MOLECULAR AND CELLULAR BIOLOGY, Dec. 1988, p. 5331–5338
0270-7306/88/125331-08$02.00/0

Vol. 8, No. 12

Differential Repair of DNA Damage in the Human Metallothionein Gene Family

STEVEN A. LEADON* AND MARGARET M. SNOWDEN

Division of Cell and Molecular Biology, Lawrence Berkeley Laboratory, University of California, Berkeley, California 94720

Received 16 June 1988/Accepted 16 September 1988

We studied the repair of UV- and aflatoxin B_1 (AFB_1)-induced damage in the human metallothionein (hMT) gene family. After exposure to either UV or AFB_1, DNA damage was initially repaired faster in the DNA fragments containing the transcribed hMT-I_A, hMT-I_E, and hMT-II_A genes than in the genome overall. By 6 h posttreatment, there was at least twice as much repair in these genes as in the rest of the genome. Repair of UV damage in the hMT-I_B gene, which shows cell-type specific expression, and in the hMT-II_B gene, which is a nontranscribed processed pseudogene, was about the same as in the rest of the genome, whereas repair of AFB_1-induced damage was deficient in these two genes. Inducing transcription of the three expressed hMT genes with $CdCl_2$ or of only the hMT-II_A gene with dexamethasone increased the initial rate of repair in the induced genes another twofold over the rate observed when they were transcribed at a basal level. The rates of repair in the hMT-I_B and hMT-II_B genes were not altered by these inducing treatments. Transcription of the hMT genes was transiently inhibited after UV irradiation. Inducing transcription of the genes did not shorten this UV-induced delay. Thus, the efficiency of repair of damage in a DNA sequence is dependent on the level of transcriptional activity associated with that sequence. However, an increased efficiency in repair of a gene itself is not necessarily coupled to recovery of its transcription after DNA damage.

An increasingly important area of investigation of DNA damage and excision repair is whether all sequences in the eucaryotic genome are repaired with the same efficiency. Evidence has been presented for a more rapid removal of pyrimidine dimers from the highly amplified dihydrofolate reductase (*DHFR*) gene both in Chinese hamster ovary cells (2, 3) and in human cells (20), for preferential repair of UV damage in the expressed c-*abl* gene but not the inactive c-*mos* locus in mouse cells (17), and for more efficient repair of UV and aflatoxin B_1 (AFB_1)-induced damage in an active and integrated pSV2-*gpt* than in the bulk of the monkey genome (12). Recent evidence indicates that the removal of UV damage from both the hamster and the human *DHFR* gene is more rapid in the transcribed strand of the gene than in the nontranscribed strand (21). The major distinction between rodent and primate cells in repair of UV damage in transcriptionally active DNA appears to be one of restricted repair versus the rate of repair. Rodent cells appear to restrict repair of UV damage to actively transcribed sequences, possibly leaving the remainder of the genome relatively excluded from the repair process. In primate cells, preferential repair is observed as a more rapid rate of repair in active DNA sequences.

In this study, we examined the repair of UV and AFB_1 damage in the nonamplified human metallothionein (hMT) gene family by using an immunological method that isolates DNA fragments containing bromouracil (BrUra) in repair patches by means of a monoclonal antibody that recognizes BrUra (12). Specific ^{32}P-labeled hybridization probes are used to detect and quantitate very small amounts of the sequence(s) of interest in the repaired fraction of the DNA. Unlike methods that rely on the loss of an endonuclease-sensitive site from a sequence, this technique (i) detects the repair synthesis event itself, thus eliminating the possible error that would be produced if the loss of an endonuclease-sensitive site reflects modification but not repair of a lesion; (ii) provides a sensitive measurement of repair since only one, not all, of the lesions in a DNA sequence need be repaired in order to detect repair in that sequence; (iii) allows direct comparison of repair of a variety of types of DNA damage; and (iv) makes it possible to simultaneously measure repair in more than one DNA sequence.

We focused on five members of the hMT gene family because of the different functional states they represent. The hMT-I_A, hMT-I_E, and hMT-II_A genes are expressed at a basal level and their transcription is inducible, but these genes show different responses to inducing agents such as heavy metals and glucocorticoids (24). The induction of hMT-I_A and hMT-I_E occurs only in the presence of Cd^{2+}, not in the presence of Zn^{2+} or dexamethasone. The hMT-I_B gene is expressed only in some cell lines and may be tissue specific (7). In a cell line where it is not expressed, the 5′-flanking region of the gene is highly methylated, whereas it is not methylated in an expressing cell line. The hMT-II_B gene is a processed pseudogene flanked by direct repeats and possessing a poly(A) sequence at the 3′ end (9). Therefore, within a single system, we can measure the repair of DNA damage in expressed genes for which transcription can also be differentially modulated, in a nontranscribed processed pseudogene, and in a gene whose expression may be tissue specific.

We found that in human cells irradiated with UV or exposed to AFB_1, the initial rate of repair of damage in DNA fragments containing the transcribed hMT-I_A, hMT-I_E, and hMT-II_A genes was more rapid than in the genome overall. Repair of UV damage in the hMT-I_B and hMT-II_B genes was about the same as in the genome overall, whereas repair of AFB_1-induced damage was deficient in these two genes. Inducing transcription of the hMT-I_A, hMT-I_E, and hMT-II_A genes with $CdCl_2$ or selectively inducing transcription of hMT-II_A with dexamethasone further increased the initial rate of repair in the induced genes but had no effect on repair

* Corresponding author.

in the genome overall or in the hMT-I_B and hMT-II_B genes. Transcription of the hMT genes was transiently inhibited after UV irradiation. Inducing transcription of the genes did not shorten this UV-induced delay. Therefore, the efficiency of repair of damage in a DNA sequence is dependent on the level of transcriptional activity associated with that sequence. However, an increased efficiency in repair of a gene itself is not necessarily coupled to the recovery of its transcription after DNA damage.

MATERIALS AND METHODS

Cell culture conditions and treatment. HT-1080, a near-diploid human fibrosarcoma cell line (4), was grown in minimal essential medium containing 10% fetal bovine serum, 2 mM glutamine, and antibiotic-antimycotic solution (GIBCO Laboratories, Grand Island, N.Y.). Cultures that were 80 to 90% confluent were used in all experiments. For prelabeling of DNA, cells were grown for 24 h in [^{3}H]thymidine (1 μCi/ml; Amersham Corp., Arlington Heights, Ill.). The medium was removed, and the cells were grown for an additional 3 days in nonradioactive medium. Cultures were washed with phosphate-buffered saline and irradiated at 254 nm, using a germicidal lamp at an incident dose rate of 0.5 J/m^2 per s, or exposed to 80 μM AFB_1 (Calbiochem-Behring, La Jolla, Calif.) in the presence of an activation buffer containing S9 microsomes (Sitek Research Laboratories) for 60 min at 37°C (13, 14). After exposure to the DNA-damaging agents, the cultures were incubated for various lengths of time in medium containing 10 μM bromodeoxyuridine (BrdUrd) and 1 μM fluorodeoxyuridine. hMT gene transcription was induced by incubation of cultures for either 12 h in the presence of 2.5 μM $CdCl_2$ or 15 h in the presence of 0.1 μM dexamethasone (Sigma Chemical Co., St. Louis, Mo.) before exposure to UV light. The same concentrations of the two inducing agents were used during the post-UV incubation.

Repair analysis. High-molecular-weight DNA was isolated from cells by lysing cultures with a solution of 10 mM Tris (pH 8), 1 mM EDTA, 0.5% sodium dodecyl sulfate (SDS), and 100 μg of proteinase K per ml for 12 to 16 h at 37°C. Lysates were treated with 100 μg of RNase A per ml for 1 h at 37°C and then gently extracted sequentially with buffered phenol, phenol-chloroform-isoamyl alcohol (24:24:1), and chloroform-isoamyl alcohol (24:1). The nucleic acids were then extensively dialyzed against 10 mM Tris (pH 8)–0.1 mM EDTA. The DNA was incubated with the endonuclease *Eco*RI (Bethesda Research Laboratories, Inc., Gaithersburg, Md.), using 8 U of enzyme per μg of DNA, for 5 h at 37°C. All digestions were checked for completion by electrophoresis of an aliquot of each sample on agarose minigels. After digestion, the DNA was centrifuged to equilibrium in a CsCl gradient to separate parental-density DNA (containing BrUra-substituted repair patches) from hybrid-density DNA (synthesized by semiconservative replication) (28). The parental-density DNA was then dialyzed extensively against 10 mM Tris (pH 8)–0.1 mM EDTA.

Unreplicated parental-density DNA was reacted with a monoclonal antibody against BrUra (supplied by M. Vanderlaan, Lawrence Livermore National Laboratory) as previously described (12). Heat-denatured DNA (50 μg) was incubated with the antibody (30 μg) in phosphate-buffered saline containing 0.1% bovine serum albumin for 1 h at 37°C. An equal volume of ice-cold saturated ammonium sulfate in phosphate-buffered saline was added, and the incubation continued overnight at 4°C. The DNA bound by the antibody was collected as a pellet by centrifugation for 10 min in a microfuge (Beckman Instruments, Inc., Fullerton, Calif.) at 4°C. The supernatant was carefully removed, and the pellet was dissolved in double-distilled water. Aliquots of the supernatant and pellet were assayed for radioactivity by liquid scintillation counting to determine the relative amount of DNA bound by the antibody.

Equal amounts of DNA, based on the ^{3}H prelabel, from the supernatant and pellet were electrophoresed on 0.7% neutral agarose gels. After electrophoresis, the DNA was transferred to a nitrocellulose membrane. Hybridizations with the ^{32}P-nick-translated probe phMT-II_3 were carried out in 6× SSPE (SSPE = 100 mM NaCl, 10 mM $NaPO_4$ [pH 7.8], 1 mM EDTA), 1× Denhardt solution, 300 μg of denatured salmon sperm DNA per ml, 0.5% SDS, and 50% formamide for 48 h at 42°C. After hybridization, the membranes were washed and exposed to XAR-5 X-ray film (Eastman Kodak Co., Rochester, N.Y.). The intensity of hybridization to the fragments of interest was measured by scanning densitometry of the autoradiogram. The value for the density of each fragment was multiplied by the amount of DNA in the bound or free fractions to obtain the total amount of each gene in each fraction. The percentage of each hMT gene bound by the antibody was then calculated from the total amount of the gene in the bound fraction divided by the total amount of the gene in the bound-plus-free fractions.

RNA isolation and analysis. Total RNA was isolated by lysing cultures in 4 M guanidinium isothiocyanate–5 mM sodium citrate (pH 7)–0.1 M 2-mercaptoethanol–0.5% sarcosine and purified by centrifugation through a cushion of 5.7 M CsCl–0.1 M EDTA (pH 7.5). The RNA pellet was suspended in 10 mM Tris (pH 7.5)–1 mM EDTA–1% SDS, extracted once with an equal volume of chloroform–1-butanol (4:1), and precipitated in ethanol. The RNA was then suspended in 10 mM Tris (pH 8)–1 mM EDTA and analyzed either by electrophoresis on 1.5% formaldehyde agarose gels and transferred to GeneScreen Plus membranes (Dupont, NEN Research Products, Boston, Mass.) (18) or by direct slot blotting of the RNA onto mRNC nitrocellulose (Schleicher & Schuell, Inc., Keene, N.H.). Hybridizations to mRNA on GeneScreen Plus membranes were carried out in a solution of 5× SSPE, 1% SDS, 200 μg of denatured salmon sperm DNA per ml, 50 μg of poly(A) per ml, and 50% formamide for 48 h at 42°C. Hybridizations to mRNA slot blotted onto nitrocellulose were carried out in a solution of 6× SSPE, 1% SDS, 250 μg of denatured salmon sperm DNA per ml, 62.5 μg of poly(A) per ml, and 50% formamide for 48 h at 42°C. The blots were probed sequentially for hMT mRNA and 18S rRNA. The levels of hMT mRNA were normalized to the amount of 18S rRNA to correct for any differences in the amount of RNA loaded.

Probes. A full-length cDNA clone of human hMT-II mRNA, phMT-II_3 (provided by M. Karin, University of California at San Diego), was used to detect the hMT genes in human genomic DNA. A *Bam*HI-*Pvu*II fragment of phMT-II_3, which contains the hMT-II coding region, was used to detect hMT mRNA, a *Pvu*II-*Pvu*I fragment of phMT-II_3, containing the 3′ untranslated region of this cDNA, was used to detect specifically the hMT-II_A mRNA (8), and an *Eco*RI fragment of pLS2 (obtained from J. Sylvester, University of Pennsylvania, Philadelphia), which contains most of a human 18S ribosomal gene, was used to detect 18S rRNA.

RESULTS

The basic approach used here to study repair in specific sequences involves the physical separation of DNA regions containing BrdUrd substitution in repair patches from all other DNA by using a monoclonal antibody against BrdUrd (12). We applied this method to examine whether the repair of damage in selected members of the hMT gene family is different from that in the genome overall. Human cell cultures, labeled in their DNA with [^{3}H]thymidine, were exposed to a DNA-damaging agent and then allowed to repair for various lengths of time in the presence of BrdUrd. Purified restriction enzyme-digested, parental-density (unreplicated) DNA was reacted with a monoclonal antibody that binds BrdUrd in DNA, and total repair was assessed by measuring the amount of ^{3}H-labeled DNA bound by the antibody. Repair in the hMT genes was determined by electrophoresing on agarose gels equal amounts of DNA from the bound and free fractions and then quantitating the amount of hybridization that occurred to a specific restriction fragment in each fraction (29, 31). The amount of hybridization was then compared with the amount of total DNA in each fraction.

Repair was examined in the hMT genes by using a full-length cDNA probe of hMT-II RNA, phMT-II$_3$. Because of the extensive homology (at least 80%) of the members of this gene family to the coding region of hMT-II, this probe hybridizes to 12 different bands when genomic DNA is digested with *Eco*RI (9). These hybridizing bands correspond to DNA fragments containing the multiple members of the hMT gene family. Our studies focused on five members of this gene family: the hMTI$_A$ and hMTI$_E$ genes, located on a single 10-kilobase (kb) fragment; the hMTI$_B$ gene, located on a 14-kb fragment; the hMTII$_A$ gene, located on a 5.9-kb fragment; and the hMT-II$_B$ gene, which, because of a polymorphic *Eco*RI site in its 5′-flanking region, is on two fragments of 4.8 and 4.6 kb, respectively (9, 16, 27). The size of the transcriptional units of hMT-I$_A$, hMT-I$_E$, hMT-I$_B$, and hMT-II$_A$ is approximately 1 kb, whereas the size of hMT-II$_B$ is approximately 0.5 kb.

Early repair of UV and AFB$_1$ damage in the hMT genes. Since preferential repair in the human *DHFR* gene was found to occur at early times after exposure to UV light (20), we initially examined repair in the hMT genes during the first 6 h posttreatment. Human cells were exposed to 10 J of UV per m^2 and allowed to repair for various lengths of time in the presence of BrdUrd. Visual comparison of the samples from the fractions of DNA bound by the antibody (i.e., containing repair patches) and from fractions of DNA free of the antibody (i.e., not containing repair patches) on agarose gels by fluorescent staining verifies that equal amounts of DNA from each fraction were electrophoresed (Fig. 1A). However, a comparison of the intensities of hybridization to restriction fragments containing the hMT genes from the same gel indicates a substantial difference in the amount of some of the genes in the bound and free fractions (Fig. 1B). The intensity of hybridization was quantitated by densitometry, and the percentage of each gene in the bound fraction per microgram of DNA was compared with the percentage of total DNA bound by the antibody (Fig. 2). An increase in the percentage of the hMT genes in the DNA bound by the antibody was found with post-UV incubation. Between 0 and 6 h post-UV, much higher percentages of the transcribed hMT-I$_A$, hMT-I$_E$, and hMT-II$_A$ genes than of total DNA were found in the repaired fraction, whereas the percentages of the hMT-I$_B$ and hMT-II$_B$ genes in the antibody-bound fraction were similar to the percentage of total DNA.

We previously demonstrated that in confluent cultures of African green monkey cells, there was a deficient repair of damage induced by AFB$_1$ in the nontranscribed alpha sequence even though repair of UV damage in this sequence was about the same as in the bulk of the genome (12, 14). To determine whether there might be a differential repair of damage depending on the type of damage introduced into the DNA sequence in the nontranscribed hMT genes, we measured repair in these after exposure of cells to AFB$_1$.

Cell cultures were exposed to 80 μM AFB$_1$ and then allowed to repair in the presence of BrdUrd. As with UV-induced damage, AFB$_1$-induced DNA damage was repaired approximately 2.5 times faster in the transcribed hMT-I$_A$, hMT-I$_E$, and hMT-II$_A$ genes than in total DNA by 6 h posttreatment (Table 1). However, in contrast to our findings with UV, repair of AFB$_1$ damage was approximately three times slower in the hMT-I$_B$ and hMT-II$_B$ genes than in the genome overall. Therefore, the efficiency of repair of damage in a nontranscribed sequence appears also to be dependent on the type of damage produced in that sequence.

Repair in the hMT genes induced with CdCl$_2$ or dexamethasone. Since an initial rapid repair of UV damage was observed in regions of the genome containing the hMT-I$_A$, hMT-I$_E$, and hMT-II$_A$ genes when they were being transcribed at a basal level, we examined the effect of the induction of transcription of the hMT genes on the efficiency of repair. Northern (RNA) and slot blot analysis showed that incubation of cells in the presence of 2.5 μM CdCl$_2$ produced a 15- to 20-fold increase in the level of hMT mRNA, which reached a plateau between 12 and 18 h. Further increasing either the concentration of CdCl$_2$ (up to 10 μM) or the length of time (up to 24 h) that the cells were exposed to it did not result in a greater induction of hMT mRNA. In fact, incubation for time periods of longer than 18 h resulted in a decrease from the maximally induced levels of mRNA. The conditions used for induction of the hMT genes did not produce any detectable DNA damage, nor did they affect the overall level of repair of UV damage in these cells (data not shown). For repair experiments, cell cultures were incubated in the presence of 2.5 μM CdCl$_2$ for 12 h before exposure to UV light and for up to 6 h during the post-UV incubation. When cells incubated with CdCl$_2$ were irradiated with UV at 10 J/m^2, higher percentages of the hMT-I$_A$, hMT-I$_E$, and hMT-II$_A$ genes were in the repaired fraction of the DNA than were in the repaired fraction under conditions in which the genes were being transcribed at a basal level (Fig. 3). Repair in the hMT-I$_B$ and hMT-II$_B$ genes was about the same as in total DNA regardless of whether hMT gene transcription was induced (data not shown).

Since the initial rate of repair of UV damage in the hMT-I$_A$, hMT-I$_E$, and hMT-II$_A$ genes was increased at least twofold when transcription of these genes is induced by incubation of the cells with CdCl$_2$, and since most of the hMT genes have been shown to be clustered on the same chromosome (8), we examined the effect of selective induction of the hMT-II$_A$ gene alone with dexamethasone on the repair of noninduced hMT genes that may be in relatively close proximity. Conditions for maximal induction of the hMT-II$_A$ gene by dexamethasone were determined by Northern and slot blot analysis of hMT-II$_A$ mRNA levels. Incubation of cell cultures with 0.1 μM dexamethasone produced a fivefold increase in the level of hMT-II$_A$ mRNA, which reached a plateau between 12 and 24 h. Treatment of cells with up to 1.0 μM dexamethasone for the same time

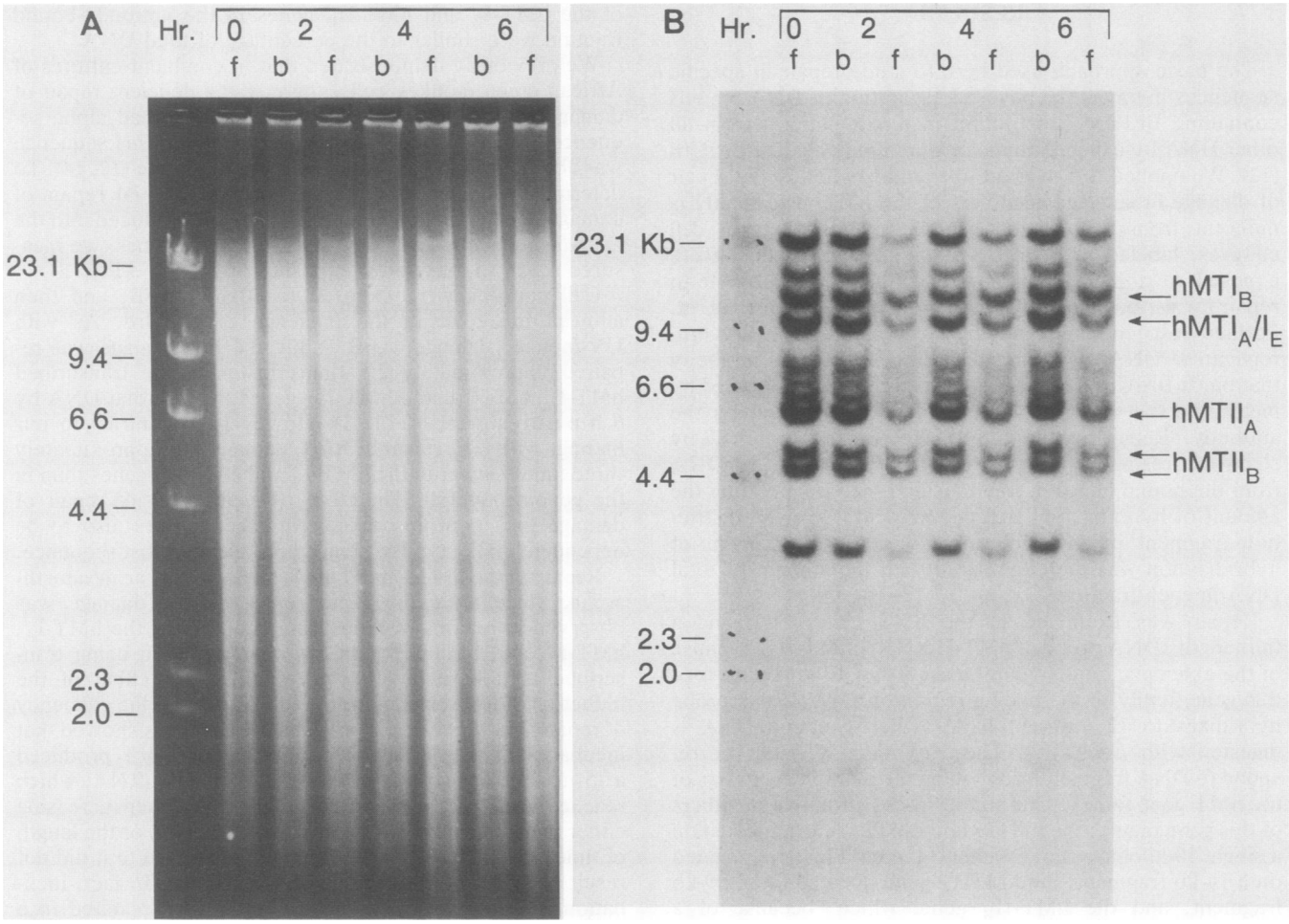

FIG. 1. Southern analysis of repair in hMT genes. Cells were exposed to UV at 10 J/m^2 and allowed to repair for various lengths of time in the presence of 10 μM BrdUrd. Genomic DNA, digested with *Eco*RI, was reacted with the antibody to BrdUrd. (A) Ethidium bromide-stained agarose gel (0.7%) of DNA from the fractions bound (b) by the antibody or free (f). (B) Autoradiogram of Southern transfer of DNA from the gels in panel A hybridized with the probe phMT-II$_3$. Positions of the hMT genes studied are identified by the arrows. Size markers are indicated.

period did not further increase the levels of mRNA. Cell cultures were incubated in the presence of 0.1 μM dexamethasone for 15 h before exposure to UV and for 6 h during the post-UV incubation. After UV irradiation of the cells at 10 J/m^2, a higher percentage of the hMT-II$_A$ gene was in the repaired fraction following the selective induction of its transcription than was found when the gene was being transcribed at a basal level (Fig. 3). Repair in DNA fragments containing the hMT-I$_A$ gene was still greater than in the total DNA but was not increased by treatment of the cells with dexamethasone. Repair in the hMT-I$_B$ and hMT-II$_B$ genes was about the same as in total DNA regardless of whether hMT-II$_A$ gene transcription was induced.

Since the transcribed hMT genes were repaired more rapidly than were the nontranscribed genes and this difference in the rate of repair was accentuated when transcription was induced above the basal levels, we examined the effect of induction on the kinetics of hMT transcription after UV irradiation. Cell cultures were incubated in either the presence or the absence of 2.5 μM $CdCl_2$ for 12 h before being irradiated with 10 J of UV per m^2 or sham irradiated. The cells were then allowed to continue to incubate with or without $CdCl_2$, and total cytoplasmic RNA was extracted at various times. Specific mRNA was detected by both Northern and slot blot analysis, using the full-length cDNA probe of hMT-II RNA contained in phMT-II$_3$, and was quantitated by densitometry of the autoradiogram. For each sample, the amount of hybridization to hMT mRNA was normalized to the amount of 18S RNA. A comparison of the amount of hMT mRNA present in irradiated and unirradiated cells showed that regardless of whether the hMT genes had been induced, hMT mRNA levels in irradiated cells decreased approximately twofold by 1 h post-UV and returned to unirradiated levels by 4 h post-UV (Fig. 4). Therefore, inducing transcription of the hMT genes did not result in a more rapid recovery of their transcription after UV irradiation. It has been suggested that the hMT genes may be part of a stress response in human cells because hMT mRNA levels were found to be induced 48 h after UV irradiation (1). In our experiments, however, no induction of hMT mRNA was observed in irradiated cells for up to 24 h post-UV.

Late repair of UV and AFB$_1$ damage in the hMT genes. Repair in the hMT genes was also examined at times later than 6 h after exposure of cells to UV (10 J/m^2) or AFB$_1$ (80 μM). Between 6 and 24 h post-UV, the rate of repair in DNA fragments containing the hMT-I$_A$, hMT-I$_E$, hMT-II$_A$, and

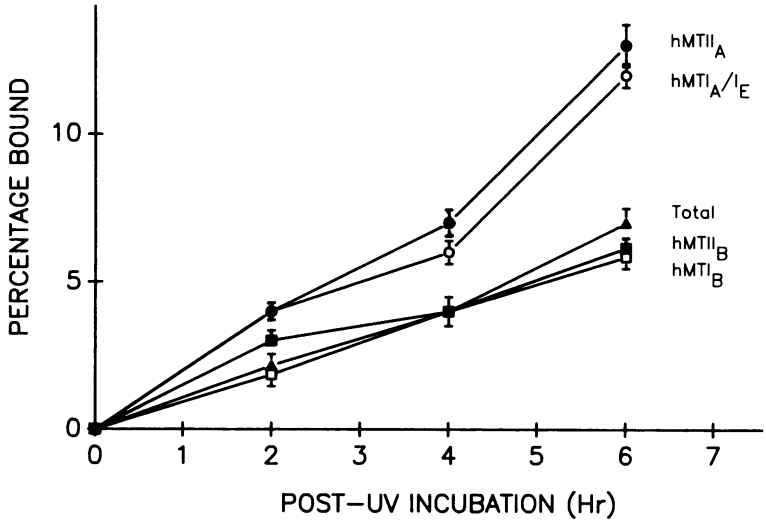

FIG. 2. Kinetics of early repair in hMT genes. Cells were exposed to UV at 10 J/m^2 and allowed to repair for various lengths of time. The percentage of total DNA bound by the antibody was determined from the ^{3}H prelabel. The percentages of the individual hMT genes in the fraction bound by the antibody were determined by densitometry of the autoradiogram. Points indicate means of seven determinations; bars indicate standard errors.

hMT-II$_B$ genes slowed down compared with the rate in total DNA, reaching a plateau by 24 h (Table 1). However, repair in the DNA fragment containing the hMT-I$_B$ gene continued to increase by 24 h, as did repair in total DNA. Between 6 and 24 h after exposure to AFB$_1$, the rate of repair in the transcribed hMT genes also began to slow down compared with the rate in total DNA and leveled out by 24 h. Rates of repair in the DNA fragments containing the hMT-II$_B$ and hMT-I$_B$ genes continued to increase during the longer post-treatment incubation but were still only 30 and 40%, respectively, of that observed in total DNA. Thus, the initial higher rate of repair found in those DNA fragments containing the transcribed hMT genes was not observed at later times.

DISCUSSION

We examined the repair of UV- and AFB$_1$-induced damage in five members of the hMT gene family that are in different functional states. We used an assay that allows direct comparison of repair in these sequences for several classes of DNA damage and found that, initially, DNA repair was more efficient in the three transcriptionally active genes than in the bulk of the genome. Repair of UV damage in a nontranscribed hMT pseudogene and in an hMT gene whose transcription shows cell-type specificity was initially about the same as in the rest of the genome, whereas repair of AFB$_1$-induced damage in these two genes was deficient compared with repair in the genome overall.

TABLE 1. Repair of AFB$_1$ and UV damage in hMT genes

Agent	Time (h)	% Bound[a]				
		Total	hMT-I$_A$–hMT-I$_E$	hMT-II$_A$	hMT-I$_B$	hMT-II$_B$
AFB$_1$ (80 μM)	6	3	7	8	1	1
	12	7	11	10	3	2
	24	13	17	15	5	4
UV (10 J/m^2)	6	5	12	13	4	5
	12	11	16	14	11	7
	24	25	20	15	23	11

[a] Values represent the means of seven experiments. Standard errors were 10% or less of the means.

In our experiments, repair is measured as the percentage of a DNA sequence that is bound by an antibody which recognizes and binds a BrUra-substituted repair patch in single-stranded DNA. We have previously shown that increasing the number of repair patches substituted with BrUra, by increasing either the initial amount of UV damage or the post-UV incubation period, increases the total amount of DNA bound by the antibody and that the percentage of the repair patches detected by the antibody is independent of fragment size for DNA fragments of between 0.2 and 15 kb (12). Therefore, we are able to measure repair in most of the hMT genes simultaneously even though they are contained on DNA fragments of more than one size. In this study, we irradiated cells with a fluence of 10 J of UV per m^2, which produces approximately 0.8 pyrimidine dimers per 10 kb (30). Based on their size, the restriction fragments containing the hMT genes should contain between 0.4 and 1.1 pyrimidine dimers. Therefore, a single repair patch within a DNA fragment will reflect complete repair of damage on that fragment. Although our assay measures the repair event itself, it does not provide information as to the initial lesion frequency; therefore, differences in repair between DNA sequences could be attributed to both differential rates of repair and a nonrandom lesion distribution. However, in our previous studies and those of others, no significant differences have been detected in the initial levels of damage produced by UV, AFB$_1$, and several other agents in a variety of DNA sequences (3, 14, 17, 21).

The repair kinetics for damage in DNA fragments containing the three transcriptionally active hMT genes (hMT-I$_A$, hMT-I$_E$, and hMT-II$_A$) showed two components: an initial fast rate of repair during the first 6 h posttreatment, followed by a much slower rate between 12 and 24 h posttreatment. The initial repair rates are independent of the size of the restriction fragments that contain the genes; however, the extent of repair observed for each gene corresponds to the overall fragment size and hence to the expected frequency of initial damage in each fragment. One explanation for the

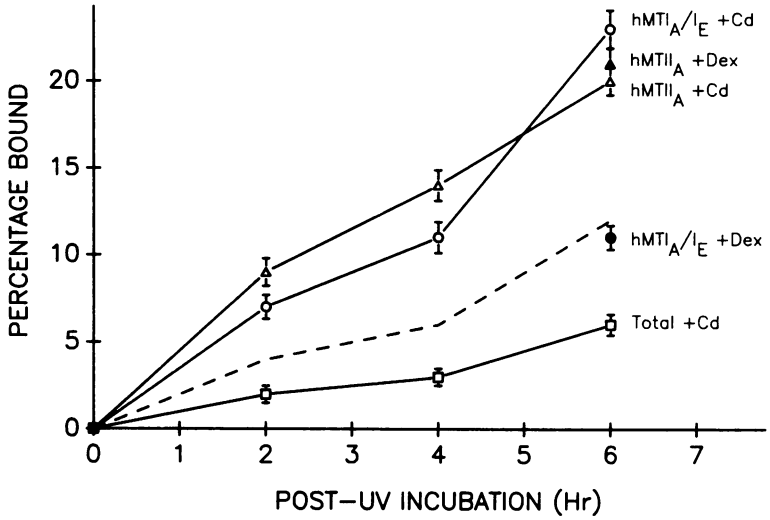

FIG. 3. Kinetics of early repair in induced hMT genes. Transcription of the hMT genes was induced before exposure of the cells to UV at 10 J/m^2 and maintained during the repair period. Points indicate means of three determinations; bars indicate standard errors. Shown are percentages of total DNA (□), hMT-I$_A$–I$_E$ (○), and hMT-II$_A$ (△) bound by the antibody after induction with 2.5 μM $CdCl_2$; hMT-I$_A$–I$_E$ (●) and hMT-II$_A$ (▲) after induction with 0.1 μM dexamethasone; and hMT-I$_A$–I$_E$ (– – –) without induction, taken from Fig. 2.

biphasic kinetics is that the initial rates reflect a very rapid repair of damage in the transcribed hMT genes, with the slower rates at later times corresponding to repair in other sequences on these fragments. Since little is known about other sequences contained on these fragments, including whether these surrounding sequences are transcriptionally active, it will be of interest to determine the repair kinetics for sequences that flank the transcriptionally active hMT genes.

In contrast to rates of repair for the hMT-I$_A$, hMT-I$_E$, and hMT-II$_A$ genes, the rate of repair for the DNA fragment containing the hMT-I$_B$ gene is linear for up to 24 h post-UV and similar to the rate of repair in total DNA. The similarity in the rate of repair of that for total DNA indicates that this gene is not being preferentially repaired and is consistent with the idea that the gene is not transcribed in this cell type. The linearity in the kinetics of repair for up to 24 h compared with the biphasic kinetics for the other hMT genes is not surprising, since this gene is contained on the largest fragment studied and should have the highest frequency of initial damage. Consequently, repair of damage is still occurring on this DNA fragment, whereas it has gone to completion on smaller fragments. The repair kinetics observed for the DNA fragment containing the nontranscribed hMT-II$_B$ pseudogene show an initial rate similar to that for total DNA, with a slower rate of repair after 6 h. Since this DNA fragment should contain the least amount of damage because of its small size, the plateau in repair at later times probably reflects the completion of repair.

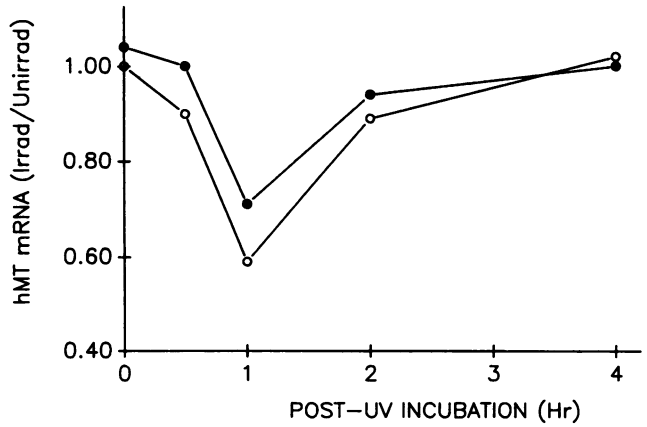

FIG. 4. Levels of hMT mRNA after UV irradiation. The amount of hMT mRNA was measured in cells, induced with 2.5 μM $CdCl_2$ or uninduced, by Northern and slot blot analysis at various times after a 10-J/m^2 dose of UV. The amount of hMT mRNA at each time point from induced (○) and uninduced (●) cells was normalized to the amount of 18S rRNA to correct for any differences in the quantity of RNA loaded and is expressed as the ratio of hMT mRNA in samples from irradiated cells to that in unirradiated cells.

There are a number of factors that not only distinguish transcriptionally active DNA from inactive regions of the genome but also are involved in the fine control of gene expression. Several of these, including the chromatin conformation associated with a DNA sequence and perhaps even the transcriptional complex itself, appear to affect the efficiency of DNA repair in mammalian cells. Several groups have shown correlations between increased repair efficiencies and various structural features of mammalian chromatin such as the nuclear matrix (22), DNA loops (5), and relaxed chromatin structures (25). The chromatin structure associated with active genes renders these sequences highly sensitive to digestion by DNase I, indicating greater accessibility of this DNA in chromatin (32). The mouse MT-I gene is highly sensitive to micrococcal nuclease digestion when it is actively transcribed but shows a sensitivity intermediate between that of active genes and nontranscribed sequences when it is in a quiescent state (11). A further increase in nuclease sensitivity is observed in the mouse MT gene after induction with cadmium (10). We find that increasing transcription of the hMT genes by induction with either a heavy metal or a glucocorticoid hormone also increases the rate of repair in the induced genes but does not affect the efficiency of repair in the uninduced genes. Increased repair in an amplified CHO MT-I gene after induction with a heavy metal has also been observed recently (23).

Induction of the MT genes by heavy metals and glucocorticoids is regulated primarily at the level of transcription initiation, although there is a small effect of glucocorticoid induction on MT mRNA stability (6, 8). The more efficient repair in the induced hMT-I_A gene appears to be relatively localized in that it does not affect the efficiency of repair in the nearby hMT-I_B gene (approximately 12 kb downstream; 24). Therefore, increased repair efficiency may be due to both the more open chromatin conformation associated with transcriptionally active DNA and a localized strand-specific repair reported by Mellon et al. (21), in which the transcribed strand of both the hamster and the human *DHFR* gene is preferentially repaired compared with the nontranscribed strand. It is hypothesized that the arrest of transcription at a lesion in the DNA selectively directs repair to the transcribed strand. If transcription or the transcriptional complex directs repair of the transcribed strand, then this process could account for the further increase in repair efficiency of a gene when its transcription is induced. Further studies are necessary to determine whether there is strand-specific repair in the transcriptionally active hMT genes, what effect inducing transcription of these genes has on that repair, and whether strand-specific repair occurs for more than one type of damaging agent.

DNA fragments containing the hMT-I_B and hMT-II_B genes did not show preferential repair of UV damage compared with the rest of the genome and in fact showed deficient repair of AFB_1 damage. This suggests that one or more of the factors that influence the efficiency of repair of a DNA sequence are altered compared with the transcriptionally active genes. The deficient repair of AFB_1 damage in these two genes suggests that they may possess chromatin conformations significantly different from those of transcribed genes. A more compact chromatin structure associated with the heterochromatic, nontranscribed alpha DNA of African green monkey cells is thought to account for the deficient repair of certain bulky chemical adducts in this DNA sequence (12, 14, 33). A more compact chromatin structure may also be associated with regions surrounding developmentally regulated genes, such as hMT-I_B, when they are in a highly methylated, nonexpressed state and with regions surrounding nonexpressed, processed pseudogenes, such as hMT-II_B. The observation that repair of AFB_1 damage in the hMT-I_B gene is deficient may also provide important insights into the processes involved in the activation of proto-oncogenes that are developmentally regulated. At times in development when these genes are not expressed, their chromatin structure may render them more prone to genetic alterations because of a deficiency in repair. Subsequent transcriptional activation of the altered gene might then contribute to the malignant phenotype.

We found that after an initial decrease in hMT mRNA levels after UV damage, hMT transcription recovered rapidly, well before significant levels of repair were detected in the genome overall. A similarly rapid recovery in total RNA synthesis has previously been reported for human cells irradiated with UV (19). The decrease probably reflects the inhibition of transcription due to damage in the DNA template (26). Since hMT mRNA has a half-life of only 2 h, a decrease would be noted relatively early after UV irradiation. However, the extent of the decrease in mRNA levels is greater than would be predicted if the inhibition were due to the transcriptional apparatus encountering a pyrimidine dimer within the gene. At a fluence of 10 J of UV per cm^2, approximately 92% of the hMT genes will not contain pyrimidine dimers in their transcribed strands. This dose, however, produces a 30% decrease in hMT mRNA levels. In addition, inducing transcription of the hMT genes did not affect the time required for transcription of the genes to recover after UV. Therefore, the target for the inhibition of RNA synthesis may be larger than the gene itself, such that more efficient repair in the gene alone is not sufficient to shorten the recovery period. DNA damage in regions outside the transcriptional unit of the gene may also be inhibiting transcription of that gene. These could be regions containing promoter or enhancer sequences upstream of the transcriptional start site that are crucial for proper assembly of promoter-binding proteins and RNA polymerase. Within this context, the return in hMT mRNA levels by 4 h probably reflects the rapid repair of damage both in the genes and in regions adjacent to the genes. It will be of interest to determine what effect DNA damage has on the binding of transcriptional factors, such as Sp1 and AP1, to controlling sequences in the 5′-flanking region of hMT-II_A (15) and what role repair in these controlling sequences might play either in the recovery of transcription after DNA damage or in facilitation of strand-specific repair. The observed decrease followed by a rapid resumption in hMT mRNA synthesis after UV irradiation may be a common feature of transcribed genes, since a similar pattern for the inhibition and recovery of transcription for the α-actin gene was also found after UV irradiation (S. A. Leadon and M. M. Snowden, unpublished observations).

ACKNOWLEDGMENTS

We thank P. K. Cooper, I. Mellon, and C. A. Smith for many helpful discussions and critical reading of the manuscript.

This work was supported by Public Health Service grant CA40453 from the National Cancer Institute, and was conducted at the Lawrence Berkeley Laboratory (Department of Energy contract DE-AC03-76SF00098 to the University of California).

LITERATURE CITED

1. **Angel, P., A. Poting, U. Mallick, H. J. Rahmsdorf, M. Schorpp, and P. Herrlich.** 1986. Induction of metallothionein and other mRNA species by carcinogens and tumor promoters in primary human skin fibroblasts. Mol. Cell. Biol. **6**:1760–1766.
2. **Bohr, V. A., D. S. Okumoto, L. Ho, and P. C. Hanawalt.** 1986. Characterization of a DNA repair domain containing the dihydrofolate reductase gene in Chinese hamster ovary cells. J. Biol. Chem. **261**:16666–16672.
3. **Bohr, V. A., C. A. Smith, D. S. Okumoto, and P. C. Hanawalt.** 1985. DNA repair in an active gene: removal of pyrimidine dimers from the DHFR gene of CHO cells is much more efficient than in the genome overall. Cell **40**:359–369.
4. **Croce, C. M.** 1976. Loss of mouse chromosomes in somatic cell hybrids between HT-1080 human fibrosarcoma cells and mouse peritoneal macrophages. Proc. Natl. Acad. Sci. USA **73**:3248–3252.
5. **Gupta, R. C., N. R. Digue, K. Randerath, and H. C. Smith.** 1986. Distribution of initial and persistent 2-acetylaminofluorene-induced DNA adducts within DNA loops. Proc. Natl. Acad. Sci. USA **82**:6605–6608.
6. **Hager, L. J., and R. D. Palmiter.** 1981. Transcriptional regulation of mouse liver metallothionein-I gene by glucocorticoids. Nature (London) **291**:340–342.
7. **Heguy, A., A. West, R. I. Richards, and M. Karin.** 1986. Structure and tissue-specific expression of the human metallothionein I_B gene. Mol. Cell. Biol. **6**:2149–2157.
8. **Karin, M., R. L. Eddy, W. M. Henry, L. L. Haley, M. G. Byers, and T. B. Shows.** 1984. Human metallothionein genes are clustered on chromosome 16. Proc. Natl. Acad. Sci. USA **81**:5494–5498.
9. **Karin, M., and R. I. Richards.** 1982. Human metallothionein genes—primary structure of the metallothionein-II gene and a

related processed gene. Nature (London) **299**:797–802.
10. **Koropatnick, J., G. Andrews, and J. D. Duerksen.** 1983. Mouse hepatic metallothionein-I gene cleavage by micrococcal nuclease is enhanced after induction by cadmium. Nucleic Acids Res. **11**:3255–3267.
11. **Koropatnick, J., and J. D. Duerksen.** 1987. Nuclease sensitivity of α-fetoprotein, metallothionein-1, and immunoglobin gene sequences in mouse during development. Dev. Biol. **122**:1–10.
12. **Leadon, S. A.** 1986. Differential repair of DNA damage in specific nucleotide sequences in monkey cells. Nucleic Acids Res. **14**:8979–8995.
13. **Leadon, S. A., R. M. Tyrrell, and P. A. Cerutti.** 1981. Excision repair of aflatoxin B_1-DNA adducts in human fibroblasts. Cancer Res. **41**:5125–5129.
14. **Leadon, S. A., M. E. Zolan, and P. C. Hanawalt.** 1983. Restricted repair of aflatoxin B_1 induced damage in alpha DNA of monkey cells. Nucleic Acids Res. **11**:5675–5689.
15. **Lee, W., A. Haslinger, M. Karin, and R. Tijan.** 1987. Activation of transcription by two factors that bind promoter and enhancer sequences of the human metallothionein gene and SV40. Nature (London) **325**:368–372.
16. **Lieberman, H. B., M. Rabin, P. E. Barker, F. H. Ruddle, U. Varshney, and L. Gedamu.** 1985. Human metallothionein-II processed gene is located in region p11-q21 of chromosome 4. Cytogenet. Cell Genet. **39**:109–115.
17. **Madhani, H. D., V. A. Bohr, and P. C. Hanawalt.** 1986. Differential DNA repair in transcriptionally active and inactive proto-oncogenes: c-*abl* and c-*mos*. Cell **45**:417–423.
18. **Maniatis, T., E. F. Fritsch, J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
19. **Mayne, L. V., and A. R. Lehmann.** 1982. Failure of RNA synthesis to recover after UV-irradiation: an early defect in cells from individuals with Cockayne's syndrome and xeroderma pigmentosum. Cancer Res. **42**:1473–1478.
20. **Mellon, I., V. A. Bohr, C. A. Smith, and P. C. Hanawalt.** 1986. Preferential DNA repair of an active gene in human cells. Proc. Natl. Acad. Sci. USA **83**:8878–8882.
21. **Mellon, I., G. Spivak, and P. C. Hanawalt.** 1987. Selective removal of transcription-blocking DNA damage from the transcribed strand of the mammalian DHFR gene. Cell **51**:241–249.
22. **Mullenders, L. H. F., A. C. van Kesteren, C. J. M. Bussman, A. A. van Zeeland, and A. T. Natarajan.** 1984. Preferential repair of nuclear matrix associated DNA in xeroderma pigmentosum complementation group C. Mutat. Res. **141**:75–82.
23. **Okumoto, D. S., and V. A. Bohr.** 1987. DNA repair in the metallothionein gene increases with transcriptional activation. Nucleic Acids Res. **15**:10021–10030.
24. **Richards, R. I., A. Heguy, and M. Karin.** 1984. Structural and functional analysis of the human metallothionein-I_A gene: differential induction by metal ions and glucocorticoids. Cell **37**: 263–272.
25. **Ryan, A. J., M. A. Billet, and P. J. O'Connor.** 1986. Selective repair of methylated purines in regions of chromatin DNA. Carcinogenesis **7**:1497–1503.
26. **Sauerbier, W.** 1976. UV damage at the transcriptional level. Adv. Radiat. Biol. **6**:49–106.
27. **Schmidt, C. J., D. H. Hamer, and O. W. McBride.** 1984. Chromosomal location of human metallothionein genes: implications for Menkes' disease. Science **224**:1104–1106.
28. **Smith, C. A., P. K. Cooper, and P. C. Hanawalt.** 1981. Measurement of repair replication by equilibrium sedimentation, p. 289–306. *In* E. C. Friedberg and P. C. Hanawalt (ed.), DNA repair: a laboratory manual of research procedures, vol. 1B. Marcel Dekker, Inc., New York.
29. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
30. **van Zeeland, A. A., C. A. Smith, and P. C. Hanawalt.** 1981. Sensitive determination of pyrimidine dimers in DNA of UV-irradiated mammalian cells: introduction of T4 endonuclease V into frozen and thawed cells. Mutat. Res. **82**:173–189.
31. **Wahl, G. M., M. Stern, and G. R. Stark.** 1979. Efficient transfer of large DNA fragments from agarose gels to diazobenzyloxymethyl-paper and rapid hybridization by using dextran sulphate. Proc. Natl. Acad. Sci. USA **76**:3683–3687.
32. **Weintraub, H., and M. Groudine.** 1976. Chromosomal subunits in active genes have an altered conformation. Science **193**:848–856.
33. **Zolan, M. E., G. A. Cortopassi, C. A. Smith, and P. C. Hanawalt.** 1982. Deficient repair of chemical adducts in alpha DNA of monkey cells. Cell **28**:613–619.