Lack of Transcription-coupled Repair of Acetylaminofluorene DNA Adducts in Human Fibroblasts Contrasts Their Efficient Inhibition of Transcription*

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The N-(deoxyguanosine-8-yl)-2-acetylaminofluorene (dG-C8-AAF) lesion is among the most helix distorting DNA lesions. In normal fibroblasts dG-C8-AAF is repaired rapidly in transcriptionally active genes, but without strand specificity, indicating that repair of dG-C8-AAF by global genome repair (GGR) overrules transcription-coupled repair (TCR). Yet, dG-C8-AAF is a very potent inhibitor of transcription. The target size of inhibition (45 kilobases) suggests that transcription inhibition by dG-C8-AAF is caused by blockage of initiation rather than elongation. Cockayne's syndrome (CS) cells appear to be extremely sensitive to the cytotoxic effects of dG-C8-AAF and are unable to recover inhibited RNA synthesis. However, CS cells exhibit no detectable defect in repair of dG-C8-AAF in active genes, indicating that impaired TCR is not the cause of the enhanced sensitivity of CS cells. These and data reported previously suggest that the degree of DNA helix distortion determines the rate of GGR as well as the extent of inhibition of transcription initiation. An interchange of the transcription/repair factor TFIIH from promoter sites to sites of damage might underlie inhibition of transcription initiation. This process is likely to occur more rapidly and efficiently in the case of strongly DNA helix distorting lesions, resulting in a very efficient GGR, a poor contribution of TCR to repair of lesions in active genes, and an efficient inhibition of transcription.

Nucleotide excision repair (NER) constitutest a versatile process capable of recognizing and eliminating a broad spectrum of DNA lesions including UV-induced photolesions, chemically induced bulky DNA adducts, and certain types of DNA cross-links (1). Two NER subpathways have been identified: the transcription-coupled repair (TCR) pathway and the global genome (GGR) repair pathway. The TCR pathway is confined to the transcribed strand of transcriptionally active genes and is dependent on ongoing transcription (2, 3), whereas the GGR pathway removes lesions from both nonexpressed DNA and transcriptional active genes. The contribution of TCR and GGR to the repair of bulky DNA lesions in active genes varies remarkably, demonstrated most strikingly for the two main UV-induced photolyses i.e. cyclobutane pyrimidine dimers (CPD) and pyrimidine 6–4 pyrimidone photoproducts (6–4 PP); briefly 6–4 PP are removed predominantly by GGR, whereas CPD are removed predominantly by TCR. Currently there is only limited knowledge concerning the factors that determine the efficiency of DNA damage recognition by the NER proteins. Studies with the purified Escherichia coli UvrABC excinuclease complex have revealed that structural determinants such as localized unwinding of the DNA helix or DNA bending or kinking influence the efficiency of NER recognition of DNA lesions (4). For TCR the potency of blocking elongation of transcription by a DNA lesion is most likely the critical factor.

Understanding the mechanisms and identification of the factors that determine DNA damage recognition requires lesion-specific information on repair kinetics, transcription blockage capacity, and structural distortion of the DNA helix. Studies with UV-irradiated mammalian cells have provided valuable insights in the repair of structural different lesions, i.e. CPD and 6–4 PP, although there are serious limitations as both types of lesions are induced simultaneously by UV light. Exposure of cells to NA-AAF provides the possibility of studying structurally different DNA lesions, for which detailed information exists on DNA conformation and transcription/replication blockage (for review, see Ref. 5). NA-AAF reacts with the guanine base of DNA inducing two major types of lesions, i.e. the deacetylated dG-C8-AF and the acetylated dG-C8-AAF. Formation of dG-C8-AAF lesions leads to a conformation change of the DNA which results in a local denaturation of about 5 base pairs (6, 7). This denaturation is caused by stacking of the amino fluorene group inside the DNA-helix, a conformation that is stabilized by the acetyl group. Such a distortion of the helix either does not occur or does so only at a low frequency when dG-C8-AAF lesions are induced (8, 9). Moreover, convincing evidence shows that a dG-C8-AAF lesion is an efficient block for transcription (10–12), whereas the dG-C8-AF lesion is a poor inhibitor of transcription (12, 13). The relative induction level of both lesions is dependent on organism, tissue, or even animal strain. In mice and cultured mammalian cells, includ-
ing human fibroblasts, the main lesion induced is the dG-C8-AF lesion (14–19), whereas in some rat strains the dG-C8-AAF lesion is induced to a substantial extent (20). The preferential induction of dG-C8-AF in most cellular systems has been related to efficient deacetylation of NA-AAF. In rat the high frequency of formation of dG-C8-AAF is caused by the presence of high levels of sulfotransferase activity, which prevents the deacetylation process by forming a stable sulfonated and acetylated compound (5). In recent studies we have focused on the repair of NA-AAF-induced dG-C8-AF lesions in normal and NA-AAF-sensitive human fibroblasts (18, 19). The repair of this lesion proceeds biphasingly and at a much slower rate than repair of UV-induced CPD or 6–4 PP in the same cell lines (21–24). In normal cells repair of dG-C8-AF is dominated by GGR, although dG-C8-AAF is target for TCR as shown in cells expressing only TCR (19). Compared with UV-induced photolesions, the effect of the dG-C8-AF lesion on transcription and cell survival is much less severe. If localized unwinding of the DNA helix and transcription blocking capacity would be critical determinants for efficient processing of DNA lesions by GGR and TCR, respectively, then one would expect dG-C8-AAF to be an extremely good target for both NER subpathways. Indeed, plasmids containing dG-C8-AAF are good substrates for NER in cell-free systems (25). The inhibition of transcription by dG-C8-AAF is more efficient than inhibition of transcription by dG-C8-AF and occurs without strand specificity, probably because the TCR is overruled by GGR. CS1AN cells remove the dG-C8-AAF lesions with kinetics similar to that of normal cells, but they show clearly enhanced sensitivity to the cytotoxic effects of paraaxon/NA-AAF treatment. Based on lesion frequencies, inhibition of transcription by dG-C8-AAF is more efficient than by UV-induced photolesions and is mediated by blockage of initiation rather than elongation.

**MATERIALS AND METHODS**

**Cell Lines and Culture Conditions—**Primary normal human (VH25D), Cockayne's syndrome complementation group B (CS1AN), and xeroderma pigmentosum complementation group C (XP21RO) fibroblasts were cultured in Ham's F-10 medium (without hypoxanthine and thymidine) supplemented with 15% fetal calf serum and antibiotics at 37 °C, using a 2.5% CO2 atmosphere. In experiments aimed at determining the frequency of adducts in defined genomic sequences, exponentially growing cells were prelabelled for 2 days with [3H]thymidine (0.06 μCi/ml, 82 Ci/mmol). For repair replication and for RNA synthesis experiments, exponentially growing cells were prelabelled with [32P] (0.3 μCi/ml) and [3H]uridine (0.02 μCi/ml, 60 mCi/mmol), respectively. In all cases the medium was replaced after the labeling period by label-free medium, and cells were allowed to grow to confluence. **Clonal Survival Studies—**Cell survival after treatment with NA-AAF was determined by measuring the colony forming ability of the treated cells relative to the untreated control. 500–2,000 cells were seeded in 94-mm Petri dishes allowing cells to attach for 16 h, and incubated with 10–8 M paraaxon (3.2 mM stock solution dissolved in 100% dimethyl sulfoxide) for 15 min before treatment with 0, 50, 100, and 150 μM NA-AAF for 30 min at 37 °C in complete medium. After incubation, the cells were washed twice with PBS, and fresh medium was added to the cells. After 7 days the medium was replaced by fresh medium; 10–14 days after plating the cells were rinsed twice with 0.9% NaCl, and colonies were stained with methylene blue.

**HPLC/EC Detection—**The nature and frequency of DNA lesions induced by the paraaxon/NA-AAF treatment were measured by HPLC analysis coupled to ECD of dG-C8-AF and dG-C8-AAF lesions. A detailed description of the methodology is published elsewhere (27). Briefly, DNA samples from the various cell lines treated with paraaxon/NA-AAF were dried and subsequently hydrolyzed in 50 mM trifluoroacetic acid at 70 °C for 45 min. After hydrolysis, H2O and ethyl acetate were added to the tubes, the DNA was extracted with ethyl acetate, and the combined ethyl acetate layers were evaporated. DNA samples were dissolved in water/methanol and injected on a Chrompack Hypersil-ODS glass cartridge column. Elution was done isocratically with 0.02 M K2PO4 and 50% methanol, pH 6.0. Detection of dG-C8-AF and dG-C8-AAF was performed by ECD detection employing a glassy carbon electrode (5000 series ANTEC).

**Measurement of DNA Repair Replication—**DNA repair replication was measured by the radioisotope and density labeling technique described by Van Zeeland et al. (28). Prelabelled confluent cells were treated with 10–7 M paraaxon for 15 min before treatment with 150 μM NA-AAF for 30 min at 37 °C. After washing the cells twice with PBS, fresh medium was added to the cells. At different time intervals after NA-AAF treatment the cells were pulse labeled with [3H]uridine (10 μCi/ml, 39.0 Ci/mmol) for 30 min at 37 °C and processed for liquid scintillation counting as described previously (18). Repair replication was expressed as H cpm/μg of DNA.

**DNA Probes—**Double-stranded DNA probes were radioactively labeled with [3H]dATP by random primer extension (29). Strand-specific single-stranded probes were radioactively labeled with [32P]dATP by a linear polymerase chain reaction, using a single primer recognizing specifically one strand (30).

**Gene-specific Analysis of NA-AAF-induced DNA Adducts—**To measure DNA adduct frequencies in defined genomic sequences as a function of dose, H-labeled confluent cells were incubated with 10–8 M paraaxon for 15 min before treatment with 0, 100, 200, 300, and 400 μM NA-AAF, in complete medium, for 30 min at 37 °C. After washing the cells twice with PBS, they were lysed. In repair experiments H-labeled confluent cells were incubated with 10–7 M paraaxon for 15 min before treatment with 300 μM NA-AAF, in complete medium, for 30 min at 37 °C. After washing the cells twice with PBS, they were either lysed immediately or incubated for various time intervals in complete medium supplemented with 10 μM BrdUrd and 1 μM FdUrd, with [3H]thymidine (5 μCi/ml, 82 Ci/mmol), and the cells were allowed to perform DNA repair at 37 °C for 24 h. Cell lysis, DNA isolation, and cesium chloride density gradient centrifugation were performed as described previously (18). Repair replication was expressed as H cpm/μg of DNA.

**Measurement of RNA Synthesis—**[14C]Uridine-prelabeled confluent cells were incubated with 10–7 M paraaxon for 15 min before treatment with 150 μM NA-AAF for 30 min at 37 °C. After washing the cells twice with PBS, fresh medium was added to the cells. At different time intervals after NA-AAF treatment the cells were pulse labeled with [3H]uridine (10 μCi/ml, 39.0 Ci/mmol) for 30 min at 37 °C and processed for liquid scintillation counting as described previously (18). Repair replication was expressed as H cpm/μg of DNA.

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**Gene-specific Analysis of NA-AAF-induced DNA Adducts—**To measure DNA adduct frequencies in defined genomic sequences as a function of dose, H-labeled confluent cells were incubated with 10–8 M paraaxon for 15 min before treatment with 0, 100, 200, 300, and 400 μM NA-AAF, in complete medium, for 30 min at 37 °C. After washing the cells twice with PBS, they were lysed. In repair experiments H-labeled confluent cells were incubated with 10–7 M paraaxon for 15 min before treatment with 300 μM NA-AAF, in complete medium, for 30 min at 37 °C. After washing the cells twice with PBS, they were either lysed immediately or incubated for various time intervals in complete medium supplemented with 10 μM BrdUrd and 1 μM FdUrd. DNA was isolated and purified as described previously (21), resuspended in 10 mM Tris-HCl, pH 8.0, 1 mM EDTA (TE), and digested overnight either with EcoRI at 37 °C or BglII at 50 °C. To separate the parental DNA from the replicated DNA, neutral cesium chloride gradient centrifugation was performed. After centrifugation, the fractions containing the parent DNA were pooled, dialyzed against 1× TE and 0.05× TE, and dried by vacuum centrifugation. Finally the samples were resolved in sterile water. The frequency of NA-AAF-induced DNA adducts was determined by in vitro incision at the sites of adducts employing the UvrABC excinuclease complex of _E. coli_ as described previously (24). Pierce et al. (25) showed that the UvrABC excinuclease incises efficiently at sites of
all dG-C8-AAF lesions in DNA fragments. Because the UvrABC complex exhibited a low but variable activity on DNA from cells not exposed to NA-AAF, a DNA sample isolated from untreated cells was also incubated with UvrABC (23). After incubation, the DNA samples were purified by phenol and chloroform extraction and subjected to 0.8% alkaline agarose gel electrophoresis followed by Southern blotting and hybridization. The $^{32}$P intensities of full size restriction fragments were quantified by performing electronic autoradiography (InstantImager, Packard). The number of UvrABC-sensitive sites/fragment was calculated from the relative band intensities of full size restriction fragments in the lanes containing the treated and mock-treated samples, assuming a Poisson distribution of lesions.

**RESULTS**

**Formation of DNA Adducts**—Because incubation of cells with NA-AAF leads primarily to the induction of dG-C8-AF, we had to develop a system of inducing dG-C8-AF. Vu et al. (20) showed that pretreatment of mice hepatocytes with paraoxon, a deacetylase inhibitor, led to the preferential induction of dG-C8-AAF in NA-AAF-treated cells. However, the concentration of paraoxon appeared to be very critical because doses that are too high abolish the formation of lesions, whereas doses that are too low lead to induction of mainly dG-C8-AF. Consistent with their results we found that because of the paraoxon treatment relatively high doses of NA-AAF had to be applied to get sufficient induction of dG-C8-AF. To determine the frequency and relative induction level of various types of DNA lesions after paraoxon/NA-AAF treatment we employed HPLC analysis combined with ECD of adducted nucleosides. With this approach the principal DNA lesions induced by NA-AAF, i.e. dG-C8-AF and dG-C8-AAF, can be analyzed quantitatively (27). As shown in Table I, the main lesion induced by the combined paraoxon/NA-AAF treatment is the acetylated dG-C8-AF (85%); this in contrast to exposure of these cells to NA-AAF alone, which induces the deacetylated dG-C8-AF exclusively (18, 19). There are no major differences in the relative frequency and the types of paraoxon/NA-AAF-induced adducts among normal, XP-C, and CS-B cells. Employing a dose of $10^{-8}$ M paraoxon alone we did not find any negative or positive effect on transcription inhibition, repair replication, or colony forming ability.

**Clonal Survival Studies**—The cytotoxic effect of the paraoxon/NA-AAF treatment was measured by determining the colony forming ability of the cells after treatment. Fig. 1 shows that the CS-B and the XP-C cells were much more sensitive to paraoxon/NA-AAF treatment than the normal human cells. Paraoxon alone had no significant effect on colony forming ability of any of the three cell lines.

**Effect of Paraoxon/NA-AAF Treatment on RNA Synthesis**—A hallmark of CS cells is the lack of recovery of inhibited RNA synthesis after treatment with DNA-damaging agents such as NA-AAF and UV light. On the contrary XP-C cells with a very reduced repair capacity are able to recover RNA synthesis after NA-AAF or UV treatment. Fig. 2A shows that RNA synthesis during the first 30 min after paraoxon/NA-AAF treatment is inhibited in a dose-dependent manner and that the level of inhibition was similar for VH25D and XP-C cells.

**TABLE I**

| Cell line | dG-C8-AF lesions/10 kb | dG-C8-AAF lesions/10 kb |
|-----------|------------------------|-------------------------|
| VH25D     | 0.05                   | 0.39                    |
| XP21RO    | 0.06                   | 0.42                    |
| CS1AN     | 0.06                   | 0.41                    |

**Induction of dG-C8-AF and dG-C8-AAF after treatment with $10^{-8}$ M paraoxon and 300 $\mu$m NA-AAF**

Lesion frequencies were measured employing HPLC/ECD analysis of DNA obtained from normal (VH25D), Cockayne’s syndrome (CS1AN), and xeroderma pigmentosum (XP21RO) cells.

Next we addressed the question of whether the three cell lines differ in their abilities to recover paraoxon/NA-AAF-inhibited RNA synthesis. Fig. 2B shows that after a dose of 150 $\mu$m NA-AAF the CS-B cells are incapable of recovering RNA synthesis, whereas the normal and the XP-C cells exhibit recovery of RNA synthesis up to 85% of the level in untreated cells 8 h after treatment.

**Repair Replication Studies**—The genome overall capacity of the three cell lines to repair paraoxon/NA-AAF-induced DNA lesions was assessed by measurement of the extent of repair replication. $^{32}$P-Prelabeled cells were treated with paraoxon and 300 $\mu$m NA-AAF and were allowed to repair for 24 h in the presence of $[^3H]$thymidine, BrdUrd, and FrdUrd. An untreated sample was included as a control to determine the specific activity of the DNA ($^{32}$P cpm/$\mu$g of DNA) from each cell strain in order to quantify the level of repair replication, allowing direct comparison of the extent of repair replication in different cell lines. The results (Fig. 3) demonstrate that the capacity of the CS cells to repair paraoxon/NA-AAF-induced DNA lesions is not substantially different from that of normal cells. In contrast, the XP-C cell line shows a reduced level of repair replication, amounting to approximately 25% of that in normal cells.

**Induction and Repair of dG-C8-AAF Adducts in Active and Inactive Genes**—The frequency of paraoxon/NA-AAF-induced DNA adducts in restriction fragments of (in)active genes as a function of the dose was determined employing the UvrABC excinuclease assay as described previously (24). The UvrABC excinuclease introduces single-stranded DNA breaks in the DNA at the site of a lesion. The frequency of these DNA breaks can be quantified at the gene level by alkaline agarose-gel electrophoresis, Southern blotting, and hybridization with (strand-specific) radiolabeled probes. The presence of DNA lesions is seen as the reduction of full size restriction fragments in lanes containing UvrABC-digested DNA compared with undigested DNA.

To determine the formation of paraoxon/NA-AAF-induced DNA lesions in active and inactive genes, lesion frequencies were measured in the 18.5-kb EcoRI fragment of the transcriptionally active ADA gene and the 14-kb EcoRI fragment of the X-chromosomal inactive 754 gene by sequential hybridization of the same membrane. A linear relationship between the dose of NA-AAF and induction of UvrABC-sensitive sites up to 400 $\mu$m was observed with an average induction frequency of 0.0017 DNA lesions/$\mu$m/10 kb for both the ADA and 754 gene (data not shown). The lesion frequency was similar in restriction fragments of active and inactive genes, and no significant differences in induction levels among the three cell lines were observed. To achieve the desirable induction of one adduct/restriction fragment for repair experiments a dose of 300 $\mu$m $\AA$F
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was chosen, inducing about 0.5 adducts/10 kb.

To detect possible differences in the kinetics of removal of dG-C8-AAF lesions between the transcribed and the nontranscribed strand of the ADA gene, we studied strand-specific repair in the 5' -located 19.9-kb BclI fragment covering a region of the ADA gene with only transcription of the ADA template strand (31). Fig. 4 is an autoradiogram representing strand-specific repair in the three cell lines. Fig. 5A shows that VH25D cells exhibit a very rapid repair of approximately 40–60% of the adducts during the first 4 h after treatment followed by repair of additional 10–20% up to 24 h. No differences in kinetics and extent of repair were observed between the transcribed and nontranscribed strands of the ADA gene in normal cells. The extent of repair of the ADA gene in CS1AN cells was virtually the same as in normal cells (Fig. 5B). Repair of the ADA gene in the XP21RO cells proceeded at a slower rate and to a lesser extent than in normal and CS cells; but in contrast to the latter cell lines, repair in XP21RO showed a clear strand specificity, i.e. repair in the transcribed strand was more efficient. After longer postincubation times repair in the transcribed strand of the ADA gene in the XP21RO cell was almost complete, e.g. 85% of the lesions were removed after 48 h. Repair of DNA adducts in the nontranscribed strand proceeded slowly but to substantial level (34%) after 48 h. Basically, the same results were obtained when repair of paraoxon/NA-AAF-induced DNA lesions was measured in the 3 ' -located 18.5-kb EcoRI fragment of the ADA gene (data not shown). When repair was assayed in the transcriptionally inactive 754 gene, a less rapid removal of lesions was observed in normal and CS cells, in particular during the first 8 h after treatment, whereas repair of 754 was virtually absent in XP-C cells.

**DISCUSSION**

NA-AAF is known to induce two types of DNA adducts with different DNA-distorting properties, i.e. dG-C8-AF and dG-C8-AAF. In the case of the dG-C8-AAF adduct, addition of the bulky acetylaminofluorene moiety to guanine is known to cause severe distortion of the DNA helix, manifested most clearly by the local denaturation of several bases at the site of the lesion resulting from stacking of the acetylaminofluorene group inside the helix (6, 7). In contrast, dG-C8-AF affects DNA conformation only marginally because the aminofluorene group resides mainly outside the helix, although no full consensus exists on this point (8, 9). Tang and co-workers (16, 25) have shown that in vitro the E. coli UvrABC excinuclease recognized the dG-C8-AAF adduct 3-fold more efficiently than dG-C8-AF.

In this study, we used NA-AAF and paraoxon to induce dG-C8-AAF in human cells and studied the repair kinetics of this lesion by GGR and TCR. In accordance with Vu et al. (20) the major lesion induced in the presence of paraoxon was dG-C8-AAF with small (15%) amounts of dG-C8-AF. No major differences were detected among normal, XP-C, and CS fibroblasts with respect to the types and frequencies of paraoxon/NA-AAF-induced DNA lesions, demonstrating that the enhanced sensitivity of XP-C and CS to paraoxon/NA-AAF cannot be attributed to higher levels of DNA adduct formation nor to different types of DNA lesions. Based on equal induction frequency, dG-C8-AAF is a much more cytotoxic lesion than dG-C8-AF (this study and Refs. 18 and 19). Interestingly, the sensitivity of XP-C cells treated with paraoxon/NA-AAF compared with normal cells is much more pronounced than after treatment with NA-AAF alone (19). This enhanced sensitivity of XP-C cells to the cytotoxic effects of paraoxon/NA-AAF is most likely caused by persistent dG-C8-AAF lesions in nontranscribed regions, blocking DNA replication. In contrast, dG-C8-AF is a poor inhibitor of replication (32, 33).

**In vitro** studies revealed that dG-C8-AAF lesions have profound transcription-blocking properties (12). This study shows that also in intact cells, dG-C8-AAF induces efficient inhibition.
of transcription with a target size of inhibition of approximately 45 kb, suggesting that less than one dG-C8-AAF lesion per transcription unit inactivates transcription and that transcription inhibition might be mediated by blocking initiation rather than elongation. Based on lesion frequencies, the inhibition of transcription by dG-C8-AAF is about 3-fold more effective than by UV-C-induced photolesions (i.e. CPD and 6–4 PP) and about 15-fold more than by dG-C8-AF. However, Petit-Frere et al. (34) showed that the immediate inhibition of RNA synthesis by UV irradiation correlates with 6–4 PP rather than the overall photolesion formation, indicating that 6–4 PP is the mediator of the inhibitory effect. Our results provide indirect evidence for the correctness of this hypothesis as the 3-fold lower effectiveness of RNA synthesis inhibition by UV-C (overall photolesion formation) mimics the 3-fold higher induction of CPD over 6–4 PP (24). Indeed, a plot of UV-induced RNA synthesis inhibition versus 6–4 PP frequency coincides with the dG-C8-AAF inhibition curve (Fig. 6).

CS cells appeared to be incapable of recovering inhibited RNA synthesis after paraoxon/NA-AAF treatment analogous to their response after UV exposure or NA-AAF treatment only (18, 35), whereas normal and XP-C cells do recover RNA synthesis. To correlate RNA synthesis recovery to efficiency and strand specificity of repair in transcriptionally active genes, we quantified dG-C8-AAF lesion frequencies in the ADA housekeeping gene. The repair of dG-C8-AAF in the ADA gene in normal and CS cells displays two characteristics: (i) repair proceeds in a biphasic manner with approximately 50% of the lesions removed within 2–4 h followed by additional repair at a slower rate, and (ii) there is no significant difference in repair between the transcribed and nontranscribed strands. Part of the slow repair at late times might be because of the presence of dG-C8-AF, which is formed as a minor fraction after the paraoxon/NA-AAF treatment. Moreover, although the majority of the dG-C8-AAF lesions resides in the syn conformation, a substantial fraction might adapt the anti formation dependent on the sequence context (36, 37); the latter is known to be less helix-distorting and therefore presumably repaired at a slower rate. In addition, sequence-dependent differences in the repair efficiency of dG-C8-AAF adducts may contribute to heterogeneity of repair since in vitro studies revealed that incision efficiency of dG-C8-AAF by UvrABC excinuclease varied substantially depending on the local sequence (38).

The absence of a strand specificity of repair in normal cells does not favor a significant contribution of TCR to repair of dG-C8-AAF in active genes. Moreover, because the repair kinetics of dG-C8-AAF in CS cells is the same as observed in normal cells, the enhanced sensitivity of CS cells to dG-C8-AAF adducts and the lack of RNA synthesis restoration in those cells cannot be attributed to defective repair of transcriptionally active genes either. Although this repair phenotype for dG-C8-AAF contrasts the defective TCR in UV-irradiated CS cells, it closely resembles the absence of strand-specific repair of the nonacetylated dG-C8-AF adduct in normal cells and normal repair kinetics of this lesion in CS cells. The enhanced sensitivity and lack of RNA synthesis recovery after induction of dG-C8-AAF fit a previous reported model (based on the repair kinetics of the dG-C8-AAF lesion) proposing that CS proteins act as repair-transcription uncoupling factors in concert with the basal transcription factor TFIH (18). TFIH is essential for initiation of RNA polymerase II-driven transcription as well as NER. We propose that upon treatment of cells with NA-AAF (and paraoxon) TFIH will be recruited for association with additional NER proteins to do repair and that CS proteins are essential for its conversion back to transcription function. In summary, the enhanced sensitivity of CS cells to NA-AAF (with or without paraoxon) and UV is not caused by a defect in TCR but rather the consequence of a defect in transcription initiation in the presence of DNA damage.

The absence of any observable strand specificity for repair of dG-C8-AAF in normal cells does not relate to a poor substrate property of this lesion for TCR; the results obtained with XP-C cells show that dG-C8-AAF is a substrate for TCR. However, repair in XP-C cells proceeds at slower rate than in normal and
CS cells and is virtually absent during the first 4 h after treatment when approximately 50% of the lesions in normal cells are repaired. The delay in repair in XP-C cells has been observed for UV-induced photolyses as well (24) and is likely to be caused by delayed transcription initiation. The results described in this and previous studies (18, 19, 23, 24) are consistent with a model in which DNA lesion conformation dictates the inhibition of transcription initiation and the efficiency of GGR. In contrast, repair of DNA lesions by TCR appears to be independent of the structure of the lesions, i.e. UV-induced CPD and 6–4 PP are repaired with same kinetics by TCR (24). However, gross differences exist between the repair rates of the various bulky adducts by GGR. Both 6–4PP and dG-C8-AAF are repaired extremely rapid (approximately 50% repair in 2 h at equal induction frequency) and much more rapid than CPD and dG-C8-AF lesions. It is tempting to speculate that the degree of damage-induced DNA helix distortion is a main determinant in the recognition of DNA damage by DNA repair proteins, i.e. XPA, XPC, and XPE. If DNA damage recognition is the critical signal for formation of incision complexes, the rate of TFIIH depletion from transcription complexes will depend largely on the efficiency by which different types of DNA damage are recognized by the relevant XP factors. In such a model the depletion of TFIIH from promoter sites will be the predominant process that causes transcription to be inhibited. We hypothesize that the conversion of TFIIH from repair to transcription is an active process for which CS proteins are required. However, efficient GGR as such cannot be the signal for this process because XP-C cells show recovery of transcription without GGR.

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