The human CSB (ERCC6) gene corrects the transcription-coupled repair defect in the CHO cell mutant UV61

David K. Orren, Grigory L. Dianov and Vilhelm A. Bohr*

Laboratory of Molecular Genetics, National Institute on Aging, National Institutes of Health, 4940 Eastern Avenue, Baltimore, MD 21224, USA

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ABSTRACT

The human CSB gene, mutated in Cockayne’s syndrome group B (partially defective in both repair and transcription) was previously cloned by virtue of its ability to correct the moderate UV sensitivity of the CHO mutant UV61. To determine whether the defect in UV61 is the hamster equivalent of Cockayne’s syndrome, the RNA polymerase II transcription and DNA repair characteristics of a repair-proficient CHO cell line (AA8), UV61 and a CSB transfectant of UV61 were compared. In each cell line, formation and removal of UV-induced cyclobutane pyrimidine dimers (CPDs) were measured in the individual strands of the actively transcribed DHFR gene and in a transcriptionally inactive region downstream of DHFR. AA8 cells efficiently remove CPDs from the transcribed strand, but not from either the non-transcribed strand or the inactive region. There was no detectable repair of CPDs in any region of the genome in UV61. Transfection of the human CSB gene into UV61 restores the normal repair pattern (CPD removal in only the transcribed strand), demonstrating that the DNA repair defect in UV61 is homologous to that in Cockayne’s syndrome (complementation group B) cells. However, we observe no significant deficiency in RNA polymerase II-mediated transcription in UV61, suggesting that the CSB protein has independent roles in DNA repair and RNA transcription pathways.

INTRODUCTION

Prokaryotic and eukaryotic cells have a variety of DNA repair mechanisms to maintain the integrity of the genetic material. Among these, nucleotide excision repair (NER) is the least specific, having the capability to remove a vast array of more or less bulky DNA adducts, including those adducts formed by ultraviolet (UV) light. Extensive research (reviewed in 1,2) into the pattern of NER within the genome has revealed that certain regions are repaired better (or at least faster) than other regions. In general, regions containing transcriptionally active genes are repaired preferentially when compared with inactive regions of the genome. Moreover, within actively transcribing genes, the transcribed strand is repaired more efficiently than the non-transcribed strand (3,4). This is widely believed to occur through a direct link of the repair machinery to the transcriptional apparatus due to the recent isolation and characterization of a transcription-repair coupling factor (TRCF) in Escherichia coli (5). The identification of certain NER genes as subunits of the basal transcription factor TFIHF (reviewed in 6) also suggests intimate connections between transcription and repair. These selective mechanisms, termed preferential and transcription-coupled repair (TCR), supposedly serve to allow early resumption of transcription and thus increase the survival of cells containing DNA damage.

The importance of preferential repair and TCR is illustrated by the human DNA repair-deficient genetic disease Cockayne’s syndrome (CS). Patients with this disease lack TCR; cells isolated from these patients repair the transcribed strand of an active gene at the same rate as non-transcribed regions of the genome in normal cells (7,8). CS patients are also thought to have variable defects in RNA polymerase II (pol II) transcription (9). Among the consequences of these defects are UV hypersensitivity, cachetic dwarfism, neurological degeneration and premature aging. The manifestations of CS are now believed to be caused by specific alterations in at least one of a number of gene products involved in DNA repair, all of which appear to either be a subunit of or interact with TFIHF.

The CSB (earlier known as ERCC6) gene, mutations in which have been shown in a CSB patient (10), was cloned by virtue of its ability to correct the UV sensitivity of UV61 (11), a Chinese hamster ovary (CHO) cell line from complementation group six of a series of UV-sensitive mutants isolated by Busch and co-workers (12). UV61 cells are sensitive to UV light, but not as sensitive as other cell lines which have been shown to be completely defective in NER (13). UV light introduces almost exclusively two types of damage to DNA, cyclobutane pyrimidine dimers (CPDs) (65–80%) and (6–4) pyrimidine-pyrimidone photoproducts (20–35%). At UV doses of 10 J/m² and above, UV61 cells can remove (6–4) photoproducts from total genomic DNA as efficiently as wild-type cells, but have a complete deficiency in the removal of CPDs from total genomic DNA (13). When CPD

* To whom correspondence should be addressed
removal was examined in the actively transcribed dihydrofolate reductase (DHFR) gene in UV61 cells, a very low level of repair (when compared with wild-type cells) of CPDs was observed in the transcribed strand, while no repair was observed in the non-transcribed strand (similar to wild-type cells) (14). Thus, the UV sensitivity of UV61 cells can be attributed to a shortfall in repair of CPDs in the transcribed strand of active genes.

Transfection of the human CSB gene into either UV61 or CSB cells both complements their UV sensitivity and restores nearly normal recovery of RNA synthesis following UV irradiation (10), suggesting that UV61 cells might have a genetically homologous defect to that of CSB cells. However, whether transfection of CSB actually restores the specific TCR defect in CSB or UV61 cells has not been demonstrated. To clarify the role of the CSB gene in repair processes, we have measured the induction and removal of CPDs in both strands of actively transcribed genes and in an inactive region from AA8 (wild-type repair), UV61 and UV61 cells transfected with the CSB gene. We have also measured in vitro pol II transcription by extracts and partially purified cell fractions of these three cell lines. Our results indicate that UV61 completely lacks TCR and that transfection of the CSB gene restores the wild-type pattern of repair. However, in contrast to CSB cells (Dianov et al., unpublished observations), transcription is not defective in UV61 (when compared with AA8 or the CSB transfectant of UV61), indicating that any role of the CSB protein in pol II transcription can be uncoupled from its role in TCR.

MATERIALS AND METHODS

Cell culture and the clonogenic survival assay

UV61 cells were obtained from D. Busch and L. Thompson. PT5, a CSB transformant of UV61 (11), was a kind gift from C. Troelstra and J. Hoeijmakers. AA8 cells, the wild-type parental strain of UV61, were obtained from the ATCC. All cells were grown in monolayer in DMEM/Ham’s F-10 (1:1) supplemented with fetal bovine serum (10%), penicillin (100 U/ml) and streptomycin (100 µg/ml).

For the clonogenic survival assay (15), AA8, UV61 and PT5 cells were seeded in complete medium at densities ranging from 10^5 to 10^6 cells/dish. After attachment of cells, the dishes were washed once with phosphate-buffered saline (PBS). After PBS was removed, the cells were irradiated with UV light (254 nm) at doses of from 0 to 14 J/m^2, then incubated in complete medium until the appearance of colonies (6–7 days). The colonies were then rinsed with PBS, fixed with methanol and stained with methylene blue (0.02%). Colonies containing greater than ∼50 cells were counted. After correction for plating efficiency, survival was determined by comparing the number of colonies in irradiated dishes to the number of colonies in unirradiated controls.

Techniques for treatment of cells in culture with DNA damaging agents and for subsequent isolation of genomic DNA were essentially as described (16). Briefly, cells were pre-incubated with [3H]thymidine (50–90 Ci/mmol) to label DNA. During exponential growth, cells were either untreated or irradiated with UV light (20 J/m^2 at 254 nm), then harvested immediately (0 h) or given fresh medium containing bromodeoxyuridine and fluorodeoxyuridine and harvested after 8 or 24 h. Lysis of cells for isolation of genomic DNA was achieved using a proteinase K, SDS, Tris, pH 8.0, solution.

DNA purification and enzyme treatments

Total genomic DNA was isolated by a NaCl extraction procedure (17). Treatment of DNA with RNase (100 µg/ml) and restriction enzyme (KpnI) was as described (16). KpnI digestion (5 U/µg DNA) yields a unique 14 kilobase (kb) fragment containing the 5′-end of the DHFR gene. Unreplicated DNA was isolated from replicated DNA (containing BrdU) after fractionation of CsCl density gradients, dialyzed against TE buffer (10 mM Tris, pH 8.0, 1 mM EDTA), ethanol precipitated and finally resuspended in TE. All DNA concentrations were determined by measuring UV absorbance at 260 nm using a Pharmacia/LKB Ultraspec III spectrophotometer.

The location of CPDs present in DNA samples isolated from UV-irradiated cells was detected by treatment of the DNA with a CPD-specific enzyme, T4 endonuclease V. Briefly, unreplicated DNA samples (5 µg) from various time points were incubated with T4 endonuclease V in buffer containing 10 mM Tris, pH 8.0, 100 mM NaCl and 10 mM EDTA for 15 min at 37°C and the reactions stopped by the addition of formamide loading dye. Parallel reactions in the same buffer minus T4 endonuclease V were performed to determine the original distribution of restriction fragments.

Southern analysis

As previously described (16), T4 endonuclease V-treated (or mock-treated) DNA samples were electrophoresed under alkaline conditions and the DNA was quantitatively transferred by posiblot (Stratagene) to nylon membranes (Oncor). Membranes were then conditioned with 2× SSPE (0.36 M NaCl, 20 mM NaH2PO4, 2.2 mM EDTA, buffered to pH 7.4 with NaOH), vacuum dried and treated with Hybrisol I (Oncor) at least 4 h prior to hybridization.

Double-stranded DNA and single-stranded RNA probes for hybridization were synthesized using a random primed DNA labeling kit (Boehringer Mannheim) with radiolabeled [α-32P]dCTP (3000 Ci/mm mol) and the T7/SP6 RNA Polymerase Transcription kit (Boehringer Mannheim) with [α-32P]CTP (3000 Ci/mm mol) respectively. A subcloned fragment was used to make the probe for the non-transcribed region 3′ of the DHFR gene (cs14DO), as previously described (18). The fragment used to synthesize the double-stranded DNA probe for exons 2 and 3 of the mouse c-myc gene was purchased from Lofstrand Labs. The transcribed and non-transcribed strand probes of the DHFR gene were synthesized as described (4). Membranes were hybridized with probe, washed to remove non-hybridized probe and then subjected to autoradiography or radioactive detection using a phosphorimager (Molecular Dynamics). By comparison of untreated and T4 endonuclease V-treated samples, the average number of CPDs per fragment was determined from the zero class of the Poisson distribution. Percent repair was calculated by dividing the amount of CPDs removed by a given time point by the initial CPD frequency.

In vitro transcription assays

In vitro transcription-competent whole cell extracts were prepared as described (19) from AA8, UV61 and PT5 cells. To enhance pol II transcription from cellular components (20), the extracts were also fractionated by phosphocellulose column chromatography, then specific fractions [PC-FI (flow-through at...
0.1 M KCl) and PC-FII (1.0 M KCl eluate) were recombined. Whole cell extracts (50 µg protein) or recombined fractions in various ratios (totaling 40 µg protein) were incubated for 1 h at 30°C with 2 µg supercoiled plasmid pML(C2AT) containing the adenovirus major late promoter (21), which directs pol II-dependent transcription of a G-less cassette (22). The reactions (50 µl) were carried out in transcription buffer which contained ATP (500 µM), CTP (500 µM), UTP (5 µM), [α-32P]UTP (20 µCi), RNase inhibitor (20 U), phosphocreatine (8 mM) and creatine kinase (500 µg). After the addition of carrier tRNA (20 µg) and RNA samples were ethanol precipitated, resuspended and heated (95°C for 2 min) in formamide dye (10 µl) and electrophoresed on a 5% polyacrylamide–7 M urea gel. Gels were washed (in H2O for 20 min) and vacuum dried prior to autoradiography or phosphorimaging analysis.

RESULTS

UV survival of AA8, UV61 and PT5

Measurement of the survival of cells following DNA damaging treatments roughly reflects their capacity to repair the inflicted damage. Cells from CHO complementation group six (which includes UV61) have intermediate levels of UV resistance when compared with NER-proficient and completely NER-deficient cell lines. Using clonogenic survival assays, the effect of UV irradiation on the survival of the AA8, UV61 and PT5 cell lines was measured (Fig. 1). As expected, AA8 has a range of UV sensitivity typical of most normal cells, while UV61 is significantly more sensitive. PT5 cells appear only slightly more UV sensitive than AA8, indicating that transfection of DNA containing the CSB gene restores a near wild-type level of UV survival to UV61.

Cyclobutane pyrimidine dimer repair in the DHFR gene

CHO cells, in general, show a low level of repair of CPDs when repair is measured over the entire genome. However, within actively transcribing strands of certain genes, the level of repair of CPDs 24 h after UV irradiation has been shown to be as high as 89% for CHO cells with normal NER capabilities (3). UV61 cells have been shown to repair (6–4) photoproducts efficiently, but have little or no repair of CPDs when measured at the level of the overall genome (13). We sought to determine whether UV61 had detectable repair of CPDs at the level of the gene and whether transfection of the CSB gene into UV61 changed the repair capability of the cells. Several previous studies have measured repair in the house-keeping DHFR gene in other wild-type and NER-deficient cells, so we felt that DHFR would be the best model gene to study. Moreover, the preparation of both transcribed and non-transcribed strand-specific probes allows the measurement of repair in each individual strand of the DHFR gene. Therefore, we measured the induction and removal of CPDs in the individual strands of the DHFR gene and in a non-transcribed region (cs14DO) downstream (3′) of DHFR in the AA8 (parental wild-type), UV61 and PT5 (CSB transfectant of UV61) cell lines. Quantitative Southern blots measuring the amount of CPDs in the individual strands of the DHFR gene over time after irradiation are shown in Figure 2. For each cell line, the number of CPDs in both strands and in the cs14DO region over time after irradiation is presented in Table 1 and the level of repair of CPDs in the transcribed strand of the DHFR gene is depicted in Figure 3A. As in other CHO repair-proficient cell lines, the repair of CPDs in AA8 appears limited to only the transcribed strand of DHFR (Table 1). Specifically, the repair of CPDs in the transcribed strand of the DHFR gene is ~70% complete by 24 h (Fig. 3A); there is no detectable repair in the non-transcribed strand after 24 h. In contrast, there is no measurable repair of either the transcribed or non-transcribed strand in the UV-sensitive UV61 cell line (Table 1 and Fig. 3A). When repair was measured in PT5, the CSB transfectant of UV61, again no repair of the non-transcribed strand of DHFR was observed. However, the transfectant had regained the ability to repair the transcribed strand of the DHFR gene. Within experimental error, both the rate and extent of repair of the transcribed strand in PT5 are similar to that of AA8 (Fig. 3A and Table 1). No repair of CPDs in the inactive region downstream of the DHFR gene was detected in any of the three cell lines (Table 1). Thus, transfection of the human repair gene (CSB) into a repair-deficient CHO cell restores the wild-type pattern of repair of CPDs.

Cyclobutane pyrimidine dimer repair in the c-myc gene

Our results showing essentially no repair of the transcribed strand of the DHFR gene contradict earlier findings by Lommel and Hanawalt (14), who reported a low level of repair (33% after 24 h) for the same DNA sequence. Since NER of CPDs in CHO cells appears to be intimately linked to transcription, the study of repair in a more highly transcribed gene might clarify the extent of the CPD repair defect in UV61. As our candidate gene we chose c-myc, which, in CHO cells, is transcribed at 5–10 times the level of the DHFR gene (23). The identical Southern blots used

Figure 1. UV survival of AA8, UV61 and PT5. The clonogenic survival assay was carried out on AA8 (□), UV61 (●) and PT5 (▲) (CSB transfectant of UV61) cells as described in Materials and Methods at UV fluences from 0 to 14 J/m2. Each dose was repeated in triplicate and survival at each dose was calculated by dividing the average number of colonies (S) by the average number of colonies of the unirradiated control (S0).
Table 1. Formation and removal of CPDs in the individual strands of the hamster DHFR gene, in cs14DO (a non-transcribed region downstream of DHFR) and in the c-myc gene

<table>
<thead>
<tr>
<th>Cell line</th>
<th>DNA fragment</th>
<th>Time post-UV irradiation (h)</th>
<th>0</th>
<th>8</th>
<th>24</th>
</tr>
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<tr>
<td>AA8</td>
<td>Transcribed</td>
<td>1.50 ± 0.05</td>
<td>0.84 ± 0.25</td>
<td>0.46 ± 0.21</td>
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<tr>
<td></td>
<td>Non-transcribed</td>
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<td>1.62 ± 0.18</td>
<td>1.58 ± 0.11</td>
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<tr>
<td>cs14DO</td>
<td>1.34 ± 0.09</td>
<td>1.38 ± 0.05</td>
<td>1.47 ± 0.22</td>
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<td></td>
</tr>
<tr>
<td>c-myc</td>
<td>0.66 ± 0.07</td>
<td>0.55 ± 0.05</td>
<td>0.42 ± 0.07</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UV61</td>
<td>Transcribed</td>
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<td>1.69 ± 0.15</td>
<td>2.06 ± 0.46</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Non-transcribed</td>
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<td>1.54 ± 0.03</td>
<td>2.04 ± 0.37</td>
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</tr>
<tr>
<td>cs14DO</td>
<td>1.63 ± 0.17</td>
<td>1.63 ± 0.20</td>
<td>2.48 ± 0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>c-myc</td>
<td>0.67 ± 0.05</td>
<td>0.74 ± 0.11</td>
<td>0.91 ± 0.19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PT5</td>
<td>Transcribed</td>
<td>1.80 ± 0.16</td>
<td>0.79 ± 0.21</td>
<td>0.36 ± 0.08</td>
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<tr>
<td></td>
<td>Non-transcribed</td>
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<td>1.84 ± 0.19</td>
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<tr>
<td>cs14DO</td>
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<td>1.40 ± 0.10</td>
<td>1.43 ± 0.19</td>
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<td></td>
</tr>
<tr>
<td>c-myc</td>
<td>0.71 ± 0.10</td>
<td>0.55 ± 0.03</td>
<td>0.36 ± 0.14</td>
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<td></td>
</tr>
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</table>

*Expressed as the number of CPDs measured in the 14 kb transcribed or non-transcribed strand fragments of the DHFR gene, in cs14DO, a non-transcribed 14 kb double-stranded fragment 3′ to the DHFR gene or in an ∼9 kb double-stranded fragment of the hamster c-myc gene. Except for cs14DO, each value represents the mean of two biological experiments, with two to three individual electrophoretic analyses for each biological experiment. Values for the cs14DO fragment were derived from multiple analyses within a single biological experiment.

Figure 2. Formation and removal of CPDs in the transcribed and non-transcribed strands of the DHFR gene in AA8, UV61 and PT5. KpnI-restricted genomic DNA (5 μg) from either unirradiated (–UV) or UV irradiated (0, 8 and 24 h time points) AA8, UV61 and PT5 cells were treated (+) with T4 endonuclease V or untreated (–), then electrophoresed though an alkaline agarose (0.5%) gel. The DNA was quantitatively transferred to a nylon membrane which was subsequently prehybridized, then hybridized with 32P-labeled riboprobes for either the transcribed or non-transcribed strand of the hamster DHFR gene. After stringency washing, individual autoradiograms were made for each probe and each cell line.

above were reprobed with a double-stranded mouse c-myc probe that hybridizes to a unique fragment of ∼9 kb in KpnI-restricted CHO genomic DNA. Results from these experiments (Table 1 and Fig. 3B) show reasonably efficient levels of CPD repair in the c-myc gene in the AA8 (wild-type) and PT5 (CSB transfectant of UV61) cell lines. The average level of damage present in both strands is detected by this probe, so the lower level of repair in AA8 and PT5 (when compared with the DHFR results) is a reflection of strong repair of the transcribed strand averaged with zero repair of the non-transcribed strand. However, no detectable repair in the c-myc gene was observed in UV61, which indicates that there was no substantial repair even in the transcribed strand of the c-myc gene. Thus, our data demonstrate that there is little or no repair of CPDs in UV61 cells.

Figure 3. CPD repair in the transcribed strand of the DHFR gene and in both strands of the c-myc gene in AA8, UV61 and PT5. At both the time of irradiation (0 h) and after various (8 and 24 h) repair intervals (see Table 1), the number of UV-induced CPDs was determined by quantitative Southern analysis (as visualized in Fig. 2) in (A) the transcribed strand of the DHFR gene or (B) the c-myc gene (exons 2 and 3) for the AA8 ( ), UV61 ( ) and PT5 ( ) cell lines. At each time point, the percent repair was calculated as the number of CPDs removed at that time point compared with the level induced by UV treatment (0 h time point) in that particular cell line.

Polymerase II transcription by extracts and partially purified fractions

CSA and CSB cells are thought to have partial defects in pol II transcription, which might account for many of the CS clinical symptoms that appear to be unrelated to the established biochemical defect in TCR (9). Thus, UV61 cells (which were complemented by transfection of the CSB gene) might also be partially defective in pol II transcription. To investigate this possibility, we used an in

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defective in NER (13). Since UV61 has normal repair of (6-4) photoproducts (13), the partial UV sensitivity of UV61 is due to the complete absence of CPD repair. Thus, both (6-4) photoproducts and CPDs contribute to UV sensitivity in CHO cells and the effect of CPDs on cell survival correlates with their persistence in the transcribed strand of active genes.

Recently, mutations in the human CSB gene were documented in cells from complementation group B of CS (10). Cells (complementation groups A and B) repair all DNA sequences at the slow rate observed for inactive regions (7,8). Specifically, TCR is absent in CS cells (26-28), which may explain their moderate UV sensitivity and slow recovery of RNA synthesis after UV irradiation (29). Transfection of a normal CSB gene into CS cells (complementation group B) complements the UV sensitivity and improves RNA synthesis recovery (10), suggesting that TCR is restored. The CSB (originally ERCC6) gene was first cloned by virtue of its ability to correct the intermediate UV sensitivity of UV61 (11). In this report, we have measured CPD repair in a CSB transfectant of UV61 and found that the wild-type repair pattern, i.e. efficient repair of CPDs only in the transcribed strand of active genes, was restored. This is the first direct demonstration that transfection of CSB corrects the specific TCR defect in either hamster or human cells and conclusive evidence that a hamster CSB homolog is defective in UV61. The complete restoration of TCR by transfection of CSB into UV61 correlates well with levels of complementation of UV sensitivity observed previously (10,11). Surprisingly, the human CSB protein appears to be completely interchangeable with the hamster protein with regard to its function in the TCR pathway. Moreover, the CSB gene product may be the functional analog of E.coli TRCF (the protein product of the mfd gene), which has been shown to directly link NER to active transcription (5). Recently, the CSB gene product has been shown to bind to the CSA protein, which, in turn, interacts with a subunit (p44) of TFIIH (30). Thus, the direct link of repair to the transcribed strand of active genes (absent in CS cells) might occur via the binding of a CSA–CSB protein complex to TFIIH.

The potential binding of a CSA–CSB complex to TFIIH (which participates in both transcription and repair) suggests that defects in human or hamster CSA and/or CSB homologs might affect transcription as well as repair. Indeed, many of the clinical manifestations of CS have been attributed to defects in transcription rather than repair (9). In agreement with this hypothesis, extracts from certain CS (groups A and B) cells display reduced levels of pol II-directed transcription (Dianov et al., unpublished observations). However, when we compared pol II-dependent transcription, using the same assay, in the putative CSB hamster mutant (UV61) with its parental cell line (AA8) and the human CSB transfectant of UV61 (PT5), no significant differences were found. Although the CSB mutation in UV61 causes a complete defect in TCR, it causes little or no change in transcription. Thus, the established role of the CSB gene in TCR and any putative role in pol II-dependent transcription appear to be independent of one another, at least in CHO cells. Intriguingly, the CSB homolog from Saccharomyces cerevisiae, RAD26, is necessary for TCR, but has no essential role in transcription (31). By analogy, the normal mammalian CSB gene may have no direct function in transcription, but certain (dominant negative) mutations could cause perturbations in transcription, perhaps through sequestration of TFIIH in the repair pathway, accounting for the clinical CS symptoms. One prediction from this hypothesis is that many CSB mutations (as in UV61) cause only defects in TCR (resulting in

**Figure 4.** *In vitro* RNA pol II-dependent transcription by complete and partially fractionated extracts from AA8, UV61 and PT5 cells. Whole cell extracts (A) or recombined, fractionated extracts (B) from PT5, UV61 and AA8 were incubated with an adenovirus major late promoter plasmid construct containing a G-less cassette in transcription buffer containing [α-32P]UTP. With the exception of AA8 extracts (150 µg), protein concentrations were 50 µg total for the extracts and 40 µg total (17.8 µg PC-FI and 22.2 µg PC-FII) for the partially purified fractions. The products were electrophoresed on a polyacrylamide/urea gel and radioactivity incorporated into the pol II-specific transcripts was visualized by phosphorimaging analysis. The RNA transcripts specified by this G-less cassette are ~400 nt.

**DISCUSSION**

Certain types of DNA damage subject to removal by NER are repaired with the highest priority when present in the transcribed strand of an active gene (TCR). For instance, in human cells, UV-induced CPDs are removed much more rapidly from the transcribed strand of active genes than from the non-transcribed strand of the same genes or from an inactive region of the genome (3,24). In an extreme example of this type of repair bias, wild-type CHO cells have efficient repair of UV-induced CPDs in the transcribed strand of active genes but little or no repair of these adducts in non-transcribed parts of the genome (3,4,25, this work). In contrast, repair of UV-induced (6-4) photoproducts is rapid over the entire genome in NER-proficient CHO cells (13). The UV sensitivity of CHO mutant UV61 is intermediate between wild-type CHO cells and CHO mutants completely defective in NER (13). Since UV61 has normal repair of (6-4) photoproducts (13), the partial UV sensitivity of UV61 is due to defective repair of CPDs. An earlier study (14) had shown a very low level of repair in the transcribed strand of the DHFR gene in UV61 cells. In contrast, the studies presented here demonstrate that UV61 cells do not detectably remove CPDs from either the transcribed strand of the DHFR gene or both strands of the highly active c-myc gene. Our results indicate that the partial UV sensitivity of UV61 cells is due to the complete absence of CPD repair. Thus, both (6-4) photoproducts and CPDs contribute to UV sensitivity in CHO cells and the effect of CPDs on cell survival correlates with their persistence in the transcribed strand of active genes.

In contrast, repair of UV-induced (6-4) photoproducts is defective in NER (13). Since UV61 has normal repair of (6-4) photoproducts (13), the partial UV sensitivity of UV61 is due to defective repair of CPDs. An earlier study (14) had shown a very low level of repair in the transcribed strand of the DHFR gene in UV61 cells. In contrast, the studies presented here demonstrate that UV61 cells do not detectably remove CPDs from either the transcribed strand of the DHFR gene or both strands of the highly active c-myc gene. Our results indicate that the partial UV sensitivity of UV61 cells is due to the complete absence of CPD repair. Thus, both (6-4) photoproducts and CPDs contribute to UV sensitivity in CHO cells and the effect of CPDs on cell survival correlates with their persistence in the transcribed strand of active genes.

Recent studies (23-25) have shown that the A-patch residue in TCR recruits the CSA protein to transcription. In a recent report, CSA recruitment to RNA polymerase II (pol II) is linked to TCR in vitro (26). We have recently shown that CSA recruitment to pol II occurs in vivo and that CSA is required for TCR (27,28). This observation, together with the results presented here, suggests that CSA recruitment to pol II may be independent of transcription and may be linked to TCR in vivo.

**Figure 4.** *In vitro* RNA pol II-dependent transcription by complete and partially fractionated extracts from AA8, UV61 and PT5 cells. Whole cell extracts (A) or recombined, fractionated extracts (B) from PT5, UV61 and AA8 were incubated with an adenovirus major late promoter plasmid construct containing a G-less cassette in transcription buffer containing [α-32P]UTP. With the exception of AA8 extracts (150 µg), protein concentrations were 50 µg total for the extracts and 40 µg total (17.8 µg PC-FI and 22.2 µg PC-FII) for the partially purified fractions. The products were electrophoresed on a polyacrylamide/urea gel and radioactivity incorporated into the pol II-specific transcripts was visualized by phosphorimaging analysis. The RNA transcripts specified by this G-less cassette are ~400 nt.

**DISCUSSION**

Certain types of DNA damage subject to removal by NER are repaired with the highest priority when present in the transcribed strand of an active gene (TCR). For instance, in human cells, UV-induced CPDs are removed much more rapidly from the transcribed strand of active genes than from the non-transcribed strand of the same genes or from an inactive region of the genome (3,24). In an extreme example of this type of repair bias, wild-type CHO cells have efficient repair of UV-induced CPDs in the transcribed strand of active genes but little or no repair of these adducts in non-transcribed parts of the genome (3,4,25, this work). In contrast, repair of UV-induced (6-4) photoproducts is rapid over the entire genome in NER-proficient CHO cells (13). The UV sensitivity of CHO mutant UV61 is intermediate between wild-type CHO cells and CHO mutants completely defective in NER (13). Since UV61 has normal repair of (6-4) photoproducts (13), the partial UV sensitivity of UV61 is due to defective repair of CPDs. An earlier study (14) had shown a very low level of repair in the transcribed strand of the DHFR gene in UV61 cells. In contrast, the studies presented here demonstrate that UV61 cells do not detectably remove CPDs from either the transcribed strand of the DHFR gene or both strands of the highly active c-myc gene. Our results indicate that the partial UV sensitivity of UV61 cells is due to the complete absence of CPD repair. Thus, both (6-4) photoproducts and CPDs contribute to UV sensitivity in CHO cells and the effect of CPDs on cell survival correlates with their persistence in the transcribed strand of active genes.

Recent studies (23-25) have shown that the A-patch residue in TCR recruits the CSA protein to transcription. In a recent report, CSA recruitment to RNA polymerase II (pol II) is linked to TCR in vitro (26). We have recently shown that CSA recruitment to pol II occurs in vivo and that CSA is required for TCR (27,28). This observation, together with the results presented here, suggests that CSA recruitment to pol II may be independent of transcription and may be linked to TCR in vivo.
probably a mild UV-sensitive phenotype) and would not be
detectable by screening for certain CS symptoms. Another
possibility is that the CSB gene product (in mammalian cells) has
an auxiliary role in transcription, and mutations in the CSB gene
can independently affect either its repair or transcription function.

Similarly, specific mutations in the XPD gene product (a subunit
of the basal transcription factor TFIH) can affect NER and
transcription differentially (32–34).

Mammalian NER is obviously very complex, with apparent
changes in mechanism and kinetics of damage removal depending
on the type of DNA adduct. Cells completely defective in NER
(CHO complementation group 2 and xeroderma pigmentosum
group A, for example) cannot repair either CPDs or (6-4)
photoproducts (35,36), indicating that both major UV adducts
are subject to NER. However, the kinetics and compartmentalization
of repair of these adducts in normal cells are very different. In wild-type
CHO cells, (6-4) photoproducts are repaired rapidly and throughout
the entire genome, while CPDs are repaired more slowly and only
in the transcribed strand of active genes. The CSB mutation in UV61 cells
eliminates the transcribed strand-specific repair of CPDs (this
work), but does not affect repair of (6-4) photoproducts (13). This
suggests that all repair of CPDs in CHO cells occurs through
coupling to transcription, explaining the lack of repair of CPDs
outside the transcribed strand of actively transcribed genes. In
contrast, repair of (6-4) photoproducts can occur (via the overall
genome repair pathway) without being coupled to transcription,
even though a recent study (37) indicates that, when overall genome
repair is deficient (in XPC cells), (6-4) photoproducts are repaired
in a strand-specific manner. Perhaps the CSA and/or CSB gene
products enhance recognition of certain bulky adducts (such as
CPDs) by linking transcription and NER processes. An analogous
situation exists in E.coli, where the low specificity of (A)BC
excinuclease for CPDs is augmented by photolyase or TRCF (5,38).

Alternatively, mutation of the CSB homolog in UV61 may affect
damage recognition subtly but directly, influencing repair of some
adducts (such as CPDs) while not affecting repair of others [6-4
photoproducts]. This explanation seems unlikely, however, based
on the close connections to transcription and the partial repair of CPDs
in the overall genome by human cells with a defective
XPC gene (7,26). Also, the CSB gene product is not required for repair of either
UV photoproducts or cholesterol adducts by human cell extracts in
in vitro repair assays (39,40). Hopefully, future characterization of the
purified CSB protein (and its interactions) will clarify its roles in
NER and transcription.

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