Cyclobutane Pyrimidine Dimers and Bulky Chemical DNA Adducts Are Efficiently Repaired in Both Strands of Either a Transcriptionally Active or Promoter-deleted APRT Gene*

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Both prokaryotic and eukaryotic cells have the capacity to repair DNA damage preferentially in the transcribed strand of actively expressed genes. However, we have found that several types of DNA damage, including cyclobutane pyrimidine dimers (CPDs) are repaired with equal efficiency in both the transcribed and non-transcribed strands of the adenine phosphoribosyltransferase (APRT) gene in Chinese hamster ovary cells. We further found that, in two mutant cell lines in which the entire APRT promoter region has been deleted, CPDs are still efficiently repaired in both strands of the promoterless APRT gene, even though neither strand appears to be transcribed. These results suggest that efficient repair of both strands at this locus does not require transcription of the APRT gene. We have also mapped CPD repair in exon 3 of the APRT gene in each cell line at single nucleotide resolution. Again, we found similar rates of CPD repair in both strands of the APRT gene domain in both APRT promoter-deletion mutants and their parental cell line. Our findings suggest that current models of transcription-coupled repair and global genomic repair may underestimate the importance of factors other than transcription in governing the efficiency of nucleotide excision repair.

The original findings of Hanawalt and colleagues (1, 2), which demonstrate that ultraviolet (UV) light-induced cyclobutane pyrimidine dimers (CPDs) are repaired much more efficiently in an actively transcribed gene, such as dihydrofolate reductase (DHFR) than in noncoding regions, and that repair is more efficient in the transcribed strand of such genes than in the nontranscribed strand, have been extended to a number of gene loci, in a wide range of prokaryotic and eukaryotic cells (3–8).

In mammalian cells, nucleotide excision repair (NER) is thought to involve two distinct subpathways: transcription-coupled repair (TCR), which selectively and very efficiently repairs transcription-blocking damage in the transcribed strand of actively expressed genes, and global genomic repair (GGR), which is responsible for repairing damage in the non-transcribed strand and the rest of the genome (2, 5–8). TCR occurs only in genes that are transcribed by RNA polymerase II. Cells from individuals with Cockayne’s syndrome (CS) are competent in GGR but deficient in TCR (9–11). Interestingly, even though CS cells are deficient in TCR, repair in actively transcribed genes is still significantly more efficient that repair in inactive regions of the genome. Cells from xeroderma pigmentosum complementation group C (XPC) patients are competent in repair of CPDs in transcriptionally active genes (with repair occurring primarily in the transcribed strand) but are defective in GGR (12, 13). Chinese hamster ovary (CHO) cells are profoundly deficient in global genomic repair of CPDs, typically showing efficient TCR of CPDs in the transcribed strand of actively transcribed genes, but little repair in the nontranscribed strand, or in nontranscribed regions of the genome (2, 14, 15).

Not all genes, however, show the characteristic pattern of preferential, TCR of transcription-blocking damage on the transcribed strand, originally described for the DHFR gene. Venema, Treolstra, van Hoffen, and colleagues (9–13) have found that, in normal human cells, CPDs in both the template and nontemplate strands of the transcriptionally active adenine deaminase (ADA) gene appear to be “preferentially” repaired. In XPC cells, CPDs in the 5′-portion of the ADA gene were found to be repaired much more efficiently in the transcribed strand than in the nontranscribed strand, but CPDs in the 3′-portion of the ADA gene were efficiently repaired in both strands (12, 13). Furthermore, in a patient with severe combined immune deficiency, in which the ADA promoter region has been deleted and the gene is not expressed, the efficiency of CPD repair was found to be only slightly reduced compared with that normally seen for the transcribed strand of an intact ADA gene (16, 17). Efficient repair of CPDs on both strands of an actively transcribed mammalian gene has been reported for two other gene loci, the human β-actin gene (18) and the Chinese hamster adenine phosphoribosyltransferase (APRT) gene (19); however, in both of these studies, the large size of the genomic fragment used to assay CPD repair, in relation to the size of the transcribed region of the gene, complicated interpretation of the results.
FIG. 1. Removal of CPDs from the APRT gene in UV-irradiated (20 J/m²) AT3-2 cells. DNAs isolated from cells after various post-irradiation incubation periods were digested with BamHI then treated with T4 endoV (protein/DNA molar ratio of 6:1). These DNAs were then denatured, separated by electrophoresis, transferred to an Oncor membrane, and hybridized with double-stranded (DS), transcribed (T) strand-specific, or nontranscribed (NT) strand-specific 32P-labeled probes for the APRT gene, as described under “Experimental Procedures.” The symbol (±) represents DNA with or without T4 endoV treatment. At the top (a) is a typical autoradiograph; at the bottom (b) are the quantitative results. The numbers of ESS (T4 endoV-sensitive sites) in the T or NT strands were calculated by the Poisson distribution, based on densitometric scanning of the autoradiographs (23). The average number of CPDs formed per 3.9-kb fragment of DNA in these experiments after 20 J/m² UV irradiation was calculated to be ~0.8.

FIG. 2. Removal of BPDE-DNA adducts from the APRT gene in AT3-2 cells. DNAs isolated from BPDE-treated (4 μM) AT3-2 cells were digested with Asp718 and treated with UvrABC nuclease (6x and 8x represent protein/DNA molar ratios of 6 and 8). The resultant DNAs were then denatured, separated by electrophoresis, transferred to an Oncor membrane, and hybridized with double-stranded (DS), transcribed (T) strand-specific, or nontranscribed (NT) strand-specific 32P-labeled probes for the APRT gene, as described under “Experimental Procedures.” The symbol (±) represents DNA with or without UvrABC nuclease treatment. At the top (a) is a typical autoradiograph; at the bottom (b) are the quantitative results. The numbers of UNSS (UvrABC nuclease-sensitive sites) in the T or NT strands were calculated by the Poisson distribution, based on densitometric scanning of the autoradiographs (23). The average number of BPDE-DNA adducts formed per 9.4-kb Asp718 fragment of DNA in these experiments after 4 μM BPDE treatment was calculated to be ~1.6.
FIG. 3. Removal of CC-1065-induced thermal-alkaline labile sites (TALS) from the APRT gene in AT3-2 cells. DNAs from CC-1065-treated (60 nm) cells were digested with Asp718 followed by thermal-alkaline treatment (22). The resultant DNAs were then denatured, separated by electrophoresis, transferred to an Oncor membrane, and hybridized with double-stranded (DS), transcribed (T) strand-specific, or nontranscribed strand-specific (NT) ²⁰P-labeled probes for the APRT gene, as described under “Experimental Procedures.” The symbol (·) represents DNA with or without thermal-alkaline treatment. At the top (a) top is a typical autoradiograph, and at the bottom (b) are the quantitative results. The numbers of TALS in the T or NT strands were calculated by the Puisson distribution, based on densitometric scanning of the autoradiographs (22). The average number of CC-1065-DNA adducts formed per 9.4-kb Asp718 fragment of DNA in these experiments after CC-1065 treatment was calculated to be ~0.8.

FIG. 4. Removal of CPDs from the DHFR gene in UV-irradiated AT3-2 cells (20 J/m²). The same DNAs described in Fig. 1 were first digested with Asp718, then treated with T4 endoV, denatured, separated by electrophoresis, transferred to an Oncor membrane, and hybridized with double-stranded (DS), transcribed (T) strand-specific, or nontranscribed (NT) strand-specific ²⁰P-labeled probes for the DHFR gene, as described under “Experimental Procedures.” The symbols, abbreviations, methods, and quantitation are the same as in Fig. 1.
In this study, we have carefully examined the kinetics, strand specificity, and transcription dependence of NER of UV-induced CPDs at the endogenous Chinese hamster APRT gene locus, in hemizygous CHO cell lines that contain only a single copy of this gene. In contrast to the preferential repair of CPDs in the transcribed strand, which is observed in the CHO DHFR gene, we have found that CPDs on both strands of the CHO APRT gene are repaired with equal efficiency. We observe a similar lack of strand specificity for repair of BPDE and CC-1065-induced DNA damage at the APRT locus. We have further found that, in two mutant cell lines in which the entire APRT promoter region has been deleted, CPDs are still efficiently repaired in both strands of the promoterless APRT gene, even though neither strand appears to be transcribed. These results, which were initially obtained by Southern analysis using a relatively small DNA fragment that includes the entire APRT gene, have been confirmed by ligation-mediated polymerase chain reaction (LMPCR) analyses in which rates of repair were determined for each CPD site along each DNA strand in exon 3 of the APRT gene, in all three cell lines. Together, these results demonstrate that the highly efficient repair observed on both strands of the Chinese hamster APRT locus is not dependent on transcription of the APRT gene.

**Fig. 5.** CPD repair in the genomic DNA of UV-irradiated CHO AT3-2 cells. Cells were grown to 50–70% of confluency, irradiated with (lanes 3–10) or without (lanes 1 and 2) UV light (20 J/m²) and incubated for different time in growth medium. The genomic DNAs were isolated from each time point and the nonirradiated control (lanes 1, 3, 5, 7, and 9). These DNAs were then digested, separated by electrophoresis in a 0.5% agarose gel, and stained with ethidium bromide. The extents of the deleted regions were determined for each CPD site along each DNA strand in exon 3 of the APRT gene, in all three cell lines. Together, these results demonstrate that the highly efficient repair observed on both strands of the Chinese hamster APRT locus is not dependent on transcription of the APRT gene.

**EXPERIMENTAL PROCEDURES**

**Cells, Cell Culture, and Carcinogen Treatment**—The Chinese hamster CHO-AT8-2 cell line is hemizygous for the endogenous APRT gene locus (20); these cells contain a single, actively transcribed APRT gene, which is located on the CHO 27 chromosome. ATS-88 and T2S-24 are two spontaneous APRT promoter deletion mutants, which were derived from CHO-AT9-2. For these experiments, cultures were grown to 50–70% confluence in 150-mm dishes, in a minimal essential medium supplemented with 10% fetal calf serum. Prior to UV irradiation, or treatment with BPDE or CC-1065, the medium was reduced and cells were washed with Dulbecco’s phosphate-buffered saline (DPBS). For UV treatments, cells were irradiated at a fluency rate of 1 J/m²/μs, using GE1518 germicidal lamps (predominate emission, 254 nm) as the UV source. For BPDE or CC-1065 treatments, either BPDE (4 μM) or CC-1065 (60 nM) was added to cell cultures in 15 ml of DPBS, and the cultures were incubated at 37 °C for 30 min (21, 22). After three rinses with fresh DPBS to remove the DNA-damaging agent, the cells were incubated in fresh medium with 5-bromo-2’-deoxyuridine (10 μM) and 5-fluorodeoxyuridine (1 μM). After incubation at 37 °C for various periods of time, the treated cells were lysed for DNA isolation.

**DNA Isolation**—Cells were washed three times with DPBS, and lysed with lysis buffer (0.5% SDS, 10 mM Tris, pH 7.5, and 0.5 mM EDTA) for 10 min. The cell lysates were clarified by centrifugation at 3,000 × g for 2 min at 4 °C. The supernatants were then incubated with 10 mg/ml proteinase K at 37 °C for 3 h. After DNAs were digested overnight with either Apa718 or BamHI (10 unit/μg of DNA) and checked for completeness of digestion by agarose gel electrophoresis. Replicated and nonreplicated DNAs were separated by the CsCl gradient centrifugation in a Ti 50 rotor (37 × 10⁰ rpm for 64 h at 21 °C) (23). Only the nonreplicated DNA was used for repair kinetics analysis.

**UvrABC Nuclease Treatment**—The UvrA, UvrB, and UvrC proteins were purified as described previously (23, 24). The UvrABC reaction was the same as previously described (21, 23). Briefly, an aliquot containing 3 μg of DNA was reacted with UvrA, UvrB, and UvrC (330 nmol) each in reaction buffer (50 mM Tris, pH 7.5, 100 mM KCl, 1 mM ATP, 10 mM MgCl₂, and 1 mM dithiothreitol) at a final volume 100 μl at 37 °C for 90 min. The protein-DNA mixture was then ethanol-precipitated (with 15 μg of tRNA, as a carrier) and resuspended in 10 μl of TE buffer.

**Thermal-alkaline Treatment**—The method was the same as previously described. To induce DNA strand breakage at the CC-1065-DNA adducts site, 3 μg of DNA was added to 40 μl of solution containing 10 mM NaOH, 75% formamide, 2.5 mM Tris, and 0.25 mM EDTA. The mixtures were heated at 90 °C for 20 min and quenched in an ice bath.

**Cleavage of CPDs**—The DNA was incubated with T4 endoV (protein/DNA molar ratio 6:1), assuming the average DNA length is 14 kb and incubated for different time in growth medium. The genomic DNAs were isolated from cells, and treated with T4 endoV (protein/DNA molar ratio of 6:1). Samples isolated from each time point and the nonirradiated control were cut with T4 endoV (lanes 2, 4, 6, 8, and 10) or mock treated (lanes 1, 3, 5, 7, and 9). These DNAs were then denatured, separated by electrophoresis in a 0.5% agarose gel, and stained with ethidium bromide (0.5 μg/ml). Note: DNAs isolated from UV-irradiated cells after different incubation times show similar sensitivity to cutting by T4 endoV, suggesting that there is very little global genomic repair of CPDs in these cells.

**Fig. 6.** Restriction maps of the APRT gene domain in the CHO AT3-2, ATS-88, and T2S-24 cell lines, showing the extents of the deleted regions in ATS-88 and T2S-24. Restriction sites: BamHI, B; EcoRI, E; EcoRV, Ev; KpnI, K; NdeI, Nd; PstI, Ps; XhoI, Xb; XhoI, X.
Fig. 7. Removal of CPDs from the APRT domain in UV irradiated (20 J/m²) ATS-88 cells (A) and T2S-24 cells (B). The symbols, abbreviations, methods, probes, and quantitation are the same as in Fig. 1.
gene, respectively, were isolated by infecting the vector-containing cells containing either the transcribed or nontranscribed strand of the vectors. These two constructs allowed us to isolate either the transcribed (T) or the nontranscribed (NT) strand of the vectors.

RNA isolation and electrophoresis—Total RNA was isolated using the guanidinium method (26). In brief, cells were lysed with a guanidinium solution (4 M guanidinium isothiocyanate, 20 mM sodium acetate, pH 5.2, 0.1 mM dithiothreitol, 0.5% N-lauroylsarcosine) and genomic DNA was sheared with a 20-gauge needle. Total RNA was isolated by centrifugation through a 5.7 M CsCl gradient, at 35°C, pH 5.2, 0.1 mM dithiothreitol, 0.5% d-lauroylsarcosine)

Detection of APRT gene transcripts or mRNAs from different CHO cell lines—Total RNA 10 μg (A) or mRNA (B) isolated from AT3-2, AT8-88, T2S-24 cells were separated by gel electrophoresis, transferred to an Oncor membrane, and hybridized with APRT strand-specific probes, as described under “Experimental Procedures” (Fig. 8). T hybridizations using a probe specific for APRT template strand transcripts, NT, hybridizations using a probe specific for transcripts from the opposite strand.

DNA Denaturation and Gel Electrophoresis—DNA was denatured in 90% formamide at 37°C for 60 min. Immediately after denaturation, the samples were electrophoresed at 5 V/cm in a 0.5% agarose gel in TBE buffer (25 mM Tris, pH 8.0, 20 mM sodium acetate, and 2.5 mM EDTA) with 0.5 μg/ml ethidium bromide. After electrophoresis, the samples were transferred to an Oncor membrane. The DNA on the membrane was then hybridized with strand-specific DNA probes.

Primer—DNA Probe—pGEM-zf1 (−)APRT and pGEM-zf1 (−)APRT vectors were constructed by inserting a 3.9 kb BamHI fragment containing the CHO APRT gene into the pGEM-zf1 (−) or (−) vectors. These two constructs allowed us to isolate either the transcribed (T) or the nontranscribed (NT) strand of the APRT gene. Single-stranded pGEM-zf1 (−)APRT or pGEM-zf1 (−)APRT DNA phages containing either the transcribed or nontranscribed strand of the APRT gene, respectively, were isolated by infecting the vector-containing cells with carrier phage M13 KO7. To generate 32P-labeled DNA probes for either the T or NT strand, 1.0 μg of template DNA was added to reaction buffer containing 50 pmol of the appropriate strand-specific primer, 5 μM dGTP/dTTP/dATP, 5 units of Klenow fragment, and 16.7 pmol of [α-32P]dTTP, and the mixture was incubated at 18°C overnight. For generation of the T strand-specific APRT probe, we used a primer with the sequence: 5′-TGGCGAAGCTGGAGCTGAAA-3′. For production of the NT strand-specific probe, we used a primer with the sequence: 5′-TGATCCATGCAGAGCCTAC-3′. To produce strand-specific probes for exon 3 of the APRT gene, a PCR-amplified fragment of the exon 3 region was used as the template and either T or NT strand-specific primer was used to perform separate linear PCR reactions.

 Autoradiographs were scanned with a Bio-Image Analyzer, using a 100 Visage-band analysis software program. The average number of damage sites per APRT fragment was calculated by the Poisson distribution equation: Poisson distribution equation: P(0) = e^(-n), where n is the number of UvrABC nuclease-sensitive sites (UNSS), or thermal-alkaline labile sites (TALS), or T4 endoV-sensitive sites (ESS). The ratio of labeling intensity of the full-length fragment from the enzyme-treated sample to that of the untreated sample is equal to P(0). For the results from LMPCR experiments, the intensities of all CPD-site bands were compared after subtraction of background values obtained from T4 endoV-treated, nonirradiated control lanes. A repair kinetics curve was generated for each CPD position, and the time required for removal of 50% of the initial CPDs at each site was then determined from this curve. Statistical analyses of the quantitated repair rate data were carried out using StatView 4.01 (Abacus Concepts, Inc.)

RESULTS

Lack of Strand Specificity of Repair in a Transcriptionally Active APRT Gene—The CHO cell line AT3-2 contains a single, actively transcribed APRT gene (20). This APRT hemizygous cell line was chosen for use in our experiments to avoid any potential complications of allelic differences in transcription or DNA repair. Exponentially growing cultures were treated with the DNA-damaging agents: UV, BPDE, or CC-1065. To assay the removal of DNA damage, cells were harvested at various times after treatment with damaging agents. For detection of UV-irradiated CPDs, DNAs isolated from UV-irradiated cells were treated with T4 endoV, which specifically cuts CPD sites (1). UvrABC nuclease was used to detect BPDE-DNA adducts in DNAs isolated from BPDE-treated cells (21), and DNAs isolated from CC-1065-treated cells were subjected to thermal-alkaline treatment to allow detection of CC-1065-DNA adducts (22). After these treatments, the resultant DNAs were denatured and separated by electrophoresis in an agarose gel; the separated DNAs were then transferred to a nylon membrane and probed with 32P-labeled, APRT-transcribed (T) or nontranscribed (NT) strand-specific probes. The results in Figs. 1–3 show that even though the kinetics for the removal of these three different types of DNA damage are quite different, the time course of CPD photoproduct, BPDE-DNA adduct, or CC-1065-DNA adduct removal is similar for both strands of the APRT gene. As a control to confirm that these cells have not lost their capacity for transcription-coupled repair, we also examined CPD repair in the T and NT strands of the DHFR gene. It has been previously shown that UV-irradiated CPDs are preferentially repaired in the transcribed strand of the DHFR gene in CHO cells (2, 21). Our results in Fig. 4 confirm that this is also the case in CHO-AT3-2 cells. Although 80% of CPDs are removed from the T strand of the DHFR gene by TCR within 24 h, only 15% of CPDs are removed from the NT strand (Fig. 4). The inefficient repair of CPDs in the NT strand of the DHFR gene seen in Fig. 4 shows that CHO-AT3-2 cells, like other CHO sublines (2, 14, 15), are deficient in global genomic repair of CPDs; this is further demonstrated in Fig. 5, which shows...
FIG. 9. Fine mapping of CPD repair in exon 3 of the APRT gene in UV-irradiated (20 J/m²) CHO cells. A, AT3-2 cells; B, ATS-88 cells; and C, T2S-24 cells. Genomic DNAs isolated from nonirradiated cells (lane 4) and UV-irradiated cells after different incubation times (0, 2, 4, 8, and 24 h in lanes 5–9) were digested with T4 endoV, and subjected to LMPCR as described under “Experimental Procedures.” The resultant DNAs were separated by electrophoresis in an 8% denatured polyacrylamide gel, transferred to a nylon membrane, and then probed with ³²P-labeled APRT DNA fragments. The positions and intensities of T4 endoV incision bands represent the sites and extents of CPD formation/repair along the sequence. Lanes 1–3 are the Maxam-Gilbert sequencing reactions for G, AG, and TC. Left panel, transcribed strand; right panel, nontranscribed strand. Regions with contiguous pyrimidines are shown at the left.
that the majority of the CPDs in bulk genomic DNA remain unrepaired even after 24 h of incubation. Thus, our results demonstrate that, although repair at the CHO APRT gene locus is much more efficient than repair in bulk genomic DNA, there is no apparent strand specificity or preferential repair of damage on the transcribed strand; similar rates and extents of repair are seen for both strands of the APRT gene.

CPDs in Both Strands of a Promoter-deleted APRT Gene Are Still Efficiently Repaired—To determine whether efficient repair of CPDs at the CHO APRT locus is dependent upon transcription of the APRT gene, we have examined CPD repair in two different CHO-AT3-2-derived, APRT promoter-deletion mutants (ATS-88 and T2S-24). In ATS-88, a 1.15-kb deletion has eliminated the entire promoter region and first two exons of the APRT gene; in T2S-24, a much larger (25 kb) 5′-extending deletion, with virtually the same 3′-breakpoint as ATS-88, has eliminated the same region (promoter and first two exons) of the APRT gene (Fig. 6). These two promoter-deletion cell lines were UV-irradiated, and their DNAs were isolated and then treated with T4 endoV, in the same manner as for their parental cells described above. Because the entire promoter region and first two exons of the APRT gene; in T2S-24, a much larger (25 kb) 5′-extending deletion, with virtually the same 3′-breakpoint as ATS-88, has eliminated the same region (promoter and first two exons) of the APRT gene (Fig. 6). These two promoter-deletion cell lines were UV-irradiated, and their DNAs were isolated and then treated with T4 endoV, in the same manner as for their parental cells described above. Because the entire APRT promoter region has been deleted from each of these mutant cell lines, if repair of the T strand of the APRT gene is coupled with or dependent upon transcription, one would expect to see a specific decrease in CPD repair in what would normally be the T strand of the APRT gene. Our results in Fig. 7, however, still show efficient repair of CPDs in both strands of the APRT gene domain in both of these promoter-deletion mutants; in each case, 80% of the CPDs in each strand are repaired within 24 h. These results suggest that CPD repair at the CHO APRT locus does not require transcription of the APRT gene, or even the presence of the APRT promoter region.

Only One Strand of the APRT Gene Is Transcribed in Wild Type Cells, and Neither Strand of This Gene Is Transcribed in ATS-88 or T2S-24 Cells—To confirm the transcription status of the APRT gene domain in the parental AT3-2 cell line, and ATS-88 and T2S-24 APRT promoter-deletion mutants, we have used strand-specific probes for the APRT gene to screen both total cellular RNA and polyadenylated mRNA isolated from each cell line. The results in Fig. 8 show that: 1) only one strand of the APRT gene is transcribed in APRT+ AT3-2 cells, and 2) neither strand of this gene appears to be transcribed in either the ATS-88 or T2S-24 promoter-deletion mutants. The 0.9-kb transcript observed in Fig. 8 represents APRT mRNA. No other RNA transcripts were detected with either T or NT strand-specific probes for the APRT gene.

Fine Mapping of CPD Repair in Exon 3 of Either a Transcriptionally Active or Inactive APRT Gene—To confirm our findings that CPDs are efficiently repaired in both the T and NT strands of the Chinese hamster APRT gene, even in the absence of transcription of this gene, we further mapped CPD repair in each strand of the exon 3 region of the APRT gene using LMPCR techniques (Fig. 9). The same DNAs used to characterize CPD repair at the defined gene fragment level were used to map CPD repair at the single nucleotide level, using the method described by Pfeifer and Dammann (28), with one modification. To correct for differential recovery due to multiple ethanol precipitations during sample preparation, a fixed amount of 32P-labeled linearized pBR322 was added to each sample before LMPCR treatment. Then, after LMPCR, a fixed amount of 32P counts was loaded for each sample to ensure equal loading of sample DNAs prior to gel electrophoresis. Typical autoradiographs are shown in Fig. 9. These results show that in APRT+ AT3-2 cells (Fig. 9A), CPDs in both the T and NT strands of exon 3 of the APRT gene are efficiently repaired. CPDs also appear to be efficiently repaired in both
strands of exon 3 in both APRT promoter deletion mutants: ATS-88 (Fig. 9B) and T2S-24 (Fig. 9C).

Quantitation of the repair kinetics for CPDs formed at sites along both DNA strands in the exon 3 region of the APRT gene in these three cell lines are shown in Fig. 10. The vertical columns at each CPD site in Fig. 10 represent the time required for removal of 50% of the CPDs formed at that particular site. Remarkably similar patterns and kinetics of repair at CPD sites on the NT strand were observed in all three cell lines. Times required for 50% removal of CPDs, determined at 25 CPD sites along the NT strand of the exon 3 region of the APRT gene, ranged from 6.0 to 13 h in AT3-2 cells, from 7.0 to 16 h in ATS-88 cells, and from 7.0 to 12.5 h in T2S-24. However, the average time (mean ± S.E.) required for removal of 50% of the CPDs formed at each site, calculated for 25 CPD sites along the NT strand of the exon 3 region of the APRT gene, was identical for all three cell lines; 9.1 ± 0.4 h in AT3-2 cells, 9.6 ± 0.5 h in ATS-88 cells, and 9.0 ± 0.3 h in T2S-24 cells.

Although a slightly faster overall rate of repair and somewhat more sequence-dependent variation in repair rates was observed for CPD sites on the T strand in AT3-2 cells, rates of repair on the T strand in ATS-88 or T2S-24 cells were virtually identical to the rates seen on the NT strand. Times required for 50% removal of CPDs, determined for 27 CPD sites along the T strand of the exon 3 region of the APRT gene, ranged from 2.1 to 10.1 h in AT3-2 cells, whereas in ATS-88 or T2S24 cells these times ranged from 5.5 to 12 h, or from 6.9 to 11.5 h, respectively. The average time (mean ± S.E.) required for removal of 50% of the CPDs formed at each site, calculated for these 27 CPD sites along the T strand of the exon 3 region of the APRT gene, was 7.7 ± 0.4 h for AT3-2 cells, 8.9 ± 0.3 h for ATS-88 cells, and 8.9 ± 0.3 h for T2S-24 cells. Interestingly, although there seems to be much less sequence-dependent variation in CPD repair rates in the APRT gene than has been reported for

Fig. 10. Quantitation of CPD repair rates in the transcribed strand (lower) and nontranscribed strand (upper) in exon 3 of the APRT gene in CHO cells. A, AT3-2; B, ATS-88; and C, T2S-24 cells. Repair rates, expressed as the time (h) required for 50% removal of CPDs, were determined for each position with a visible signal above background, from autoradiographs such as those shown in Fig. 9. Comparison of the intensities of CPD-site bands after subtraction of background values obtained from T4 endoV-treated, nonirradiated control lanes allowed us to establish time-course repair kinetics curves for each CPD site. The time required for removal 50% of the initial CPDs formed at each site was then determined from these curves. Vertical columns represent repair rates at each CPD site. These data represent the average of two experiments for each DNA strand.
several other genes (28, 31, 32), we did find four CPD sites on the T strand of exon 3 (at bases 56, 73, 129, and 134 of this exon) that appeared to be repaired two to four times more rapidly in AT3-2 cells than in either ATS-88 or T2S-24 cells.

DISCUSSION

NER in mammalian cells is thought to involve two distinct subpathways: a TCR pathway, which selectively and very efficiently repairs transcription-blocking damage in the transcribed strand of actively expressed genes, and a GGR pathway, which is responsible for repairing damage in the nontranscribed strand and the rest of the genome (2, 5–8). However, several discordant lines of evidence suggest that NER in mammalian cells may not be that simple. For example, CS cells exhibit no deficiency in the repair of helix-distorting dG-C8-AAF adducts, which are very effective blocks to RNA polymerase II transcription, suggesting that these adducts must be removed by GGR, not by TCR (29). Second, recovery of RNA synthesis from the middle of the DHFR gene following UV irradiation appears to be much faster than can be accounted for by the kinetics of removal of UV-induced DNA lesions from the 5′-half of this gene (30). Third, LMPCR studies of repair in the human JUN gene (31, 32) have found very rapid repair of CPDs on both strands in the vicinity of the transcription initiation site (between nucleotides −40 and +100), where >90% of the dimers are repaired within 4 h, and a gradient of repair efficiency along the gene, with faster repair at the 5′-end and diminished repair at the 3′-end. Interestingly, CS cells, which are clearly deficient in repair of the transcribed DNA strand of the JUN gene, still efficiently repair CPDs on both strands near the transcription initiation site (31). Because TCR is presumed to be initiated by an RNA polymerase II stalled at a helix-distorting lesion in the transcribed strand during transcription (2, 5–8, 30), the rapid repair observed at sequence positions upstream of the initiation site and on the nontranscribed strand in the 5′-portion of the JUN gene is unlikely to represent TCR.

Efficient repair of CPDs on both DNA strands has been previously reported for two human genes, ADA and β-actin (9, 13, 17, 18). Although, the efficient repair of both template and nontemplate strands observed at these loci has been ascribed to the possible occurrence of TCR on both strands, either as a result of internal antisense transcription or overlapping convergent transcription from a downstream gene, little evidence has been presented to substantiate such suggestions. Vreeswijk and co-workers (19) have also reported a lack of strand bias of CPD repair at the APRT locus in V79 cells. However, their study examined repair of CPDs in an 18.2-kb BclI fragment, upon which the entire APRT gene transcribes (transcription initiation site to polyadenylation site) occupies only −2.2 kb. They did not determine the transcription status of the APRT alleles present in the V79 cell line or whether any other transcribed sequences were present within the BclI fragment that could have contributed to the overall pattern of repair that was observed.

In this study we demonstrate that the highly efficient repair observed on both strands of the APRT locus does not require transcription of the APRT gene and is unaffected by deletion of the entire APRT promoter region. CHO cells are profoundly deficient in expression of p48 UV-DDB (14, 15), and GGR of CPDs in these cells is extremely inefficient, as evidenced by the very slow repair of CPDs in the nontranscribed strand of the DHFR locus and in bulk genomic DNA. Thus, the patterns of CPD repair we have observed at the APRT gene locus are not readily explainable by either TCR or enhanced levels of GGR. Our findings of similar CPD repair efficiencies in both strands of the intact APRT gene, as well as in transcriptionally inactive, promoterless APRT genes, suggest the possibility that efficient repair of some actively expressed genes may not necessarily depend on transcription, per se, but may be due to unique chromatin structure or to chromatin changes that precede transcription.

A variety of modifications of chromatin structure, including histone acetylation, phosphorylation, ubiquitination, or methylation might affect the rate of NER (8, 33–35). Changes in the chromatin structure of active genes can involve both cis-acting sequences (such as enhancer, silencer, or locus control regions), and trans-acting transcription factors or nonhistone proteins such as HMG14 and HMG17. However, the involvement of these factors in NER has yet to be established. Because nucleosome-free DNA is known to be repaired much more rapidly than nucleosomal DNA (35, 36), we wondered whether efficient repair of damage on both DNA strands of the APRT gene might reflect a lack of nucleosome formation at this locus. However, we have obtained preliminary evidence, based on micrococcal nuclease digestion, that the APRT gene in AT3-2 cells contains nucleosome structure (data not shown).

Interestingly, deletion of the APRT promoter region has no apparent effect on replication timing; we have recently obtained results suggesting that in all three cell lines examined in this study, the APRT gene domain is replicated early in S-phase. In contrast, Dijkstra and Hamlin (37) have reported that replication of the DHFR locus in a DHFR promoter-deletion mutant occurs 5–6 h later in S-phase than replication of the normal, actively transcribed DHFR locus. To our knowledge, no one has examined CPD repair in the DHFR domain of their DHFR promoter-deletion mutant.

In summary, the results presented in this paper clearly demonstrate that the highly efficient repair observed on both strands of the APRT locus does not require transcription of the APRT gene or even the presence of the APRT promoter region. Our findings suggest that current models of transcription-coupled and global genomic nucleotide excision repair may underestimate the importance of factors other than transcription in governing the efficiency of repair and their contribution to the heterogeneity of NER that has been observed in mammalian cells.

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