Association between Transcriptional Activity, Local Chromatin Structure, and the Efficiencies of Both Subpathways of Nucleotide Excision Repair of Melphalan Adducts

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The repair of melphalan-induced N-alkylpurine monoadducts and interstrand cross-links was examined in different repair backgrounds, focusing on four genes (β-actin, p53, N-ras, and δ-globin) with dissimilar transcription activities. Adducts were found to be substrates for both global genome repair (GGR) and transcription-coupled repair (TCR), with TCR being less efficient than GGR. In nucleotide excision repair–deficient cells, adducts accumulated to similar levels in all four genes. The repair efficiency in different gene loci varied in a qualitatively and quantitatively similar way in both GGR-deficient and TCR-deficient backgrounds and correlated with transcriptional activity and local chromatin condensation. No strand-specific repair was found in GGR/TCR cells, implying that GGR dominated. Adducts were lost over two sharply demarcated phases: a rapid phase resulting in the removal within 1 hour of up to ~80% of the adducts, and a subsequent phase with t1/2 ~ 36 to 48 hours. Following pretreatment of cells with α-amanitin, the rate of transcription, the state of chromatin condensation, and the repair efficiencies (both TCR and GGR) of the transcribed β-actin, p53, and N-ras genes became similar to those of the nontranscribed δ-globin gene. In conclusion, a continuous, parallel variation of the state of transcription and local chromatin condensation, on one hand, and the rates of both GGR and TCR, on the other hand, have been shown. [Cancer Res 2009;69(10):4424–33]

Introduction

Melphalan [4-[bis(2-chloroethyl)amino]-1-phenylalanine] is a nitrogen mustard used in the management of multiple myeloma (1). It reacts with DNA, producing mostly N-alkylpurine monoadducts, a small proportion of which goes on to form interstrand cross-links (ICL), which probably play a major role in cytotoxicity (2–4). Melphalan monoadducts are repaired by nucleotide excision repair (NER), whereas at higher adduct levels, there was no difference in repair efficiency of GGR and TCR, on the other hand, have been shown.

Research Article

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For a variety of DNA lesions, NER takes place more efficiently in transcriptionally active DNA owing to the action of the transcription-coupled repair (TCR; refs. 8, 9). When both TCR and global genome repair (GGR) are present, the overall efficiency of repair of the transcribed strand is not necessarily additive. For example, the N-(deoxyguanosine-8-yl) adducts induced by N-acetoxy-2-acetylaminofluorene, known to be substrates for TCR and GGR, are removed without strand specificity from the active adenosine deaminases (ADA) gene of normal and TCR-deficient Cockayne’s syndrome (CS) human fibroblasts (10), indicating that repair is dominated by GGR.

There is evidence that other factors, in addition to the interplay of GGR and TCR, contribute to the genomic heterogeneity of DNA repair. For example, evidence of preferential repair of potentially active genes, even in the absence of active transcription, was obtained with a human cell line in which transcription of the ADA gene was abolished by promoter deletion (9). Furthermore, in CS cells, GGR of N-acetoxy-2-acetylaminofluorene–induced adducts in an inactive locus occurs much more slowly than in the active ADA gene (10). These results are in line with other studies showing that lesions known to undergo GGR are repaired considerably more slowly in X-chromosome loci than in active genes, and that DNA damage present in subnuclear compartments or chromatin domains within which transcription occurs is preferentially repaired by GGR (11–14).

Conflicting data have been reported about the mechanism of repair of nitrogen mustard–induced N-alkylpurines. Thus, in Chinese hamster ovary (CHO) cells, repair of such lesions occurred faster and with a slight bias in favor of the transcribed strand in the expressed diphosphatase reducetase (DHFR) gene relative to a noncoding 3′-flanking region (15, 16). On the other hand, repair was reported to occur at the same rate in the transcriptionally repressed and the actively transcribed alleles of the c-myc gene in Burkitt’s lymphoma cells (17). A similar independence of repair of these adducts from transcriptional state was reported in the metallothionein gene of CHO cells, with and without zinc-induced transcription activation (18). Finally, Larminat and colleagues (19) reported that, in CHO cells, at low adduct levels, preferential repair occurred in the DHFR gene as compared with an inactive region, whereas at higher adduct levels, there was no difference in repair rates due to an increase in repair efficiency of the inactive DNA.

We have previously shown that, in peripheral blood mononuclear cells (PBMC) of multiple myeloma patients treated with melphalan, accumulation of N-alkylpurines in the p53 gene correlates with better therapeutic response (20–22), and that repair in different genes correlated with the gene transcriptional activity and the degree of local chromatin condensation (23). To further investigate the mechanistic basis of melphalan adduct repair, we have now conducted studies in cells with varying repair activity. For this purpose, we used four primary human
fibroblast cell lines derived from normal, CS complementation group B, and xeroderma pigmentosum (XP) complementation group C and group A subjects, whose DNA repair characteristics have been previously characterized and shown to reflect specific DNA repair genotypes.

**Materials and Methods**

**Cell lines and culture conditions.** Primary fibroblasts from a normal individual (VH25; GGR+/TCR+), CS complementation group B (CS1AN; GGR+/TCR+), XP complementation group C (XP21RO; GGR+/TCR+) and XP complementation group A (XP25RO; GGR/C0/TCR+) were cultured at 37°C, in 5% CO2 atmosphere in DMEM containing 10% fetal bovine serum and 1% penicillin, streptomycin.

**Cell survival studies.** Cell survival was determined by measuring colony-forming ability (15). A total of 500 cells were seeded in Petri dishes, allowed to attach for 16 h, and incubated with melphalan for 1 h at 37°C in complete medium. After incubation, fresh medium was added and, 10 to 14 d after plating, colonies were stained with methylene blue.

**Cell treatment with melphalan in the absence or presence of α-amanitin.** To measure adduct frequencies in defined genomic sequences, confluent cells were exposed to melphalan (10 μg/mL for 1 h, unless otherwise indicated) in complete medium (23). For time course experiments, cells were subsequently incubated in drug-free medium for various times, harvested, and stored at ~70°C. In some experiments, confluent cells were exposed to 10 μg/mL α-amanitin for 6 h and subsequently treated as above, except that α-amanitin was always present in the culture medium.

**Measurement of gene-specific damage and repair.** For the measurement of total N-alkylpurines (monoadducts plus ICL), DNA was digested with the appropriate restriction enzyme and N-alkylated purines were converted to apurinic sites by heating and then to single-strand breaks with NaOH. The DNA was finally size-fractionated by gel electrophoresis, Southern blotted, and hybridized with appropriate probes (23). For the measurement of ICL, following restriction enzyme digestion, DNA was denatured and subjected to gel electrophoresis and Southern blotting. The frequency of ICL was directly derived from the latter samples, whereas the frequency of monoadducts was calculated by subtracting this value from that of total N-alkylpurines.

**Figure 1.** A, cytotoxic effects of melphalan after 1-h treatment. B, inhibition and recovery of total RNA synthesis after treatment with 10 μg/mL melphalan for 1 h. The zero time point corresponds to the end of the melphalan exposure. Bars, SD. The data shown are based on two independent experiments with at least three analyses each. C, steady-state RNA levels (lane 1) and nuclear run-off transcription (lane 2) in different genes of untreated normal cells (as representative of all cell lines). D, autoradiograms showing micrococcal nuclease sensitivity of different genes from CS-B nuclei in the absence (lanes 1–4) or presence (lanes 5–8) of α-amanitin pretreatment: β-actin (lanes 1 and 5), p53 (lanes 2 and 6), N-ras (lanes 3 and 7), and δ-globin (lanes 4 and 8). Lanes 9 and 10, ethidium bromide staining of the agarose gel of DNA from micrococcal nuclease–digested nuclei in the absence or presence of α-amanitin pretreatment, respectively. Lane 11, DNA from undigested nuclei. M, D, and T, positions of nucleosome monomers, dimers, and trimers, respectively.
For the analysis of strand-specific repair, riboprobes were used (16, 23). Recombinant plasmids containing the appropriate DNA fragment were digested with BamH1 (when using T7 RNA polymerase) or Kpn1 (when using SP6 RNA polymerase) to generate templates for strand-specific riboprobes. Reactions with T7 and SP6 RNA polymerases were carried out with [32P]CTP using the Boehminger Mannheim SP6/T7 transcription kit, and radiolabeled riboprobes were hybridized to DNA samples immobilized on nitrocellulose.

Overall genome repair was determined by gel electrophoresis using densitometer scanning of ethidium bromide–stained agarose gels (23, 24).

Measurement of RNA synthesis. For the analysis of total RNA synthesis, cells were treated in triplicate with [5,6-3H]uridine during the last hour of incubation (23, 25). Cells were subsequently suspended in ice-cold trichloracetic acid; the resulting acid-insoluble material was collected onto Millipore GS/0.22-μm filter disks and radioactivity was counted.

For slot-blot analysis, total cellular RNA was slot-blotted on nitrocellulose and hybridized to 32P-labeled probes; the expression of cellular genes was evaluated by densitometric comparison (23, 26).

Results

Cytotoxicity. Following a 1-hour melphalan treatment, normal cells showed the highest resistance and XP-A the highest sensitivity (Fig. 1A). Loss of TCR (CS-B) had a greater effect on resistance than did loss of GGR (XP-C) regardless of the remaining repair background, whereas loss of both subpathways resulted in additional sensitization (i.e., the effects of the two subpathways were complementary).

Effects on total RNA synthesis. Figure 1B shows that total RNA synthesis was inhibited by melphalan treatment in all four cell lines and that cells with at least one NER subpathway active were capable of recovering RNA synthesis after 24 hours. In contrast, in NER-deficient cells no recovery occurred. Interestingly, the sensitivity of the different cell lines to inhibition of RNA synthesis parallels their sensitivity to melphalan cytotoxicity (Fig. 1A).

Transcriptional activity and chromatin condensation at different gene loci. We focused our studies of repair on different genomic loci corresponding to the β-actin, p53, N-ras, and δ-globin genes. The steady-state RNA levels of these genes were measured by RNA slot-blot analysis (Fig. 1C, lane 1), whereas transcription rates were measured using a run-off transcription assay (Fig. 1C, lane 2). The results of both assays (Table 1) consistently showed that transcription varies in the order β-actin > p53 > N-ras in all cell lines, whereas no δ-globin gene transcripts were detected. This order of variation is in agreement with our previous findings in human PBMC (23).

Table 1. Steady-state RNA levels and nuclear run-off transcription rates of the genes expressed as a ratio to that of β-actin

<table>
<thead>
<tr>
<th>Expression ratio (mean value ± SD)</th>
<th>VH25</th>
<th>CS-B</th>
<th>XP-C</th>
<th>XP-A</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-Actin/p53 (steady state)</td>
<td>1.8 ± 0.2</td>
<td>2.0 ± 0.2</td>
<td>1.6 ± 0.2</td>
<td>1.9 ± 0.3</td>
</tr>
<tr>
<td>β-Actin/N-ras (steady state)</td>
<td>2.5 ± 0.3</td>
<td>3.1 ± 0.3</td>
<td>2.7 ± 0.3</td>
<td>3.0 ± 0.3</td>
</tr>
<tr>
<td>β-Actin/p53 (run-off)</td>
<td>1.6 ± 0.2</td>
<td>1.5 ± 0.2</td>
<td>1.3 ± 0.2</td>
<td>1.7 ± 0.2</td>
</tr>
<tr>
<td>β-Actin/N-ras (run-off)</td>
<td>1.9 ± 0.2</td>
<td>1.8 ± 0.2</td>
<td>2.1 ± 0.2</td>
<td>2.0 ± 0.2</td>
</tr>
</tbody>
</table>

Adduct kinetics in different repair backgrounds. Monoadducts and ICL were measured following cell treatment with 10 μg/mL melphalan for 1 hour. This concentration is of biological relevance because it gives rise to adduct levels similar to those detected in multiple myeloma patients following therapeutic treatment (23, 28). In all cases, monoadduct levels were observed 2 hours following the end of treatment, and in XP-A cells, they reached very similar levels in all genes examined (Fig. 2). On the other hand, substantial differences in XP-A cells, they reached very similar levels in all genes examined (Fig. 2). On the other hand, substantial differences among the different genes were observed in the remaining cell lines, even when examined immediately after the end of treatment (t = 0 in Fig. 2), indicating that substantial repair occurred during the 1-hour treatment period. The differences in the rates of subsequent adduct loss in the different cell types and gene loci were, by comparison, minor. ICL levels reached maximal levels within 8 hours after the end of treatment, showed no evidence of repair in XP-A cells, and were approximately 10% to 30% reduced by 24 hours posttreatment in the remaining cell lines (results not shown).

Focusing on strand-specific repair, for all active genes monoadduct kinetics in the transcribed strand (TS) and nontranscribed strand (NTS) of GGR-proficient normal and CS-B cells were very similar (Fig. 2A–C), showing maximal levels 2 hours posttreatment followed by slow loss with t1/2 ∼36 to 48 hours. In these cells, monoadduct levels, immediately after the end of melphalan treatment, were lowest (i.e., early repair

5 Unpublished results.
most efficient) in the β-actin gene, followed by p53, then N-ras, and finally δ-globin (only NTS in the last case). In XP-C cells, the corresponding monoadduct levels in the TS were also substantially reduced relatively to those in XP-A, but were slightly higher than those seen in the corresponding genes in GGR-proficient cells, indicating that GGR is more efficient than TCR at repairing the TS. The variation of the efficiency of early TCR between the different genes paralleled that described above for GGR.

Figure 2. Monoadduct kinetics in different repair backgrounds. Cells were exposed to 10 μg/ml melphalan for 1 h and strand-specific monoadduct levels were measured in the β-actin (A), N-ras (B), p53 (C, left), and δ-globin (D, left) genes during the following 24 h. The zero time point corresponds to the end of treatment. The data shown are based on two independent experiments with at least three analyses each. Bars, SD. C and D, right, autoradiograms reflecting total adduct levels in the transcribed strand of p53 (C, right; representative of the three active genes) and in one strand of the silent δ-globin gene (D, right), from which the data shown on the left were calculated. S1 and S2, the two strands of δ-globin gene; 0/0, control, nontreated samples.
Little if any repair was observed in both strands of all genes in XP-A cells and in the NTS in XP-C cells (Fig. 2). In all transcribed genes, the presence of TCR only (i.e., in XP-C) was associated with slightly higher monoadduct levels. As for the nontranscribed \( \delta \)-globin gene, as expected, similar monoadduct kinetics were observed for both strands in normal and CS-B cells, whereas little, if any, adduct loss from either strand was seen in XP-C and XP-A cells (Fig. 2D).

Substantial differences were observed between the levels of ICL in different genes and cell lines (data not shown), which paralleled closely those seen for monoadducts. Because the latter are the precursors of ICL, no conclusions about ICL repair can be drawn from this variation. Beyond the 8-hour peak, limited loss of ICL (probably reflecting unhooking) was observed, which tended to vary in the order normal cells > CS-B > XP-C > XP-A and \( \beta \)-actin > \( p53 \) > \( N-ras \) > \( \delta \)-globin.

To further investigate the extensive repair occurring during the 1-hour melphalan treatment, cells were treated with a higher concentration of melphalan (100 \( \mu \)g/mL) for only 5 minutes and, subsequently, repair was followed for up to 2 hours (Fig. 3). The results confirmed that, in the absence of NER, similar adduct levels accumulated in all four genes (Fig. 3D), whereas in all repair-proficient backgrounds, a substantial fraction (~60–75%) of the adducts formed underwent rapid repair. Repair kinetics in normal and CS-B cells were very similar (Fig. 3A and B), with adducts being lost with \( t_{1/2} \) ranging from <15 minutes to ~2 hours for different genes (Fig. 3B). Rapid initial repair was also observed in XP-C cells with \( t_{1/2} \) ranging ~30 minutes to ~2 hours and with a similar variation as seen for GGR. As expected, no repair was observed in the \( \delta \)-globin gene (Fig. 3C). Early repair was slower in XP-C cells than in CS-B, confirming that TCR is less effective than GGR.

**Effect of dose on repair efficiency.** To explore whether the lower efficiency of TCR might result from inhibition of initiation of RNA synthesis, CS-B and XP-C cells were treated with various melphalan doses (2–100 \( \mu \)g/mL) for 1 hour, and monoadducts measured in \( p53 \) (taken as representative of the transcribed genes). In CS-B cells, a linearly dose-dependent increase in adduct levels, reflecting constant repair efficiency, was observed throughout the dose range used (Fig. 4A). In XP-C cells, a parallel dose-response curve was observed, with higher adduct levels (i.e., lower repair

![Figure 3](image-url)

Figure 3. Early kinetics of monoadduct repair. Cells were exposed to 100 \( \mu \)g/mL melphalan for 5 min and monoadducts were measured in normal (A), CS-B (B), XP-C (C), and XP-A (D) cells during the following 2 h. The zero time point corresponds to the end of treatment. Bars, SD. The data presented here are based on two independent experiments with at least three analyses each.
efficiency) up to a dose of 20 \( \mu \text{g/mL} \), beyond which an abrupt increase in the slope occurred, reflecting reduced TCR (Fig. 4A). To understand the molecular basis of this effect, total as well as p53-specific RNA synthesis was measured under the same conditions. Dose-dependent inhibition of total RNA synthesis until 8 hours posttreatment, followed by slow recovery at 24 hours, was observed at all doses (data not shown). On the other hand, in both cell lines, with up to 20 \( \mu \text{g/mL} \) melphalan, a dose-dependent, partial inhibition of p53-specific RNA synthesis was observed, whereas at higher doses (at which decreased TCR occurred) complete inhibition and no recovery up to 24 hours were seen (Fig. 4B and C). We conclude that any effect on transcription of melphalan at low doses does not modify the relative efficiencies of TCR and GGR. Strikingly, under these conditions, the gene-to-gene variations of the efficiencies of GGR and TCR were similar not only qualitatively but also quantitatively (Fig. 4D).

**Effect of \( \alpha \)-amanitin on gene-specific repair.** \( \alpha \)-Amanitin is an inhibitor of RNA polymerase II that can also induce condensation of chromatin (29, 30). To examine the dependence of repair on transcription and chromatin structure, we compared the rates of adduct repair in CS-B and XP-C cells without or with prior treatment with \( \alpha \)-amanitin. With the use of slot-blot and run-off transcription assays, we confirmed that treatment with 10 \( \mu \text{g/mL} \) \( \alpha \)-amanitin for 6 hours completely blocked transcription (data not shown). Using micrococcal nuclease digestion (Fig. 1D, lanes 9 and 10), we found that \( \alpha \)-amanitin induced some chromatin condensation at the total DNA level, whereas, at the level of specific genes, chromatin condensation of the active genes in CS-B cells occurred, leading to a picture very similar to that observed for the nonexpressed \( \delta \)-globin, for which no change was observed (Fig. 1D, lanes 5–8).

Following \( \alpha \)-amanitin treatment, the efficiency of both repair subpathways in the active genes was significantly reduced and became similar to that observed for \( \delta \)-globin, which was not affected by \( \alpha \)-amanitin (Fig. 5). In accordance with the increased condensation of total chromatin (Fig. 1D, lanes 9 and 10), \( \alpha \)-amanitin also led to marginally higher overall genome N-alkylpurine accumulation (i.e., lower repair efficiency). Furthermore, in agreement with the

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**Figure 4.** Effect of melphalan dose on the efficiency of TCR and GGR. A, total adduct levels in p53 (as representative of the active genes) in CS-B and XP-C cells treated with various doses of melphalan for 1 h. p53-specific RNA synthesis in XP-C (B) and CS-B (C) cells, expressed relative to the corresponding untreated cells. The zero time point corresponds to the end of the 1-h melphalan exposure. The data presented here are based on two independent experiments with at least three analyses each. Bars, SD. D, correlation between the efficiencies of GGR and TCR in different gene loci, with adduct levels remaining 2 h following the end of treatment in CS-B and XP-C cells (taken from Fig. 2).
results of the gene-specific repair experiments, in the absence of α-amanitin, the efficiency of the overall genome N-alkylpurine repair was lower in XP-C than in CS-B cells (Fig. 5A and C).

**Discussion**

To further investigate the mechanism of repair of melphalan-induced N-alkylpurines, primary fibroblasts with varying repair activities, derived from normal, CS complementation group B, and XP complementation group C and group A subjects, were used. The DNA repair phenotypes of these cells have been previously shown to be similar to those of other cells derived from patients belonging to the same complementation groups. Thus, similar repair kinetics toward UV-induced photolesions have been shown for normal human cell lines (VH25 and VH16; refs. 31, 32), XP-C cell lines (XP21RO, XP1TE, and XP6RO; refs. 31, 32), CS-B cell lines (CS1AN and 11961; ref. 33), and XP-A cell lines (XP12BE and XP25RO; ref. 34). Further support for a causal association between the repair phenotypes of our cell lines and specific gene defects in the NER pathway has been provided by complementation studies (35, 36).

In accordance with previous reports (17, 20, 23), monoadduct accumulation continued for 2 hours beyond the end of the 1-hour melphalan treatment (Fig. 2). This was probably caused by the very rapid, active uptake of the drug (37, 38), which exceeds the rate of its intracellular breakdown, in combination with the relatively slow DNA repair beyond 1 hour. In contrast, after a 5-minute incubation, lower drug uptake and much faster first-phase repair prevent further accumulation of adducts (Fig. 3). ICL accumulated more slowly and reached maximal levels within 8 hours, reflecting a slow reaction of monoadducts with a site on the opposite strand. In the present study, in the absence of NER, the maximal levels of ICL corresponded to ~15% of those of monoadducts (compare ~5–10% reported with naked DNA; refs. 23, 39).

In NER-deficient cells, adducts accumulated to similar levels in all four genes, indicating that the state of transcription and local chromatin condensation did not affect their formation. On the other hand, substantial differences were observed immediately following the end of the 1-hour melphalan treatment, during which a large fraction of the total adducts expected to have been formed were lost. Adduct loss subsequent to this time point was relatively slow (t1/2 ~36–48 hours) and more or less similar in the different genes and cell lines. The importance of the initial phase of repair in determining the overall differences between the different gene loci was confirmed by a more detailed examination of repair kinetics following a 5-minute pulse of melphalan. This confirmed the operation of very rapid repair (t1/2 ranging from

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**Figure 5.** Effect of α-amanitin on DNA repair. The kinetics of gene-specific monoadducts as well as overall genome N-alkylpurines in CS-B (A and B) and XP-C (C and D) cells following melphalan (A and C) or melphalan plus α-amanitin (B and D) exposure are shown. Bars, SD. The data presented here are based on two independent experiments with at least three analyses each.
The cytotoxic effect of melphalan was greater in CS-B than in XP-C cells. Melphalan reflect an inherently lower efficiency of TCR and/or the limited inhibition of RNA synthesis, no slowdown of TCR occurred. (Fig. 4A–C). However, at lower melphalan doses, causing only limited inhibition of RNA synthesis, no slowdown of TCR occurred. This indicates that the reduced efficiency of TCR relative to GGR and its inability to compete with the latter pathway after 10 μg/mL melphalan reflect an inherently lower efficiency of TCR and/or the different adduct specificities of the two subpathways. Although the efficiency of GGR was greater than that of TCR, the cytotoxic effect of melphalan was greater in CS-B than in XP-C cells (Fig. 1A). Examination of the effects on RNA synthesis showed a greater degree of inhibition and slower recovery in the former than in the latter cell line (Fig. 1B), suggesting that TCR is more important for survival than GGR by permitting faster transcription recovery.

A notable finding of the present study is that the efficiencies of both GGR and TCR varied in the order \( \beta\text{-actin} > p53 > N\text{-ras} > \delta\text{-globin} \) (Fig. 3). This order of variation parallels that of the transcriptional activity and local chromatin condensation at the different gene loci (Fig. 1). The close association between chromatin structure and GGR was further underlined by the results of our studies involving \( \alpha\text{-amanitin} \): Following pretreatment of CS-B cells with \( \alpha\text{-amanitin} \), chromatin condensation and the efficiency of GGR in the \( \beta\text{-actin}, p53 \), and N-ras genes became very similar to those of the silent \( \delta\text{-globin} \) gene (in which no change was observed), whereas the overall repair of N-alkylpurines was marginally reduced. Taken together, these results suggest that increased accessibility of the DNA in transcribed chromatin enhances GGR. Interestingly, biphasic repair kinetics and inhibition of the early, rapid phase by \( \alpha\text{-amanitin} \) were also observed for \( O^2\text{-methylguanine} \) (25), which is repaired by a mechanism entirely different from NER.

An influence of the state of transcription and of local chromatin condensation on TCR and GGR has been previously reported. For example, UV-induced CPDs in both the TS and NTS of exon 1 of the \( \text{DHF}r \) gene of CHO cells are repaired more efficiently than adducts in exons 2 and 5, and this variation qualitatively parallels the level of local transcriptional activity (47, 48). Ours is the first study in which a continuous, parallel variation, covering four different loci, of the state of transcription and local chromatin condensation on one hand, and the rates of both GGR and TCR on the other hand, has been shown. Strikingly, the efficiencies of GGR and TCR varied from gene to gene in a manner that was similar not only qualitatively but also quantitatively (Fig. 4D). The association of high transcriptional activity with increased TCR efficiency may be attributed to higher numbers of RNA polymerase II complexes acting as lesion detectors (49, 50). On the other hand, the modulation of local GGR efficiency by the state of transcription may be the result of active transcription being associated with an open chromatin structure, which permits easier access to DNA repair factors. The correlation shown in Fig. 4D suggests that features of chromatin structure associated with transcription may affect one or more factors involved in both NER subpathways.

These data are in agreement with the report of Bedoyan and colleagues (51) suggesting that transcription, nucleosome stability, and DNA repair in a yeast minichromosome may be mechanistically interdependent.

ICLs are among the most cytotoxic lesions because they effectively prevent the separation of DNA strands and therefore block essential cellular processes. Whereas the combined action of NER and recombination has been proposed for the repair of ICL in \( E\text{-coli} \) and yeast (7). ICL repair in mammalian systems is poorly understood. De Silva and colleagues (52) found that XPF and ERCC1 mutants (defective in NER) and XRCC2 and XRCC3 mutants (defective in Rad51-related homologous recombination) were highly sensitive to the nitrogen mustard mechlorethamine. Furthermore, Rothfuss and Grompe (53) provided evidence for a refined model in which ICLs are recognized and rapidly incised by ERCC1/XPF ("unhooking" step) independent of DNA replication. However, the incised ICLs are then processed further and double-strand breaks form exclusively in the S phase. In the present study, which involved confluent cells most of which were in G\(_0\)-G\(_1\),...
evidence for ICL unhooking was obtained as reflected by a slow adduct decrease in cells active in either TCR or GGR. It is uncertain whether this initial step of ICL repair in G0-G1 cells has a significant role in resistance to melphalan toxicity.

In conclusion, our study has shown a parallel variation in the transcriptional activity, chromatin condensation, and efficiency of both TCR and GGR in different genomic loci and suggests that features of chromatin structure that influence the local transcriptional rate also affect accessibility to both GGR- and TCR-related repair factors.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Acknowledgments
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5. Edler M, Jakubowski N, Linscheid M. Quantitative evidence for ICL unhooking was obtained as reflected by a slow adduct decrease in cells active in either TCR or GGR. It is uncertain whether this initial step of ICL repair in G0-G1 cells has a significant role in resistance to melphalan toxicity.

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5. Edler M, Jakubowski N, Linscheid M. Quantitative evidence for ICL unhooking was obtained as reflected by a slow adduct decrease in cells active in either TCR or GGR. It is uncertain whether this initial step of ICL repair in G0-G1 cells has a significant role in resistance to melphalan toxicity.

In conclusion, our study has shown a parallel variation in the transcriptional activity, chromatin condensation, and efficiency of both TCR and GGR in different genomic loci and suggests that features of chromatin structure that influence the local transcriptional rate also affect accessibility to both GGR- and TCR-related repair factors.

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Association between Transcriptional Activity, Local Chromatin Structure, and the Efficiencies of Both Subpathways of Nucleotide Excision Repair of Melphalan Adducts

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