cross-link/100 kb). Table 1 includes data for the processing of psoralen cross-links in the DHFR and extragenic fragments. With time, the cross-linking frequency decreased in the DHFR fragment (Figure 2A) but remained relatively constant (at least for the 0-, 4-, and 8-h time points) in the extragenic fragment (Figure 2B). Figure 3 depicts the removal of HMT cross-links from the DHFR fragment and the nontranscribed extragenic fragment as a function of time. Within 4 h, over 50% of HMT cross-links were removed from the DHFR fragment. Within the same period, almost no cross-links were removed from the extragenic fragment. Even after 8 h, when nearly all of the HMT cross-links had been removed from the DHFR fragment, less than 5% of the cross-links had been removed from the extragenic fragment. However, removal of HMT cross-links in the extragenic fragment was significant after 24 h, by which time about 50% had been removed.

Repair of HMT Monoadducts. Comparing re-irradiated and denatured lanes in panels A and B of Figure 2 shows that the rate and extent of repair of cross-linkable monoadducts in the DHFR and extragenic fragments were nearly identical. Although the re-irradiated and denatured lanes are actually the sum of cross-links and cross-linkable monoadducts, the contribution from cross-links is so small that the reduction in band intensity seen is due almost exclusively to the removal of cross-linkable monoadducts. Table 1 also includes data

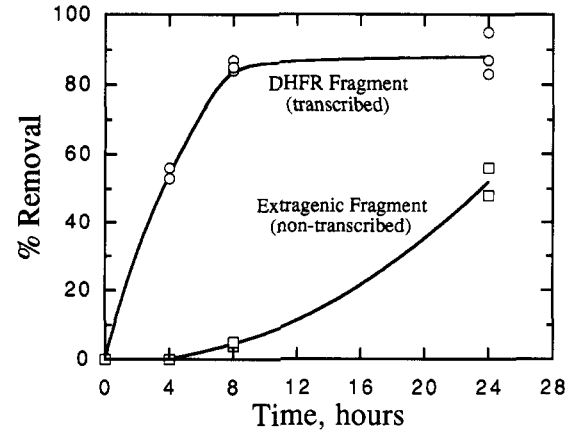

FIGURE 3: Removal of HMT cross-links from the CHO DHFR fragment and the extragenic fragment. Cells were treating with 1.0 μg/mL (3.8 μM) HMT and UVA [0.6 kJ/(m^2 min^{-1})] for 5 min. Open circles show the cross-link removal in the transcribed DHFR fragment. Open squares show the cross-link removal from the nontranscribed extragenic fragment.

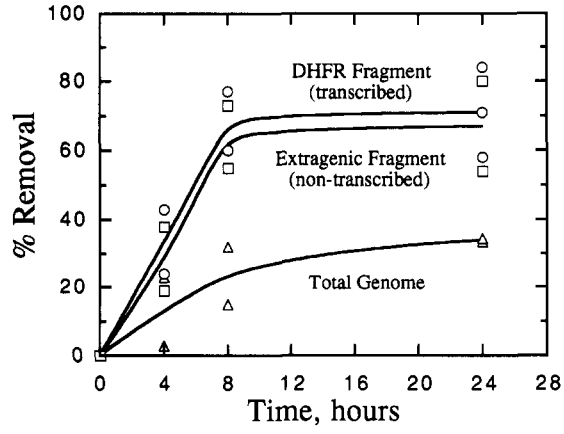

FIGURE 4: Removal of HMT-cross-linkable monoadducts from the DHFR fragment and the extragenic fragment and removal of HMT monoadducts from the total genome. The repair of monoadducts from the total genome (open triangles) was determined by measuring the removal of [^3H]HMT from the monoadducted DNA separated from cross-linked DNA as described in Materials and Methods. Open circles show the removal of cross-linkable monoadducts from the DHFR fragment, Open squares show the removal of cross-linkable monoadducts form the extragenic fragment.

for the repair of HMT cross-linkable monoadducts in the DHFR and extragenic fragments as well as data for the repair of HMT monoadducts in the entire genome. Figure 4 illustrates these data. The repair characteristics of the DHFR fragment and the extragenic fragment are essentially the same; within 4 h, approximately 30% of the cross-linkable monoadducts are removed from both fragments. By 8 h, the repair of cross-linkable monoadducts appears to reach a plateau with over 60% of cross-linkable monoadducts removed.

In order to measure the repair of psoralen monoadducts in the entire genome, cells were treated with ^3H-labeled HMT and the disappearance of the ^3H label (normalized to ^{32}P-prelabeled DNA) as a function of time was taken as a measure of the repair of all HMT monoadducts (cross-linkable furan-side monoadducts and non-cross-linkable pyrone-side monoadducts). The repair of total HMT monoadducts in the entire genome occurred at a slightly slower rate and to a lower extent than the repair of cross-linkable monoadducts in the two *Kpn*I fragments (Table 1). Only approximately 30% of monoadducts were removed within 24 h.

DISCUSSION

Our results clearly demonstrate that two qualitatively different types of bulky chemical adducts, the psoralen monoadduct and the interstrand cross-link, can be processed with very different efficiencies in different regions of the genome. Cross-links were removed much more rapidly from the transcribed DHFR fragment than from the nontranscribed fragment. In contrast, most of the cross-linkable monoadducts were removed rapidly from both fragments.

Processing of Psoralen Cross-Links. The removal of HMT cross-links from the transcribed DHFR fragment in CHO B_{11} cells was very rapid, with nearly all of the cross-links removed within the first 8 h after damage induction. These results are consistent with previous reports of rapid removal of cross-links from the DHFR gene in these cells (Vos & Wauthier, 1991; Wauthier et al., 1990). However, we found that fewer than 5% of the HMT cross-links were removed from the nontranscribed extragenic fragment within 8 h. The fragment is downstream from the DHFR transcription unit and is not transcribed, although it may contain a region in which DNA replication initiates (Dijkwel & Hamlin, 1988). By 24 h, approximately 50% of the HMT cross-links were removed from the extragenic fragment; thus, these CHO cells are not totally deficient in the processing of psoralen cross-links in this nontranscribed fragment. In contrast, nearly a complete lack of repair of UV-induced cyclobutane pyrimidine dimers in this fragment has been observed (Bohr et al., 1985; Ho et al., 1989). Our data suggest that psoralen cross-links are preferentially removed from actively transcribed regions of the genome, consistent with previously published observations for human cells (Islas et al., 1991). A previous study of cisplatin adducts showed no preferential removal of cross-links but some preferential removal of monoadducts (Jones et al., 1991). However, in that study, relatively high initial levels of adducts were used, in contrast to the experiments reported here in which relatively low initial adduct frequencies were used. Because cross-links comprise approximately 5% of the total adducts, the probability of having a cross-link affect monoadduct repair is therefore extremely small.

Psoralen cross-links are absolute blocks to DNA replication because they prevent dissociation of the parental strands. Therefore, one cross-link in the entire genome would in principle be sufficient to prevent completion of replication and cell division. Thus, if completion of the cell cycle and progression toward cell division were primary factors in the removal of DNA lesions, one might expect psoralen cross-links to be removed equally efficiently throughout the genome. Our findings do not support that hypothesis. Since cross-links are being processed in the actively transcribed DHFR gene and since psoralen cross-links are also absolute blocks to transcription, it would appear that transcription is the definitive functional event for the preferential removal of psoralen cross-links. Indeed, some component involved or associated with the transcriptional machinery may direct the repair machinery to recognize psoralen cross-links as it does for CPDs. Selby and Sancar have characterized a protein that apparently is essential for coupling the repair machinery to the transcription machinery in *E. coli* (Selby & Sancar, 1991, 1993; Selby et al., 1991). Alternatively, a blocked RNA polymerase complex at a psoralen cross-link may be sufficient to "direct" the repair machinery to incise the cross-link without the need for a physical "coupling factor". In any event, the processing of psoralen cross-links in the DHFR gene is very efficient and may reflect a high affinity for transcription-blocking cross-links by the repair machinery in the cells.

The kinetics of removal of cross-links from the nontranscribed extragenic fragment were strikingly different from those seen in the DHFR fragment. This result suggests that, in the absence of transcription, the rate of psoralen cross-link recognition and incision is poor. It should be noted, however, that by 24 h approximately 50% of HMT cross-links had been removed from this fragment. Thus, although the intrinsic recognition and/or binding affinity of the cellular repair complex for these cross-links is poor, most of the cross-links are ultimately recognized and processed. This is in sharp contrast to the removal of UV-induced cyclobutane pyrimidine dimers in these cells; few if any of these lesions are removed from the extragenic fragment within 24 h (Bohr et al., 1986; Ho et al., 1989). Indeed, CHO B_{11} cells appear to be deficient in the repair of CPDs except in the transcribed strands of actively transcribed genes (Hanawalt, 1991). We conclude that the repair system can recognize HMT cross-links more readily than CPDs, but probably less well than 6–4 pyrimidine-pyrimidone photoproducts (see below).

Repair of HMT Monoadducts. The repair of HMT-cross-linkable monoadducts in the transcribed DHFR fragment was nearly identical with that in the nontranscribed extragenic fragment in both rate and extent. The rate and extent of repair of total monoadducts in the entire genome were slightly lower than the rate and extent of repair of cross-linkable monoadducts in the DHFR and extragenic fragment and may reflect a difference in the repair efficiency of furan vs pyrone monoadducts. Our estimate of repair of cross-linkable monoadducts in the DHFR fragment is in agreement with that previously published (Vos & Wauthier, 1991). Furthermore, the lack of differential repair between the two restriction fragments is similar to the report for removal of dG-C8-AF adducts from these fragments (Tang et al., 1989). Although the initial lesion frequency for psoralen monoadducts in our experiments is an order of magnitude lower than that reported for the aminofluorene adducts, the repair characteristics for both lesions in both fragments are similar. Although the rate and extent of repair of HMT monoadducts are slightly greater than those of dG-C8-AF adducts, the differences observed could be due to differences in the assays used.

As the results for the extragenic fragment illustrate, transcription per se is not necessary for the removal of HMT-cross-linkable monoadducts. As discussed above, if CHO cells are deficient in the ability to recognize and repair HMT cross-links, and to a greater extent CPDs, in nontranscribed sequences, then clearly that deficiency is not sufficient to prevent them from recognizing and repairing HMT-cross-linkable monoadducts and dG-C8-AF adducts. The observation that the repair machinery in mammalian cells has different affinities for different adducts is consistent with the observation that a revertant of xeroderma pigmentosum complementation group A cells (XP129) has regained a wild-type resistance to killing by psoralen cross-links but is as sensitive to monoadducts as the parental XPA cell line (Vuksanovic & Cleaver, 1987). Presumably the reversion in the XPA protein at least partially restored its ability to repair cross-links but not monoadducts. Furthermore, the revertant cell line showed rapid removal of UV-induced 6–4 photoproducts in the entire genome as well as strand-specific removal of CPDs from the transcribed DHFR gene but not from bulk DNA (Lommel & Hanawalt, 1993; Mitchell, 1988). The XPA complementation protein (XPAC) in XP129 has a missense mutation (compared to the wild-type protein), suggesting that subtle changes in the amino acid sequence could dramatically affect the stability of the protein or its affinity for various lesions, resulting in different

repair rates for various lesions. This may be the case for CHO cells as well. The XPAC analog in CHO cells may recognize psoralen monoadducts very efficiently, irrespective of transcription, but have such a low affinity for CPD lesions that they are only recognized when they block transcription. That is, transcription may facilitate the rapid recognition and removal of cross-links, but in the absence of transcription, the repair machinery may still recognize and incise cross-links although at a much lower rate. It is interesting to note that a psoralen furan-side monoadduct on the transcribed strand of a synthetic oligonucleotide blocks transcription by *E. coli* and T7 RNA polymerases *in vitro* (Shi et al., 1987, 1988). Thus, although it is clear that both cross-links and monoadducts block transcription, the mechanism involved in blocking RNA polymerase is not clear. Indeed, cross-links may block an RNA polymerase in a fundamentally different way than monoadducts. While a monoadduct at a thymidine base may block an RNA polymerase by preventing it from reading the base, a psoralen cross-link could block an RNA polymerase in this same manner; but it could also block polymerization by preventing localized unwinding of the DNA strands ahead of the polymerase. It may be that how an RNA polymerase is blocked (e.g., inhibition of torsional unwinding, active site inhibition, etc.) is critical to understanding whether a lesion may be removed in a transcription-dependent manner.

Recent studies in both normal human fibroblasts and CHO fibroblasts have revealed that mutations from psoralen cross-links are preferentially targeted to nontranscribed strands of genes (Papadopoulo et al., 1993; Sage et al., 1993; Yang et al., 1994). This is consistent with the notion that psoralen cross-links are being preferentially repaired through a transcription-coupled repair pathway. Moreover, it appears that mutations caused by psoralen monoadducts may also be targeted to the nontranscribed strand (Guillouf et al., 1993). This is consistent with earlier *in vitro* evidence for the strand-specific removal of monoadducts in *E. coli* extracts (Selby & Sancar, 1991). Although our data do not address directly the issue of strand-specific repair, they do strongly suggest, because of the nearly identical repair seen in the DHFR and nontranscribed fragments, that, at least in CHO cells, repair may be independent of transcription. However, in relating lesion repair to mutagenic potency, other factors such as effects on cell cycle regulation may also come into play. Clearly, further information on whether psoralen cross-links and monoadducts are incised in a strand-specific manner is needed, but that awaits the development of a reliable and quantitative *in vivo* assay for the strand-specific processing of psoralen adducts.

What is clear from this study is that psoralen monoadducts and cross-links are processed in fundamentally different ways. Psoralen cross-links are more rapidly removed in a transcriptionally active sequence than in an inactive one; however, cross-linkable monoadducts, being efficiently repaired in the DHFR and extragenic fragments, show no transcription dependence.

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