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Cells from XP-D and XP-D-CS patients exhibit equally inefficient repair of UV-induced damage in transcribed genes but different capacity to recover UV-inhibited transcription

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ABSTRACT

Xeroderma pigmentosum (XP) is a rare hereditary human disorder clinically associated with severe sun sensitivity and predisposition to skin cancer. Some XP patients also show clinical characteristics of Cockayne syndrome (CS), a disorder associated with defective preferential repair of DNA lesions in transcriptionally active genes. Cells from the two XP-patients who belong to complementation group D and exhibit additional clinical symptoms of CS are strikingly more sensitive to the cytotoxic effects of UV-light than cells from classical XP-D patients. To explain the severe UV-sensitivity it was suggested that XP-D-CS cells have a defect in preferential repair of UV-induced 6–4 photoproducts (6–4PP) in active genes. We investigated the capacity of XP-D and XP-D-CS cells to repair UV-induced DNA lesions in the active adenosine deaminase gene (ADA) and in the inactive 754 gene by determining (i) the removal of specific lesions, i.e. cyclobutane pyrimidine dimers (CPD) and 6–4PP, or (ii) the formation of BrdUrd-labeled repair patches. No differences in repair capacity were observed between XP-D and XP-D-CS cells. In both cell types repair of CPD was completely absent whereas 6–4PP were inefficiently removed from the ADA gene and the 754 gene with similar kinetics. However, whereas XP-D cells were able to restore UV-inhibited RNA synthesis after a UV-dose of 2 J/m^2, RNA synthesis in XP-D-CS cells remained repressed up to 24 h after irradiation. Our results are inconsistent with the hypothesis that differences in the capacity to perform preferential repair of UV-induced photolesions in active genes between XP-D and XP-D-CS cells are the cause of the extreme UV-sensitivity of XP-D-CS cells. Rather, the enhanced sensitivity of XP-D-CS cells may be associated with a defect in transcription regulation superimposed on the repair defect.

INTRODUCTION

Nucleotide excision repair (NER) is a versatile DNA repair pathway taking care of the removal of a variety of structurally unrelated DNA lesions. There is clear evidence that NER can operate via two subpathways. In one of these, transcription-coupled repair (TCR), NER is directly coupled to RNA polymerase II driven transcription, which results in more rapid repair of lesions in the transcribed strand of active genes when compared to the non-transcribed strand or inactive DNA (1–4). The second repair pathway is global genome repair (GGR), which acts with variable efficiency on damage across the genome.

Three rare hereditary human disorders have been shown to be associated with defective NER: xeroderma pigmentosum (XP), Cockayne syndrome (CS) and the photosensitive form of trichothiodystrophy. The clinical and cellular features of CS pose challenging questions about the molecular mechanism underlying repair defect and disease. Clinical symptoms of CS include growth retardation, neurological abnormalities and cutaneous photosensitivity (5). In spite of normal levels of repair, CS cell strains are sensitive to the lethal effects of UV and unable to recover UV-inhibited DNA and RNA synthesis (6). The lack of RNA synthesis recovery has been related to a specific defect in TCR (3,7–9), i.e. the absence of accelerated repair of DNA lesions in the transcribed strand of active genes in CS cells. The finding that CS cells were unable to perform TCR suggested that a transcription-repair coupling factor, equivalent to the factor identified in *Escherichia coli* (10),

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might be defective in these cells. Recently, this hypothesis has been challenged by results of studies with N-acetoxy-2-acetylaminofluorene: CS cells are more sensitive to NA-AAF than normal cells although these cells exhibit a normal level of repair of transcribed strands. As an alternative model, we proposed that the deficient recovery of DNA damage-inhibited RNA synthesis *per se* might be the crucial defect in CS causing the inability to perform TCR (11). Defects in transcription have also been observed in CS cells unrelated to exogenous DNA damage, i.e. CS cells show a reduced level of RNA polymerase II transcription (12) possibly related to the presence of oxidative DNA damage. Oxidative DNA damage is known to be a substrate for TCR and this requires the CS and XP-G gene products (13). In addition, CS proteins might be required for enhancement of transcription elongation at sites of base damage or structural perturbations (14).

In addition to the classical forms of XP and CS, some patients show characteristics of both diseases. Complementation studies have revealed that these patients belong to XP group B, D or G (1,15–18). For most of the XP complementation groups and for both CS groups the responsible genes have been identified. The XPB and XPD genes code for subunits of basal transcription initiation factor TFIIH (19–21), which has a dual function in NER and transcription, but no clear evidence has been reported for a functional association of CS gene products with TFIIH (22).

Mutations in the XPD gene can result in three clinical phenotypes, XP, XP/CS and TTD (trichothiodystrophy), and different specific mutations are associated with the different clinical phenotypes (23). Broughton *et al.* (1) and Takayama *et al.* (24) described the molecular and cellular characteristics of cells derived from two XPD patients with both XP and CS symptoms. XP8BR is a compound heterozygote mutated in the XPD gene with a frame shift at amino acid 669, which is likely to inactivate the protein completely. The second allele contains a mis-sense Gly675Arg mutation (1). In XP-CS-2 only one allele is expressed and it has a mis-sense Gly602Asp mutation (24).

Strikingly, the XP-D-CS cell strains are much more sensitive to cytotoxic effects of UV-light than XP-D cells but the level of excision repair, measured as unscheduled DNA synthesis (UDS) or repair replication was 30–40% of that in normal cells, which is close to the maximum levels found in XP-D cells (1,25). It has been shown that XP-D cells are completely deficient in excising cyclobutane pyrimidine dimers (CPD) and have a strongly reduced capacity to excise 6–4 photoproducts (6–4PP) (26,27). Broughton *et al.* (1) also showed that RNA synthesis recovery was absent in the XP-D-CS cells as well as in XP-D cells. The authors suggested that XP-D cells were able to excise 6–4PP from active genes, whereas in XP-D-CS cells this ability was totally abolished. This might underlie the extreme UV-sensitivity of the cells and account for the clinical symptoms of CS that were associated with the patients.

In this paper we report results of experiments that were performed to test this hypothesis. Repair of CPD and 6–4PP was measured in the active adenosine deaminase (ADA) gene from XP-D and XP-D-CS cells that exhibited similar levels of UDS. Both cell types were completely deficient in the removal of CPD, confirming the results of other investigators (26,27) and in 24 h removed only a small fraction of 6–4PP from the active ADA gene. We did not find indications for TCR of 6–4PP in active genes in XP-D cells, nor for a specific defect in removal of 6–4PP from active genes in XP-D-CS cells. Since we were unable to find clear differences in repair between XP-D and XP-D-CS cells, we investigated the effect of low dose UV-irradiation on transcription in these cells. In contrast to XP-D cells that showed moderate recovery of UV-inhibited RNA synthesis after 24 h, the XP-D-CS cells could not restore their transcriptional activity. These results are consistent with a specific defect in transcription initiation in XP-D-CS cells in addition to the repair defect which XP-D and XP-D-CS cells have in common.

MATERIALS AND METHODS

Cell culture, irradiation conditions and isolation of DNA

The cell strains used in this study were primary human fibroblasts: VH25 (normal); XPITE and XP21RO (XP-C); XP25RO (XP-A); XP3NE, XP1NE and XP1DU (XP-D); XP-CS-2 and XP8BR (XP-D-CS) (1,15,17). All cell strains were cultured in Ham's F10 medium (without hypoxanthine and thymidine) supplemented with 15% fetal calf serum and antibiotics, in a 2.5% CO_2 atmosphere. For immunochemical measurement of repair the cells were prelabeled with ^{3}H-thymidine (0.05 μCi/ml, 80 Ci/mmol) and 1 μM thymidine. Prior to irradiation, cells were grown to confluence in 90 mm Petri dishes. For RNA synthesis measurements cells were prelabeled with ^{14}C-thymidine (0.01 μCi/ml, 60 mCi/mmol) and prior to irradiation seeded into 60 mm Petri dishes and grown to confluence. Cells were rinsed with PBS, irradiated with UV (Philips TUV lamp, predominantly 254 nm) at a dose rate of 0.2 W/m^2 and incubated in conditioned medium for up to 24 h; for immunochemical measurements of repair UV-irradiated cells were incubated in the presence of BrdUrd (10^{-5} M) and FdUrd (10^{-6} M) to allow separation of replicated and non-replicated DNA. DNA was isolated and purified as described previously (3).

Determination of CPD frequencies in specific DNA sequences

High molecular weight DNA was digested with *Eco*RI, purified by phenol and chloroform extractions, precipitated with ethanol and dissolved in Tris-EDTA (TE). Equal amounts of DNA were either treated or mock-treated with the CPD-specific T4 endonuclease V and electrophoresed in 0.6% alkaline agarose gels. The DNA was transferred to Hybond N$^+$ membranes (Amersham) by vacuum Southern blotting (Pharmacia-LKB Vacugene 2016) and hybridized with ^{32}P-labeled gene-specific probes. Radioactivity in full size fragments was quantified using an InstantImager Electronic Autoradiography System (Packard). The number of CPD was calculated from the relative radioactivities of the bands in the lanes containing DNA that was either treated or mock treated with T4 endonuclease V, using the Poisson expression (3).

Determination of 6–4PP frequencies in specific sequences

High molecular weight DNA was restricted with *Eco*RI and processed for 6–4PP measurements as described previously (28). Briefly, CPD were removed from the DNA using photolyase derived from *Anacystis nidulans* (kindly provided by Dr A. Eker, Erasmus University, Rotterdam, The Netherlands) (29) and visible light. After purification, the photoreactivation

of CPD was checked for completeness by treatment of the DNA with T4 endonuclease V and subsequent Southern analysis. Equal amounts of photoreactivated DNA were treated or mock-treated with UvrABC endonuclease (2 pmol of each subunit per μg DNA) (30), purified, subjected to electrophoresis in alkaline agarose gels and treated as described for CPD. The incision frequency calculated for each sample, was corrected for the non-specific cutting of the enzyme complex by including in each experiment a DNA sample from non-irradiated cells that had been processed simultaneously with the UV-irradiated samples (28).

Determination of repair patches in specific sequences

The quantification of repair patches in specific sequences was performed as described previously (31). Briefly, the DNA was restricted with either *Eco*RI (Pharmacia) or *Bcl*I (Biolabs). After digestion the DNA was centrifuged to equilibrium in CsCl density gradients. Gradients were fractionated and fractions containing parental density DNA were pooled, dialyzed against a buffer containing 10 mM Tris, 1 mM EDTA (TE, pH 8.0) and precipitated with ethanol. The concentration of DNA in the samples was determined by absorption measurement at 260 nm. The DNA was denatured and incubated with a monoclonal antibody against BrdUrd (Partec). This mixture was incubated with a biotinylated Goat-anti-Mouse antibody and subsequently with polymeric magnetic particles coated with streptavidin (Dynabeads M-280, Dynal). BrdUrd-containing DNA was separated from non-BrdUrd-containing DNA by using a magnetic particle concentrator (MPC-E, Dynal). Bound (BrdUrd-containing, repaired DNA) and unbound fractions were blotted to a nylon membrane (Hybond N^+, Amersham) using a minifold II slotblot apparatus (Schleicher and Schuell). The membranes were hybridized with ^{32}P-labeled double-stranded or strand-specific probes for detection of the 754 gene or the ADA gene and radioactivity on the blots was quantified using an InstantImager Electronic Autoradiography System (Packard). In order to correct the data for the efficiency of the immuno extraction, filters were immunostained for BrdUrd as described previously (31). To compare the extraction efficiency of fragments of different size, the amount of DNA that would have been bound if all fragments were of the same size (i.e. 10 kb) was calculated using the Poisson expression.

Preparation of ^{32}P-labeled double- or single-stranded probes

The *Pst*I fragment Bo of the human ADA cDNA (exons 6–11) recognizing the 18.5 kb *Eco*RI fragment of the human ADA gene (32) and a 2.0 kb *Hin*dIII genomic fragment of the 754 gene recognizing a 14.5 kb *Eco*RI fragment of the 754 gene (33) were used to prepare randomly labeled DNA probes.

The *Pst*I fragment Ba of the human ADA cDNA (exons 1–5) recognizing the 19.9 kb *Bcl*I fragment of the human ADA gene was used for the preparation of strand-specific probes by linear PCR (34).

RNA synthesis recovery

For RNA synthesis measurements ^{14}C-thymidine prelabeled confluent cells were washed with PBS, irradiated with UV and incubated in conditioned medium for up to 24 h. After incubating the cells for various periods of time, the medium was replaced by medium containing ^{3}H-uridine (20 μCi/ml, 43 Ci/mmol) and cells were allowed to incorporate this label for 30 min. The cells were washed twice with PBS and lysed in 150 mM NaCl, 10 mM Tris, 1 mM EDTA, 0.5% SDS and 100 μg/ml proteinase K at 37°C. Lysates were TCA precipitated, filtered on GF/C glass fibre filters and radioactivity of acid insoluble material was measured by liquid scintillation counting.

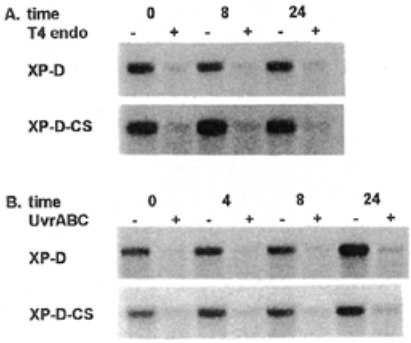

Figure 1. Autoradiograms showing the induction (0 h) and repair of CPD (**A**) and 6–4PP (**B**) at various times after UV (4, 8, 24 h) in the active ADA gene in XP1DU (XP-D) and XP-CS-2 (XP-D-CS) cells. For determination of CPD and 6–4PP frequencies, cells were exposed to 10 and 30 J/m^2, respectively.

RESULTS

Repair of CPD in XP-D and XP-D-CS cells

The induction and repair of UV-induced CPD (UV dose 10 J/m^2) were measured in confluent fibroblasts from classical XP-D patients (XP3NE, XP1DU and XP1NE) and from XP-D patients that exhibited CS symptoms as well (XP-CS-2 and XP8BR), employing techniques that have been described previously (3). These cell strains were selected based on similar UDS levels (1,25). Prior to this study we checked the UV-sensitivity of the cell strains by clonal survival studies. We confirmed the previously published (1,25) hypersensitivity of the XP-D-CS cells to UV when compared to XP-D cells (data not shown).

Repair was measured in a 3' located 18.5 kb *Eco*RI fragment of the active ADA gene, in which both strands are transcribed (35). The initial induction frequency of CPD amounted to 0.065 CPD/10 kb/J/m^2 in both XP-D and XP-D-CS strains, which is close to the frequency found in normal human fibroblasts (3). In all cell lines that were investigated, repair of CPD appeared to be completely deficient. Representative autoradiograms are shown in Figure 1A. Thus, there is no difference in gene-specific repair of CPD between XP-D-CS cells and classical XP-D cells.

Repair of 6–4PP in XP-D and XP-D-CS cells

Removal of UV-induced 6–4PP from the active ADA gene was measured employing UvrABC endonuclease treatment to introduce DNA strand breaks specifically at the sites of 6–4PP in the DNA. A high UV dose (30 J/m^2) had to be applied in order to induce sufficient levels of 6–4PP for gene-specific analysis, i.e. 1–2 6–4PP per restriction fragment of ~20 kb. Representative autoradiograms are shown in Figure 1B. Quantification of the intensity of full-size restriction fragments

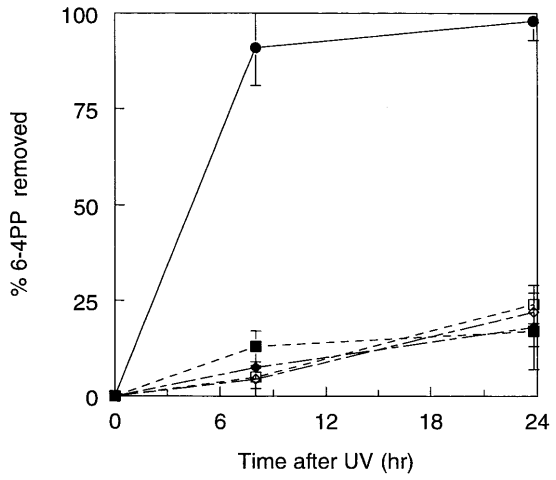

Figure 2. Removal of 6–4PP from the ADA gene in normal fibroblasts VH25 (circle), XP-D fibroblasts XP1DU (closed square) and XP3NE (open square) and XP-D-CS fibroblasts XP-CS-2 (open diamond) and XP8BR (closed diamond) irradiated with 30 J/m^2 UV-light. For each cell line mean values are shown from at least three experiments. Bars represent standard errors of the mean (SEM).

of DNA samples obtained from cells that were lysed immediately after UV-irradiation revealed comparable induction frequencies of 6–4PP in the ADA gene for all cell lines investigated, i.e. 0.015 6–4PP/10 kb/J/m^2. The three XP-D cell strains that were investigated as well as the two XP-D-CS cell strains removed UV-induced 6–4PP from the *Eco*RI fragment of the active ADA gene slowly and inefficiently. Repair levels after 24 h were <25% (Fig. 2). When compared to XP-D cells, XP-D-CS cells did not exhibit significant differences in removal of 6–4PP from the *Eco*RI fragment of the ADA gene, in which both strands are transcribed.

Immunochemical measurement of repair in normal, XP-A, XP-C and XP-D cells

The hypothesis of Broughton *et al.* (1) was based on the assumption that 6–4PP were repaired strand specifically in XP-D but not in XP-D-CS cells. Since the method for 6–4PP detection employing UvrABC and Southern blot analysis requires a relatively high UV dose (30 J/m^2), and since XP-D cells and XP-D-CS cells are very UV-sensitive, one could argue that the high dose could impair TCR of 6–4PP in XP-D cells and mask the differences between XP-D and XP-D-CS cells. In order to investigate whether XP-D cells are able to perform preferential repair of 6–4PP in the transcribed strand of active genes at lower doses, we applied an immunochemical detection method, which enabled us to measure the relative amount of repair synthesis in specific sequences. Repair proficient and several repair deficient cell strains were irradiated with a UV dose of 10 J/m^2 and incubated with BrdUrd to label repair patches. DNA was isolated, purified and treated with restriction enzymes. BrdUrd-containing DNA was extracted with specific antibodies, bound and unbound fractions were blotted to nylon membranes and analyzed using gene-specific radioactively labeled probes. Autoradiograms of these experiments are shown in Figure 3 and the data are plotted in Figure 4. In previous publications (4,28) we have shown that the kinetics of repair of CPD (by TCR) and 6–4PP (by GGR) in both (transcribed) strands of the *Eco*RI fragment and the transcribed strand of the *Bcl*I fragment of the ADA gene are similar. Therefore, we have plotted in Figure 4 the average percentages of bound DNA from both strands of the *Eco*RI fragment plus the transcribed strand of the *Bcl*I fragment as being the values for the transcribed strand. The plotted values represent the percentage of specific sequences that would have been bound if all fragments were of a size of 10 kb.

In normal cells no differences in repair of the transcribed and non-transcribed strand were observed during the first 4 h after UV-irradiation, but at later times a larger percentage of the transcribed strand was bound compared to the non-transcribed strand or the inactive 754 gene (Fig. 4A). In DNA from NER deficient XP-A cells, no ADA or 754 sequences could be detected in the 'bound' fraction at any time point after UV-irradiation (Fig. 4B). In XP-C cells, which are able to remove lesions from the transcribed strand of active genes only (28,36) the transcribed strand of the ADA gene was selectively bound by the antibodies. The non-transcribed strand of the ADA gene and the 754 gene were bound with low efficiency, showing that the immuno extraction method can be applied to detect strand-specific repair (Fig. 4C).

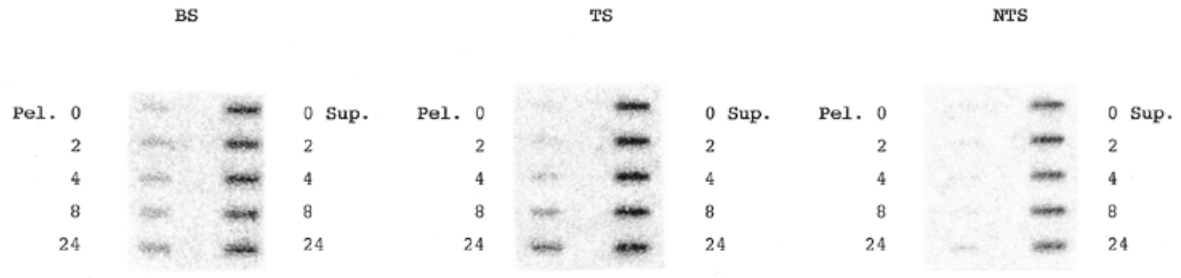

Figure 3. Immunochemical detection of BrdUrd-containing repair patches in DNA isolated from XP1TE cells at various times after irradiation with 10 J/m^2 UV-light. The fraction bound by the BrdUrd antibody (Pel.) was compared with the non-BrdUrd-containing DNA (Sup.) for the presence of both strands (BS), the transcribed strand (TS) or non-transcribed strand (NTS) of the ADA gene. The numbers represent the repair times in hours.

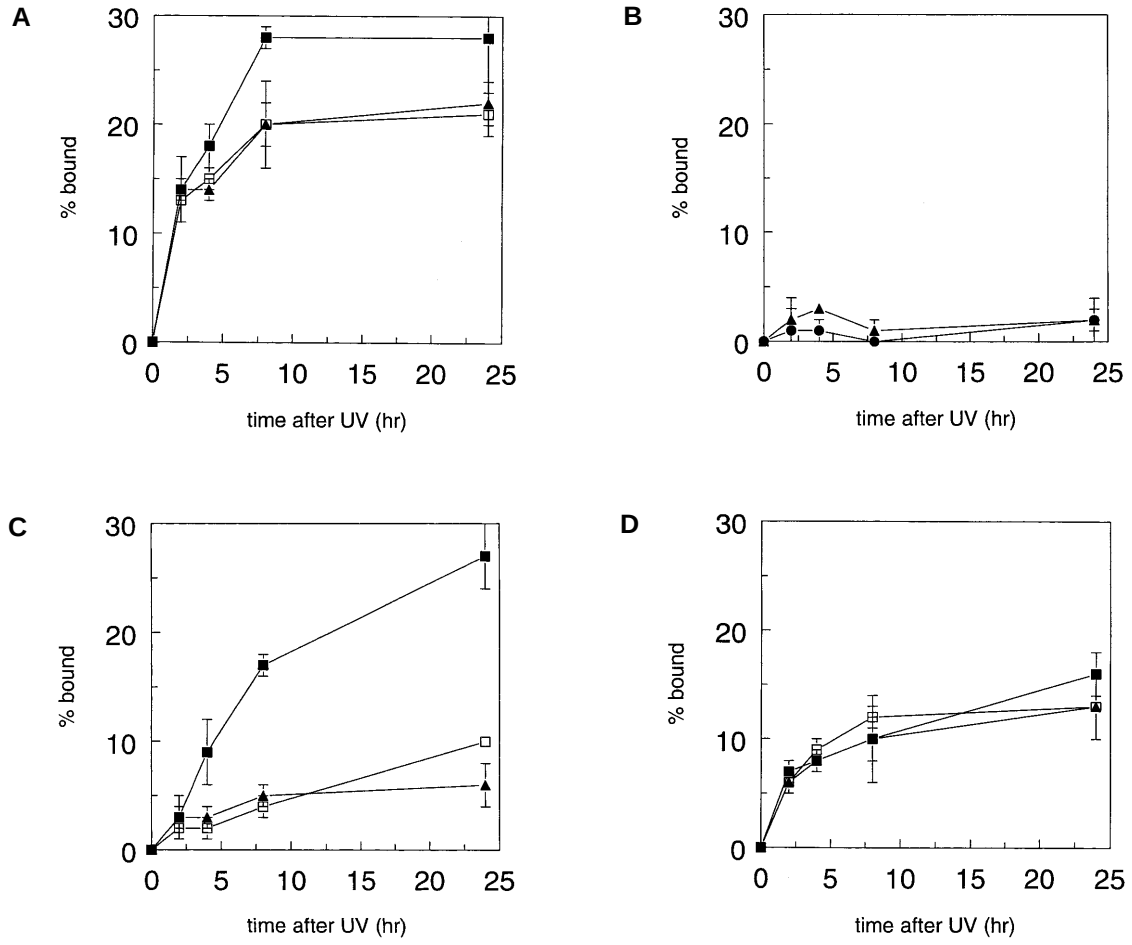

Figure 4. Quantification of the relative frequencies of defined 10 kb DNA sequences in BrdUrd-containing DNA from normal human fibroblasts VH25 (**A**), XP-A fibroblasts XP25RO (**B**), XP-C fibroblasts XP1TE (**C**) and XP-D fibroblasts XP3NE (**D**) at various times after UV-irradiation (10 J/m^2). For all cell lines, three independent experiments were performed except for XP25RO (for which two experiments were performed). Bars represent SEM. Closed square, ADA gene transcribed strand; open square, ADA gene non-transcribed strand; circle, ADA gene both strands (*Eco*RI fragment); triangle, 754 gene both strands.

In DNA from XP-D cells, increasing amounts of ADA and 754 fragments were detected during the time course after UV-irradiation. But compared to the bound fraction in normal cells, the levels of BrdUrd-containing DNA in XP-D cells were clearly lower (Fig. 4D). Analysis of the bound fractions for the presence of the transcribed and non-transcribed strand of the ADA gene did not reveal any differences between the two strands. The inactive 754 gene and the ADA gene fragments were bound with similar efficiencies. Since in XP-D cells at this UV dose (10 J/m^2) no CPD are repaired, all repair synthesis must be due to repair of 6–4PP. Our results suggest that in XP-D cells, after a high UV dose of 30 J/m^2 (see above) and a relatively low UV dose of 10 J/m^2, there is neither preferential nor strand-specific repair of 6–4PP and that repair of 6–4PP in XP-D cells is less efficient than in normal cells.

RNA synthesis recovery

We investigated the capacity of XP-D and XP-D-CS cells to recover UV-inhibited RNA-synthesis. As a control we used XP-C cells since XP-C cells are able to restore UV-inhibited transcription to the level of non-irradiated cells. We used a low UV-dose of 2 J/m^2 and compared the incorporation of ^{3}H-uridine in irradiated cells at various times after UV with the incorporation in non-irradiated cells (Fig. 5). We note that agarose gel electrophoresis of labeled RNA revealed that the majority of ^{3}H-uridine (80%) is incorporated into heterogeneous nuclear RNA (hnRNA) and that this incorporation is sensitive to UV and to α-amanitin. The latter suggests that the incorporation reflects merely RNA polymerase II activity (data not shown). XP-C cells rapidly restored RNA-synthesis to the level of non-irradiated cells. After this low UV dose, XP-D cells showed transcription recovery albeit less rapid than XP-C cells (100% after 24 h). However, XP-D-CS cells were not able to restore their transcription activity to the level of non-irradiated cells within 34 h, even at this very low dose of 2 J/m^2, at which inhibition of transcription was only 30%.

DISCUSSION

We have investigated repair of UV-induced DNA lesions in cells derived from classical XP-D patients and from XP-D patients who additionally exhibited CS symptoms. Cells from

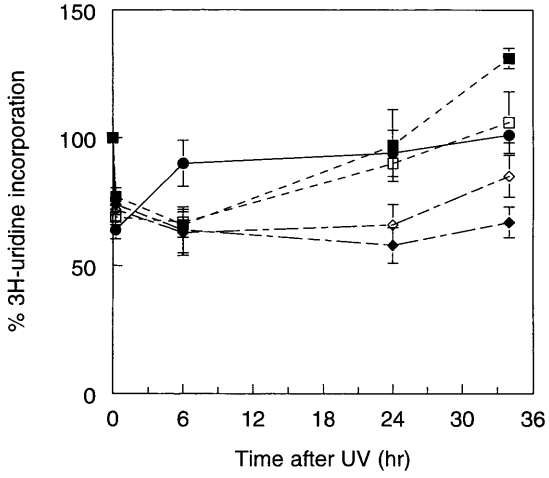

Figure 5. RNA synthesis in XP-C fibroblasts XP21RO (circle), XP-D fibroblasts XP1DU (closed square) and XP3NE (open square) and XP-D-CS fibroblasts XP-CS-2 (open diamond) and XP8BR (closed diamond) irradiated with 2 J/m^2 UV-light and incubated with ^{3}H-uridine for 30 min at various times after irradiation. For each cell line mean values are shown from at least three experiments. Bars represent SEM.

the latter are extremely sensitive to UV-light, but the levels of UV-induced UDS are comparable to that in XP-D cells indicating no gross differences in genome overall repair between the cell lines. To explain the difference in UV-sensitivity between XP-D and XP-D-CS cells, Broughton *et al.* (1) suggested that the severe cytotoxic effects of UV in XP-D-CS cells could be due to impaired ability to remove 6–4PP from active genes. In this model, classical XP-D cells should have at least a residual capacity to remove lesions preferentially from active genes, leading to higher resistance to the lethal effects of UV.

The results of the current study are not consistent with this hypothesis. They demonstrate that XP-D and XP-D-CS cells are completely deficient in repair of CPD in active and inactive genes (measured at a UV dose of 10 J/m^2), confirming observations previously reported by other investigators (26,27). Even at a dose of 5 J/m^2, XP-D cells did not show removal of CPD from the genome overall (unpublished data). The repair of 6–4PP in XP-D and XP-D-CS fibroblasts exposed to 30 J/m^2 UV-light is strongly reduced when compared to normal cells. During a 24 h repair period only 20–25% of these lesions are removed from the active ADA gene. No evidence could be found for more efficient repair of lesions in the active ADA gene in XP-D cells when compared to XP-D-CS cells. In contrast, XP-C cells which are only able to carry out TCR, are able to repair a significant proportion of 6–4PP, even at the high dose of 30 J/m^2 TCR (28). This demonstrates that TCR occurs even at a high dose of UV.

The data on 6–4PP repair in XP-D and XP-D-CS cells, however, are not entirely conclusive with respect to the role of TCR in repair of 6–4PP and a possible defect in XP-D-CS cells. To achieve frequencies of 6–4PP sufficiently high to allow reliable gene-specific repair measurements, cells were irradiated with a high UV-dose (30 J/m^2). In a previous study we showed that in normal human fibroblasts exposed to such a high dose, TCR makes only a minor contribution to repair of CPD and 6–4PP in active genes as it is overruled by very efficient GGR (28). In order to measure repair of 6–4PP at a lower UV-dose (10 J/m^2) we applied an immunochemical assay which is based on selective extraction of DNA fragments containing BrdUrd-labeled repair sites. In principle this method is not lesion-specific, but as a consequence of the lack of CPD repair in XP-D cells exposed to 10 J/m^2, BrdUrd-labeled repair patches in these cells will result entirely from repair of 6–4PP. In XP-D cells fewer BrdUrd-containing fragments were detected during the time-course after irradiation than in normal cells. However we note here that the level of repair patches in XP-D cells is high considering the lack of CPD repair and the reduced level of 6–4PP repair in these cells. The same phenomenon refers to the high levels of UDS in XP-D cells in general in spite of the low level of repair of photoproducts (25). Nevertheless, it may indicate that repair of 6–4PP at a low dose is more efficient in XP-D cells than expected from the repair data measured at 30 J/m^2. However, the similar levels of repair synthesis in XP-D and XP-D-CS cells indicate that 6–4PP repair is affected to the same extent. No differences in repair rates were observed between the transcribed and non-transcribed strand of the ADA gene, nor between the ADA gene and the 754 gene. Since in XP-D cells all BrdUrd-containing repair patches must be due to repair of 6–4PP, the equal distribution of repair patches over active and inactive genes, transcribed and non-transcribed strands, indicates that 6–4PP are not preferentially repaired in XP-D cells at a dose of 10 J/m^2. Thus our results do not support the suggestion by Broughton *et al.* (1) that defective preferential repair of 6–4PP in active genes might be the cause of the extreme UV-sensitivity of XP-D-CS cells. However, we cannot completely exclude the possibility that at very low UV-doses at which a reasonable percentage of XP-D cells can survive, the inability to perform TCR makes XP-D-CS cells more sensitive to the cytotoxic effects of UV. An alternative explanation for the severe sensitivity of XP-D-CS cells to UV refers to oxidative damage. Mutations in CS genes are known to impair TCR of oxidative damage (13). However, it is unlikely that defective repair of oxidative damage would underlie the sensitivity of XP-D-CS cells as thymine glycols and hydrates are induced at 100-fold lower frequency than CPD by UVC light and we did not detect significant levels of these types of base damage in UVC irradiated human fibroblasts (28).

In a recent study we postulated that the sensitivity of CS cells is not primarily due to deficient TCR, but to the inability to restore UV-inhibited RNA-synthesis (11). The lack of (initiation) of transcription in UV- or NA-AAF-treated cells would cause cellular sensitivity to these DNA damaging agents and, since transcription is inhibited, TCR of DNA lesions cannot occur. The results presented here are consistent with this hypothesis. The only additional defect we could detect in XP-D-CS cells when compared to XP-D cells, was strongly delayed recovery of UV-inhibited RNA synthesis. XP-D cells were able to restore their transcription activity after a low dose of UV almost to normal levels. The inhibition of transcription after induction of DNA damage might be due to recruitment of the transcription initiation protein complex TFIIH by the NER machinery. In this model the CS proteins are involved in transferring TFIIH from the repair machinery back to transcription initiation complexes in order to restore transcription of essential genes and enable TCR to occur. A mutation in the CS

protein could disturb its ability to effect this transfer, and thereby abolishing reinitiation of DNA damage inhibited transcription. On this model, the specific mutations in the XPD subunit of TFIIH that result in the XP/CS phenotype (1,24) could hinder the transfer of TFIIH from repair to transcription mode, thereby giving rise to the clinical and cellular characteristics of CS.

In conclusion, our results suggest that the enhanced UV-sensitivity of XP-D-CS cells may be associated with a defect in transcription regulation superimposed on the repair defect.

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