

# Effects of genomic context and chromatin structure on transcription-coupled and global genomic repair in mammalian cells

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## ABSTRACT

It has been long recognized that in mammalian cells, DNA damage is preferentially repaired in the transcribed strand of transcriptionally active genes. However, recently, we found that in Chinese hamster ovary (CHO) cells, UV-induced cyclobutane pyrimidine dimers (CPDs) are preferentially repaired in both the transcribed and the non-transcribed strand of exon 1 of the dihydrofolate reductase (*DHFR*) gene. We mapped CPD repair at the nucleotide level in the transcriptionally active *DHFR* gene and the adjacent upstream *OST* gene, both of which have been translocated to two chromosomal positions that differ from their normal endogenous positions. This allowed us to study the role of transcription, genomic context and chromatin structure on repair. We found that CPD repair in the transcribed strand is the same for endogenous and translocated *DHFR* genes, and the order of repair efficiency is exon 1 > exon 2 > exon 5. However, unlike the endogenous *DHFR* gene, efficient repair of CPDs in the non-transcribed strand of exon 1 is not observed in the translocated *DHFR* gene. CPDs are efficiently repaired in the transcribed strand in endogenous and translocated *OST* genes, which indicates that efficient repair in exon 1 of the non-transcribed strand of the endogenous *DHFR* gene is not due to the extension of transcription-coupled repair of the *OST* gene. Using micrococcal nuclease digestion, we probed the chromatin structure in the *DHFR* gene and found that chromatin structure in the exon 1 region of endogenous *DHFR* is much more open than at translocated loci. These results suggest that while transcription-coupled repair is transcription dependent, global genomic repair is greatly affected by chromatin structure.

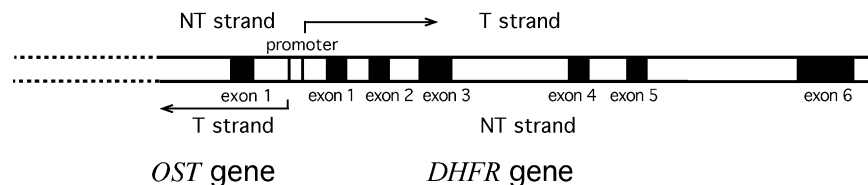
## INTRODUCTION

Nucleotide excision repair (NER) is a versatile repair system that repairs a wide range of bulky DNA lesions, including UV light-induced cyclobutane pyrimidine dimers (CPDs) (1–7). Two distinct NER subpathways—transcription-coupled repair (TCR) and global genomic repair (GGR)—have been found in mammalian cells (3–8). It is generally believed that the TCR pathway is responsible for the repair of DNA damage in the transcribed (T) strand of transcriptionally active genes, and that the GGR pathway is responsible for the repair of DNA damage occurring in locations other than the T strand of transcriptionally active genes in the genome. In general, TCR is more efficient than GGR. It has been found that human cells are proficient in both TCR and GGR of CPDs, while rodent cells lack GGR of CPDs (9).

The basic enzymology of NER in mammalian cells has been well determined *in vitro* using naked DNA as substrates; more than 30 proteins are involved in NER in mammalian cells (10–13). However, the molecular mechanism of NER on its natural substrate, chromatin, remains largely unknown, especially the mechanism of TCR in mammalian cells. Increasing evidence suggests that NER in mammalian cells is much more complex than our current understanding of TCR and GGR would indicate (14–19). Recently, we found that in Chinese hamster ovary (CHO) AT3-2 cells, CPDs in the T strand of the *DHFR* gene are efficiently repaired and CPDs in the non-transcribed (NT) strand of the first exon of the *DHFR* gene are also efficiently repaired. In contrast, CPDs in exons 2 and 5 of the NT strand of the *DHFR* gene are poorly repaired (19). These results raise the possibility that the efficient repair in the first exon of the NT strand of the endogenous *DHFR* gene may result from two mechanisms. The first mechanism is the extension of TCR from the *OST* gene (20,21) to the *DHFR* gene. The *OST* gene is located immediately 5' of the *DHFR* gene, shares the same promoter region with the *DHFR* gene and is transcribed in the direction opposite from the *DHFR* gene (20,21) (Fig. 1). The second possible mechanism is that the genomic context and/or chromatin structure of exon 1 is different from that found in exons 2 and 5. To test these possibilities, we mapped the CPD repair at nucleotide

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**Figure 1.** Map of the endogenous *DHFR* and *OST* gene domains in CHO cells. The *DHFR* and *OST* genes share a common promoter region and are transcribed (arrow indicates the transcription direction) divergently from this common promoter region.

resolution in the *DHFR* and *OST* genes in three CHO cell lines, AT3-2, C26 and C38, using the T4 endonuclease V (T4 endo V) incision method in combination with ligation-mediated PCR (LMPCR) (18,19). We also probed the chromatin structure in the *DHFR* gene by determining the kinetics of micrococcal nuclease (MN) digestion. The *DHFR* and *OST* genes are located in different genomic contexts in these three cell lines: the AT3-2 cells contain *DHFR* and *OST* genes located in their normal endogenous positions, while in the C26 and C38 cells, the entire endogenous *DHFR* gene and at least 10 kb of the 5' portion of the *OST* genes are deleted, and a fragment containing a single copy of the intact *DHFR* gene and 8 kb of its upstream region containing the *OST* gene has been introduced into different positions of the chromosome in these two cell lines (22). Since the *DHFR* genes in C26 and C38 are located at chromosome positions differing from the endogenous *DHFR* gene position in AT3-2 cells but are still transcriptionally active, these cells provide us with tools for studying the effects of genomic context, transcription and chromatin structure on TCR and GGR in this gene.

## MATERIALS AND METHODS

### Cell lines and culture conditions

AT3-2 cells contain diploid *DHFR* loci, and both loci are transcriptionally active. Both C26 and C38 cells contain a single copy of a transcriptionally active *DHFR* gene; they were constructed by transfection of CHO-DG44, a double deletion (>115 kb) mutant lacking both copies of the entire *DHFR* gene and at least 10 kb of the 5' portion of the *OST* gene, with a cosmid containing a 41 kb DNA fragment from Chinese hamster genomic DNA containing the intact *DHFR* gene and flanking sequences (~8 kb upstream containing the *OST* gene and 7 kb downstream) (22). As determined by fluorescence *in situ* hybridization, the chromosomal position of integration differed from the endogenous *DHFR* locus (22) and the site of integration was also different in these two transfectants. Northern blot and RT-PCR methods were used to confirm that the *DHFR* gene in these three cell lines is transcriptionally active. Cells were grown in  $\alpha$ -minimum Eagle's medium supplemented with 10% fetal calf serum.

### UV irradiation and genomic DNA isolation

For UV irradiation, AT3-2, C26 and C38 cells were grown to 50–70% confluence in 150 mm dishes. Prior to UV irradiation, the culture medium was removed, the cells were washed with

phosphate-buffered saline (68 mM NaCl, 1.94 mM KCl, 1.07 mM  $\text{KH}_2\text{PO}_4$ , pH 7.4) and the cells were then UV irradiated at a fluence rate of 1 J/m<sup>2</sup>/s for 15 s using GE15118 germicidal lamps (predominant emission 254 nm) as the UV source. After irradiation, the cells were incubated in fresh medium containing 10  $\mu\text{M}$  5-bromo-2'-deoxyuridine and 1  $\mu\text{M}$  5-fluorodeoxyuridine for various periods of time to allow DNA repair for repair kinetic analysis. After incubation, cells were lysed with lysing buffer (0.5% SDS, 10 mM Tris, pH 7.8, 10 mM EDTA, 10 mM NaCl, 100  $\mu\text{g}/\text{ml}$  proteinase K) at room temperature for 1 h. Protein was removed by repeated phenol extractions followed by diethyl ether extractions. DNA was then ethanol precipitated and resuspended in TE buffer (10 mM Tris, pH 7.5, 1 mM EDTA). RNA was removed by treatment with RNase A (50  $\mu\text{g}/\text{ml}$ ) for 1 h followed by repeated phenol and diethyl ether extractions. DNA was then ethanol precipitated and resuspended in TE buffer (pH 7.5). Replicated and non-replicated DNA were separated by CsCl gradient centrifugation in a Ti 50 rotor ( $3.7 \times 10^4$  r.p.m. for 72 h at 21°C). Only the unreplicated DNA was used for repair kinetics analysis (14,15,19).

### Cleavage of CPDs with T4 endo V

A known quantity of purified genomic DNA (10  $\mu\text{g}$ ) was treated with T4 endo V (protein:DNA molar ratio 6:1, assuming the average DNA length was 14 kb) in a solution of 100 mM NaCl, 10 mM Tris, pH 7.5, and 0.5 mM EDTA at 37°C for 60 min to cleave CPDs. *Escherichia coli* photolyase (0.5  $\mu\text{g}/\mu\text{g}$  DNA) was then added and the mixtures were irradiated with 366 nm UV light (Sylvania 15 watt F15T8) for 60 min at room temperature in the presence of 10 mM dithiothreitol for photoreactivation. The reactions were stopped by repeated phenol and diethyl ether extractions. The resultant genomic DNA was then precipitated by ethanol, resuspended in TE buffer (pH 7.5) and subjected to LMPCR (14,15,19).

### Mapping the repair of CPDs at the nucleotide level with LMPCR

To investigate the repair of CPDs in the *DHFR* and *OST* genes, a known quantity (1  $\mu\text{g}$ ) of T4 endo V-treated genomic DNA was subjected to LMPCR to map the distribution of CPDs along exons 1, 2 and 5 of the *DHFR* gene and the T strand of exon 1 in the *OST* gene. Control genomic DNA was subjected to Maxam–Gilbert sequencing (23) followed by LMPCR, in order to serve as a sequencing ladder. The LMPCR method was the same as previously described (14,15,18,19). The oligonucleotide primers (Midland Certified Reagent Co., Midland, TX) used for LMPCR analysis of exons 1, 2 and 5

**Table 1.** Synthetic oligomer primers for LMPCR analysis of the exon 1 transcribed strand of the *OST* gene

| Primer | Sequence              | $T_m$ (°C) |
|--------|-----------------------|------------|
| 1      | ACTCCGCCTCCACCAG      | 48.1       |
| 2      | CGCCCAGTCCGGCGTGGC    | 66.4       |
| 3      | CGCTCCAGGCGGGGTAGT    | 65.0       |
| 4      | GGTAGACGCTGGGGGCGTGAG | 65.6       |

of the *DHFR* gene were the same as previously described (19), and the primers used for LMPCR analysis of the T strand of exon 1 in the *OST* gene are shown in Table 1. Oligonucleotide primer 1 was used in the first primer extension step of LMPCR, primer 2 was the PCR primer, and primer 3 was used to make the single-stranded hybridization probe. The template used for hybridization probe synthesis was prepared by PCR amplification from CHO genomic DNA with primers 3 and 4. The resultant LMPCR products were separated by electrophoresis in 8% denaturing polyacrylamide gels and electrotransferred to GeneScreen nylon membranes (NEN, Boston, MA). Blots were hybridized with  $^{32}\text{P}$ -labeled DNA probes specific for exons 1, 2 or 5 of the *DHFR* gene and exon 1 of the *OST* gene in hybridization buffer (0.25 M  $\text{Na}_2\text{HPO}_4$ , pH 7.2, 1 mM EDTA, 7% SDS, 1% bovine serum albumin) at 60°C for 12 h. The membranes were then exposed to a Cyclone Storage Phosphor screen (Packard, Meriden, CT), and the intensities of T4 endo V incision bands were quantified with the Cyclone Storage Phosphor System (Packard). Approximately 20 000 d.p.m. of  $^{32}\text{P}$ -labeled linearized pBR322 plasmid DNA was added to each genomic DNA sample at the beginning of the LMPCR as an internal standard to monitor sample recovery. After LMPCR, equivalent counts of  $^{32}\text{P}$ , as measured by a liquid scintillation counter (LKB-Wallac, Turku, Finland) and representing equivalent amounts of sample DNA, were loaded into each lane of the sequencing gel to separate DNA fragments of different sizes.

#### Isolation of nuclei and digestion of chromatin by micrococcal nuclease

Methods for isolation of nuclei and subsequent MN digestion were the same as described previously (19,24). Briefly, AT3-2, C26 and C38 cells were harvested and nuclei were immediately isolated. The freshly isolated nuclei ( $1 \times 10^7$ ) of each cell line were immediately digested with MN (Amersham Pharmacia Biotech, Piscataway, NJ) (1 U) in 100  $\mu\text{l}$  of digestion buffer (100 mM Tris, pH 8.0, 50 mM NaCl, 3 mM  $\text{MgCl}_2$ , 1 mM  $\text{CaCl}_2$ ) for 1, 2, 5 or 10 min at 37°C. The digestion was stopped by adding an equal volume of stop solution (200 mM Tris, pH 8.0, 200 mM NaCl, 20 mM EDTA, 2% SDS, 200  $\mu\text{g}/\text{ml}$  proteinase K). The control was an undigested, freshly lysed sample of nuclei. Genomic DNA was purified as described above and then separated by electrophoresis in 1.5% agarose gels. After staining with ethidium bromide, the separated DNA was transferred to nylon membranes and hybridized with a  $^{32}\text{P}$ -labeled probe specific for *DHFR* exon 1. After de-probing, the membranes were further hybridized with a  $^{32}\text{P}$ -labeled probe specific for *DHFR* exon 2.

## RESULTS

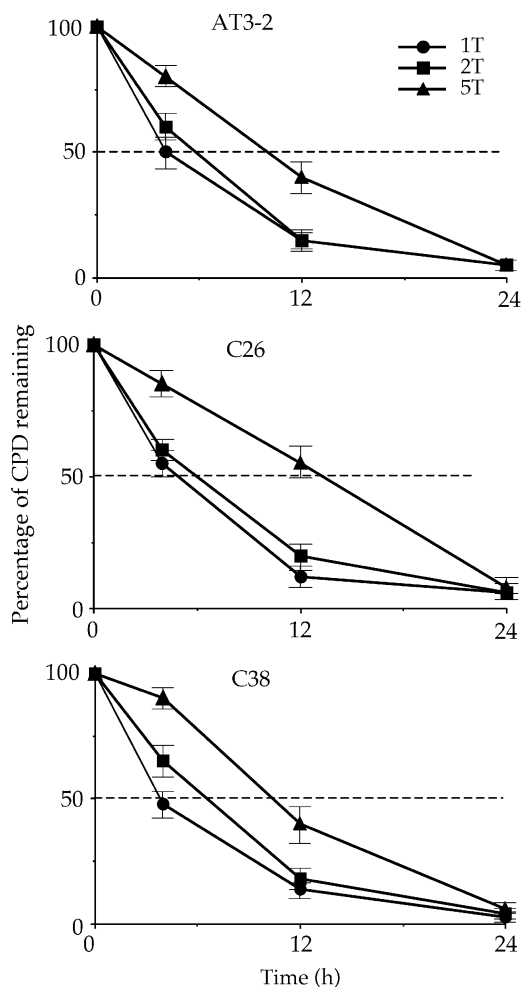
### CPD repair in the transcribed strand of the *DHFR* gene in different genomic contexts is the same

The repair of CPDs was mapped at the sequence level using the T4 endo V incision method in combination with LMPCR. CPD repair in the T strand of the *DHFR* gene in these three cell lines is shown in Figures 2 and 3. The kinetics of CPD repair in the T strand of exons 1, 2 and 5 in the translocated *DHFR* gene in C26 and C38 cells are very similar, if not identical, to the kinetics of repair in the endogenous *DHFR* gene in AT3-2 cells. The initial repair rate of CPDs along the T strand of the translocated *DHFR* genes showed a 5' to 3' polarity effect similar to that observed in the endogenous *DHFR* gene in AT3-2 cells: the initial repair rate was faster in exon 1 than in exon 2, and much faster in exons 1 and 2 than in exon 5. In addition, there were no significant differences in the initial repair rates along the T strand between these three cell lines. The time required for 50% CPD removal ( $T_{1/2}$ ) in exons 1, 2 and 5 of the *DHFR* gene in these three cell lines was ~4, 6 and 12 h, respectively. However, 24 h post-irradiation, the CPDs were almost completely removed in these three exons of the *DHFR* gene in all three cell lines.

### CPD repair in the non-transcribed strand of the *DHFR* gene in different genomic contexts is not the same

The efficient CPD repair in the NT strand of exon 1 in the endogenous *DHFR* gene in AT3-2 cells could be due to the DNA sequence effects; it is possible that the sequence of exon 1 of *DHFR* may intrinsically allow more efficient CPD repair. It is also possible that the same mechanism facilitating efficient repair in the T strand of exon 1, such as the transcription process of the *DHFR* gene, may also facilitate efficient repair in the NT strand. In either case, our findings that CPD repair in the T strand is the same in translocated and endogenous *DHFR* genes led us to expect that CPD repair in the NT strand of exons 1, 2 and 5 of the translocated *DHFR* gene in C26 and C38 cells should be the same as that observed in the endogenous *DHFR* gene in AT3-2 cells. To test this possibility, we mapped the CPD repair in the NT strand of exons 1, 2 and 5 of the translocated and endogenous *DHFR* genes in these three cell lines. The results in Figures 4 and 5 show that efficient repair was not observed in the NT strand of exon 1 of the translocated *DHFR* gene in either transfectant cell line. CPDs along the NT strand were poorly repaired in exon 1 as well as in exons 2 and 5 in the translocated *DHFR* genes, and >90% of CPDs remained unrepaired 24 h after UV irradiation. In contrast, CPDs along the NT strand of exon 1 in the endogenous *DHFR* gene were efficiently repaired and were almost completely removed 24 h after UV irradiation. The CPD repair in the NT strand of exons 2 and 5 was the same in both the endogenous and translocated *DHFR* genes. These results exclude the possibility that DNA sequence causes efficient CPD repair in the NT strand of exon 1 and strongly suggest that the mechanism facilitating the efficient CPD repair in the T strand of exon 1 in the *DHFR* gene in different genomic contexts differs from the mechanism facilitating the efficient repair in the NT strand of the endogenous *DHFR* gene. Since the *DHFR* genes in these three cell lines are all transcriptionally active, these results





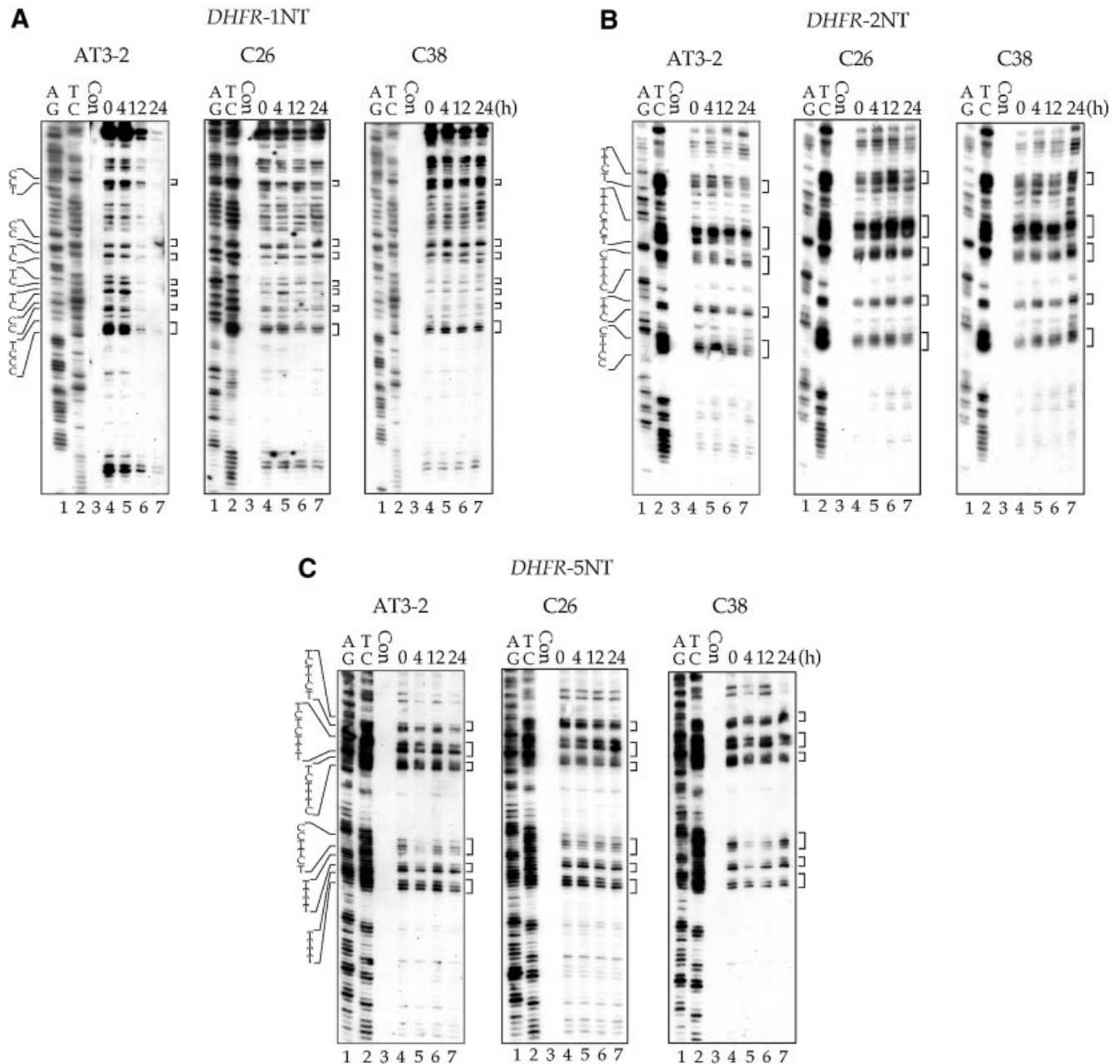
**Figure 3.** The kinetics of CPD repair in the transcribed strand of the *DHFR* gene in AT3-2, C26 and C38 cells. The relative amount of CPD formed at the dipyrimidine sites (bracketed) along the T strand of exons 1 (1T), 2 (2T) and 5 (5T) of the *DHFR* gene for each time point shown in Figure 2 was quantified with a Cyclone Storage Phosphor System. The percentage of CPDs remaining in the T strand of each exon was plotted as a function of repair time. The results represent three independent experiments.

cells (28). The *OST* gene is transcribed in the direction opposite to that of the *DHFR* gene; the DNA strand used as the NT strand of the *DHFR* gene is the T strand of the *OST* gene (Fig. 1) (20,21,29). It has been shown that in the *OST* gene, CPDs are preferentially repaired in the T strand and poorly repaired in the NT strand (30). These findings raise the possibility that the preferential repair of CPDs in the NT strand of exon 1 in the endogenous *DHFR* gene in AT3-2 cells comes from the extension of TCR in the T strand of the *OST* gene. If this is the case, the disappearance of the preferential repair of CPDs in exon 1 of the NT strand in the translocated *DHFR* gene in C26 and C38 cells should be accompanied by the disappearance of TCR from the T strand of the *OST* gene, which could be caused by the translocation of the fragment containing the intact *DHFR* gene and ~8 kb of the *OST* gene 5' end, including the promoter region. To test this possibility, CPD repair in exon 1 of the *OST* gene was mapped at the nucleotide level in the AT3-2, C26 and C38 cell lines. The

results in Figure 6 show that in both endogenous and translocated *OST* genes, the CPDs in the T strand of exon 1 are efficiently repaired, and CPDs are almost completely removed 12 h after UV irradiation. Furthermore, the repair efficiency of CPDs in the T strand of the *OST* gene in these three cell lines is very similar. These results exclude the possibility that the efficient repair of CPDs in the NT strand of exon 1 of the endogenous *DHFR* gene in AT3-2 cells comes from the extension of TCR from the upstream *OST* gene.

### The kinetics of MN digestion in the exon 1 region are slower in the translocated *DHFR* genes than in the endogenous *DHFR* gene

It is known that packaging of eukaryotic DNA into chromatin affects all aspects of DNA processing, including DNA repair, because it modulates access of proteins to DNA (31–33). Several recent studies have demonstrated that nucleosomal structure can inhibit repair of CPDs and <6–4> photoproducts in *in vitro* NER assays using mononucleosome or dinucleosome systems, suggesting that the assembly of nucleosomes may restrict the access of DNA repair proteins to the damaged DNA bases (34–36). We previously found that the exon 1 region of the endogenous *DHFR* gene is more sensitive to MN digestion than the exon 2 and 5 regions, which indicates that the exon 1 region has a more open chromatin structure than the exon 2 and 5 regions (19). These results raise the possibility that the more open chromatin structure in exon 1 may contribute to the preferential CPD repair in the NT strand of exon 1 in the endogenous *DHFR* gene in AT3-2 cells. If this is the case, then we would expect the chromatin structure of exon 1 in the translocated *DHFR* gene to be different from that found in exon 1 of the endogenous *DHFR* gene, since we have found that CPD repair in the NT strand of exon 1 is much more efficient in the endogenous than in the translocated *DHFR* gene. To test this possibility, we probed the chromatin structure of exons 1 and 2 in both the endogenous and translocated *DHFR* genes by determining their sensitivity to MN digestion. Nuclei were isolated from AT3-2, C26 and C38 cells, and subjected to digestion with MN for different time periods. The results in Figure 7 show that the chromatin structure of the exon 1 region of the endogenous *DHFR* gene in AT3-2 cells was much more sensitive to MN digestion than in the translocated *DHFR* gene in C26 and C38 cells. After 3 min of MN digestion, a significant amount of the endogenous *DHFR* gene exon 1 was in tri-, di- and mononucleosome structures. In contrast, most of exon 1 in the translocated *DHFR* genes remained in trinucleosome and higher nucleosome structures. After 5 min of MN digestion, there were more di- and mononucleosomes in exon 1 of the endogenous *DHFR* gene than that in exon 1 of the translocated *DHFR* genes. However, the kinetics of MN digestion for exon 2 in the endogenous and translocated *DHFR* genes are very similar if not identical. These results indicate that the exon 1 region of the endogenous *DHFR* gene in AT3-2 cells has a much more open chromatin structure than that of the translocated *DHFR* genes in C26 and C38, which strongly suggests that the more efficient repair of CPDs in the NT strand of the exon 1 region of the endogenous *DHFR* gene is due to its more open chromatin structure.

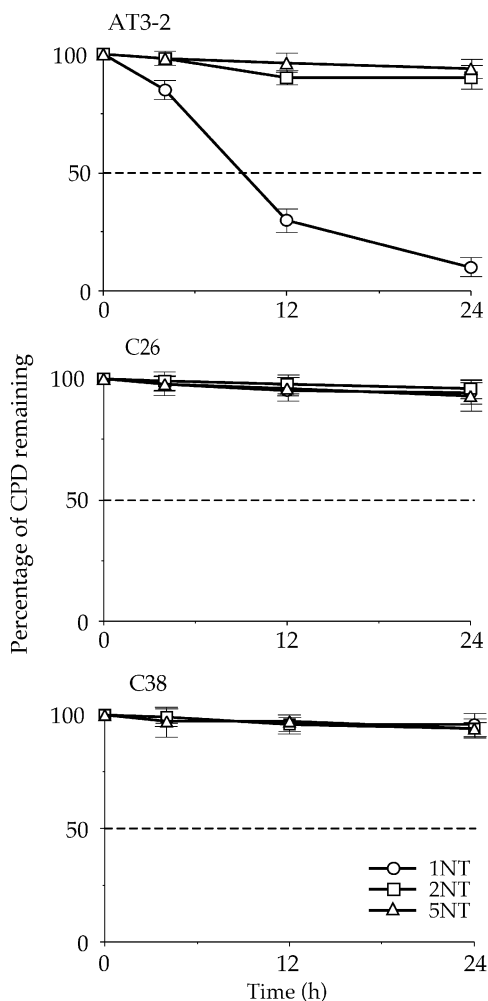


**Figure 4.** The time course of CPD repair in the non-transcribed strand of exon 1 (A), exon 2 (B) and exon 5 (C) of the *DHFR* gene in AT3-2, C26 and C38 cells. Cultured cells were UV irradiated ( $15 \text{ J/m}^2$ ) and then incubated for various periods of time. Genomic DNA was isolated, treated with T4 endo V followed by photoreactivation, and then subjected to LMP-PCR. A + G and T + C represent Maxam–Gilbert sequencing reactions. Sequences of contiguous pyrimidines with the potential to form CPD are indicated on the left, and T4 endo V incision sites are indicated on the right (bracketed). Lanes 4–7 show the relative frequency of T4 endo V cutting at dipyrimidine sites along each sequence at different post-UV repair time points (0, 4, 12 and 24 h). Lane 3 (Con) represents DNA isolated from unirradiated control cells treated with T4 endo V. Very similar results were obtained from three independent experiments.

## DISCUSSION

It is generally accepted that NER consists of two pathways: TCR and GGR. While the TCR pathway repairs DNA damage in the T strand of actively transcribed genes, the GGR pathway repairs DNA damage in the NT strand of actively transcribed genes and non-coding DNA (3–8). Most of our knowledge about TCR and GGR is derived from the results of mapping CPD repair in defined regions of the *DHFR* gene in rodent and

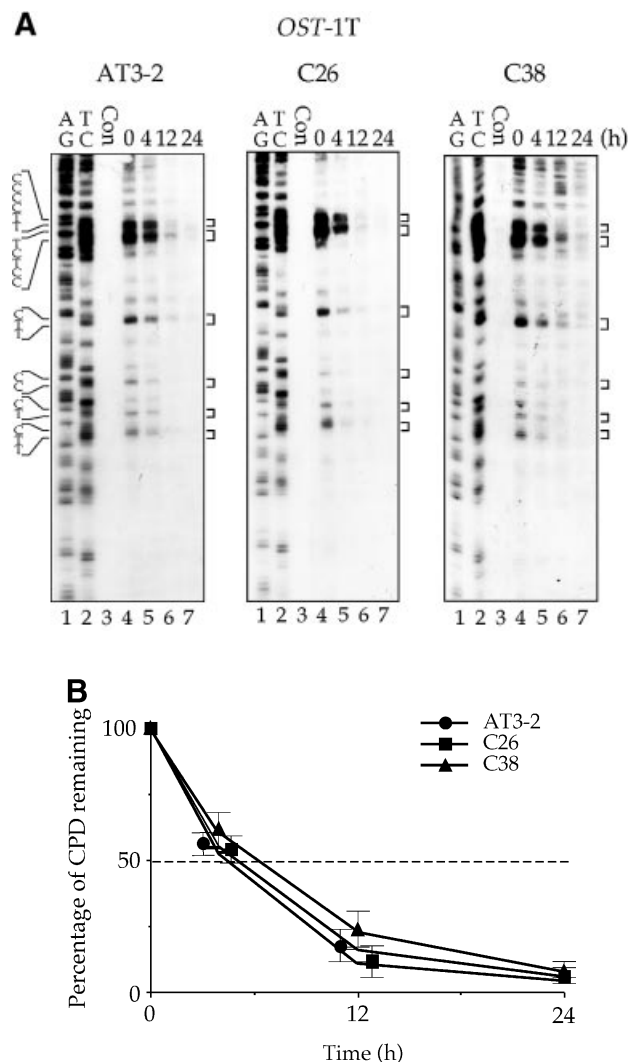
human cells (8,30,37–40). Ample evidence has demonstrated that in both rodent and human cells, CPD repair is much faster in the coding region of this gene than in the 3' downstream non-coding region, and is also much faster in the T strand than in the NT strand (8,30,37–40). Based on these results, the concept of two subpathways for the repair of bulky DNA damage by NER has emerged during the past decade. Hereditary defects and somatic mutations that lead to defects in either subpathway have also been found (41–45). Human



**Figure 5.** The kinetics of CPD repair in the non-transcribed strand of the *DHFR* gene in AT3-2, C26 and C38 cells. The relative amount of CPD formed at the dipyrimidine sites (bracketed) along the non-transcribed strand of exons 1 (1NT), 2 (2NT) and 5 (5NT) of the *DHFR* gene for each time point shown in Figure 4 was quantified with a Cyclone Storage Phosphor System. The percentage of CPD remaining in the non-transcribed strand of each exon was plotted as a function of repair time. The results represent three independent experiments.

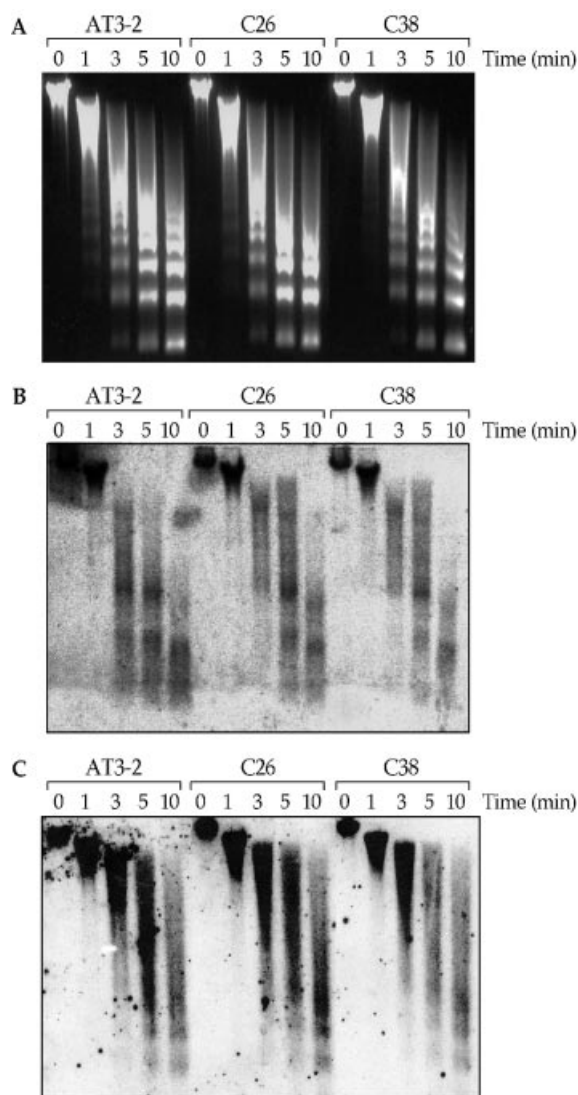
cells are proficient in both pathways. Cultured rodent cells, however, are proficient in TCR but are deficient in GGR of CPDs (9). It has been found that human xeroderma pigmentosum complementation group C (XPC) cells are deficient in GGR, while Cockayne syndrome (CS) cells and rodent ERCC6 cells are deficient in TCR (39,40,44–47). Interestingly, despite great efforts by many laboratories, the mechanism of TCR in mammalian cells remains unclear. In *E. coli* cells, not only has the *mfd* gene, which regulates TCR, been identified, but also the role of the *mfd* protein in TCR has been elucidated in a cell-free system (48,49). In contrast, even though all the major NER factors, including CS proteins, have been purified and efficient NER can be reconstituted *in vitro* (13), the TCR phenomenon has not yet been seen in a cell-free cell lysate system.

The current model proposed in the literature suggests that DNA damage that serves as a substrate for TCR should block



**Figure 6.** The time course of CPD repair in the transcribed strand of exon 1 of the *OST* gene in AT3-2, C26 and C38 cells. Cultured cells were UV irradiated ( $15 \text{ J/m}^2$ ) and then incubated for various periods of time. Genomic DNA was isolated, treated with T4 endo V followed by photo-reactivation, and then subjected to LMPCR. (A) Typical autoradiographs. A + G and T + C represent Maxam–Gilbert sequencing reactions. Sequences of contiguous pyrimidines with the potential to form CPDs are indicated on the left, and T4 endo V incision sites are indicated on the right (bracketed). Lanes 4–7 show the relative frequency of T4 endo V cutting at dipyrimidine sites along each sequence at different post-UV repair time points (0, 4, 12 and 24 h). Lane 3 (Con) represents DNA isolated from unirradiated control cells and treated with T4 endo V. Very similar results were obtained from three independent experiments. (B) The kinetics of CPD repair in the transcribed strand of exon 1 of the *OST* gene. The relative amount of CPD formed at the dipyrimidine sites (bracketed) along the transcribed strand of exons 1 of the *OST* gene for each time point shown in (A) was quantified with a Cyclone Storage Phosphor System. The percentage of CPD remaining in the transcribed strand was plotted as a function of repair time. The results represent three independent experiments.

transcription; this blockage gives rise to a special signal that attracts NER factors to the damaged site and subsequently allows NER to proceed (50). However, it has been found that in some cell-free cell lysate systems, blockage of the transcription process by DNA damage actually hinders NER (51,52). This finding led to the proposition that the blocked



**Figure 7.** The MN digestion sensitivity of exons 1 and 2 of the *DHFR* gene in AT3-2, C26 and C38 cells. Nuclei were isolated from the three CHO cell lines and digested with MN (1 U/100  $\mu$ l) for different times (0, 1, 3, 5 and 10 min). Genomic DNA was isolated, separated by electrophoresis in a 1.5% agarose gel, stained with ethidium bromide (A) and then transferred to a nitrocellulose membrane and hybridized with  $^{32}$ P-labeled probes specific for exon 1 (B) or exon 2 (C) of the *DHFR* gene.

transcription machinery may retreat from the damaged site to allow the attraction of NER factors to the site (4), which raises the possibility that TCR could occur more often at the 5' end of the gene if significant amounts of abortive transcription take place. It is also possible that the transcriptionally active genes, because of transcription factor binding, exist in a 'state' of chromatin structure that is more susceptible to NER, thereby allowing bulky DNA damage along the transcriptionally active genes to be evenly repaired in the T strand. It is well established that the 5' end of the *DHFR* gene is transcribed more frequently than the 3' end of the gene because of abortive transcription (53). Controversial results, however, have been reported with regard to the repair pattern of CPDs along the T strand of the *DHFR* gene. Using a Southern blot-based DNA repair assay, both uniform repair and a 5' to 3' polarity effect

on CPD repair along the T strand of the *DHFR* gene have been reported (30,37). In this study, a much more sensitive method—T4 endo V incision in combination with LMPCR—was used to measure CPD repair in exons 1, 2 and 5 of the *DHFR* gene at nucleotide resolution. Our finding clearly demonstrates that CPD repair in the T strand of the *DHFR* gene is subject to a polarity effect; CPDs are repaired more quickly at the 5' than at the 3' end of the gene in different chromosomal positions that exhibit different chromatin structures, which suggests that blockage of transcription triggers the process of TCR. It is likely that the transcription process *per se* opens up a limited area, thus allowing the repair process to take place. Our results, however, are unable to determine whether the blocked transcription machinery retreats from the DNA damage site.

We have found that 90% of CPDs of genomic DNA in UV (15 J/m<sup>2</sup>)-irradiated CHO cells remained unrepaired after 24 h of post-irradiation incubation. These results are consistent with the notion that cultured rodent cells are deficient in GGR of CPDs (9). The finding that CPDs in the NT strand of exon 1 of the endogenous *DHFR* gene are repaired as efficiently as in the T strand is intriguing. We have previously demonstrated that the NT strand of exon 1 of the *DHFR* gene is not transcribed; therefore, the repair in this region is not due to TCR of an immediately upstream transcription unit (19). In this study, we demonstrate that neither the DNA sequence nor the TCR of the upstream *OST* gene contributes to the efficient repair of CPDs in the NT strand of exon 1 of the endogenous *DHFR* gene. Our results also suggest that the efficient repair is not due to the transcription of the *DHFR* gene since the *DHFR* genes in these three cell lines are all transcriptionally active. However, we have found that chromatin in the exon 1 region of the endogenous *DHFR* gene is much more sensitive to MN digestion than chromatin in exons 2 and 5 (19). We have also found that in the translocated *DHFR* gene, the disappearance of efficient CPD repair in the NT strand of exon 1 is accompanied by the disappearance of sensitivity to MN digestion in this region. Together, these results suggest that certain open chromatin structures in rodent cells allow efficient repair to occur in the NT strand without the help of the transcription process. The effect of this open chromatin structure on NER may be similar to the effect of blocked transcription on NER and most probably is able to attract NER factors to the damaged sites and allows NER to proceed. Recently, Sancar and colleagues have shown that the chromatin-remodeling factor SWI/SNF can greatly enhance NER on mononucleosome core substrates, but has no effect on NER on a naked DNA substrate (54). Perhaps the chromatin at exon 1 of the endogenous *DHFR* gene is favored to associate with this type of remodeling factor and/or is remodeled by histone acetylation or phosphorylation, which consequently allows NER to take place.

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