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Gene-Specific Repair of 6-4 Photoproducts in Trichothiodystrophy Cells

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TTD1BI cells are non-hypersensitive to UV irradiation and perform normal genome repair of pyrimidine dimers but fail to excise 6-4 photoproducts and, concomitantly, are unable to restore RNA synthesis levels following UV irradiation. This pointed to a defect in gene-specific repair and this study was undertaken to examine repair of 6-4 photoproducts at the gene-level. The results indicated a defect in gene-specific repair of 6-4 photoproducts in active genes, although strand-specificity of 6-4 photoproduct removal was essentially similar to that of normal cells. These findings indicate that the near normal UV resistance of TTD1BI cells may be due to the inability of these cells to remove DNA lesions preferentially, as well as to the cells opting out of the cell cycle to repair damage before resuming replication.

Keywords: DNA repair, Gene-specific repair, Trichothiodystrophy, UV sensitivity,

Introduction

Trichothiodystrophy (TTD), or PIBIDS as it is sometimes referred to, represents a central pathologic dysplasia associated with several disorders in organs derived from ectoderm and neuroectoderm and is inherited as an autosomal recessive trait (Price *et al.*, 1980). TTD patients demonstrate phenotypical heterogeneity in DNA repair and UV sensitivity (Lehmann *et al.*, 1988). In this study, we focussed on the TTD phenotype which is not photosensitive and demonstrates survival levels similar to those of normal cells. Although mutation frequencies are not elevated, there is a reduced rate of repair synthesis in these cells (for both pyrimidine dimers and 6-4 photoproducts) but only at early times after UV irradiation (Lehmann *et al.*, 1988; Eveno *et al.*, 1995).

Despite the apparent normal ability of TTD1BI cells to remove pyrimidine dimers from the overall genome, RNA synthesis levels in non-dividing cells were reported to be severely depressed following irradiation (Lehmann *et al.*, 1988). This inability to restore RNA synthesis is usually attributed to dimers acting as blocks to transcription. In normal cells, recovery of RNA synthesis is a result of gene-specific repair of transcriptionally active genes (Kantor *et al.*, 1990; Venema *et al.*, 1990a). Petit Frere *et al.* (1996) showed evidence that suggested the 6-4 photoproduct as the mediator of the immediate inhibition of DNA and RNA synthesis.

This phenomenon of gene-specific or preferential repair of transcriptionally active genes has been studied most thoroughly for UV-induced DNA damage. UV-induced pyrimidine dimers in human and animal cells have been shown to be preferentially repaired from active genes and this preference is due to a rapid removal of adducts from the transcribed strand (TS) (Friedberg *et al.*, 1994; van Hoffen *et al.*, 1995; Vreeswijk *et al.*, 1998). Thomas *et al.* (1989) and van Hoffen *et al.* (1995) have also demonstrated gene-specific repair of 6-4 photoproducts in normal cells. However, no differences in strand-specific repair of these adducts were observed (van Hoffen *et al.*, 1995).

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In an earlier preliminary study, we have reported that gene-specific repair of pyrimidine dimers in the UV-resistant TTD cells was defective, but only at early times following UV irradiation, and this has been attributed to a defect at early times in performing strand-specific repair (Nathan *et al.*, submitted). Thus, to determine if poor gene-specific repair of 6-4 photoproducts contributed to the TTD1BI phenotype, we examined for repair in the constitutively expressed housekeeping genes, adenosine deaminase (*ADA*) and dihydrofolate reductase (*DHFR*), and compared their levels of repair to the untranscribed 754 locus. We demonstrate that in these cells, gene-specific repair of 6-4 photoproducts (like pyrimidine dimers) was defective, but only at early times following UV irradiation, attributable to a defect at early times in performing strand-specific repair.

Materials and Methods

Cells strains and maintanence Diploid normal human MRC5 (Huschtscha and Holliday, 1983) and trichothiodystrophy TTD1BI (Lehmann *et al.*, 1988) fibroblasts were cultured in DMEM supplemented with 15% fetal calf serum and antibiotics in 5% CO_2 at 37°C. Cells were routinely harvested in trypsin solution and reseeded in petri dishes when required.

UV-irradiation of cells and isolation of DNA Cells were seeded at a density of 4×10^6 cells per 120-mm petri dish and incubated for 4–6 d. Cells were then irradiated with UV light at a fluence of 30 Jm^{-2} (254 nm) and either lysed immediately (t = 10 h) in 1 ml SDS Lysis-mix (1% SDS, 25 mM EDTA, 200 mM Tris·Cl; pH8.0) with 100 μg/ml Proteinase K per plate or incubated for various times up to 24 h in complete medium and then lysed as above. Following an incubation of lysate for 4 h at 37°C, DNA was further purified by phenol and chloroform extractions. After ethanol precipitation and centrifugation, pellets were dissolved in TE buffer [10 mM Tris (pH 8.0), 1 mM EDTA] and digested with 50 μg/ml RNase for 1 h at 37°C.

Restriction digestion of DNA Irradiated DNA was digested overnight with *Eco*RI or *Hin*dIII (6 U/μg) under conditions recommended by the manufacturer and all digestions were checked for completion. DNA was then purified by phenol and chloroform extractions, concentrated by butanol extraction before ethanol precipitation, and dissolved in TE to a final concentration of 1 μg/μl.

Quantitation of 6-4 photoproduct repair Irradiated DNA was photoreactivated with *Anacytis nidulans* photolyase enzyme (Eker *et al.*, 1990). The reaction mixture contained the enzyme (1:100 dilution in PRE buffer) and 10× PRE buffer (100 mM K_2HPO_4, 1 M NaCl, 50 mM Mercaptoethanol, and 1 mg/ml BSA) (to give a final concentration of 1× PRE) in a volume of 300 μl. The reaction was kept in the dark for 5 min in a flat-bottomed 24-well plate (Falcon). The DNA samples were then irradiated with white light (425 nm) for 60 min at room temperature. 0.1% SDS and 0.1 μg/ml Proteinase K were added to remove traces of enzyme and the samples were further incubated for 15 min at 37°C. The DNA was then phenol-chloroform extracted, ethanol precipitated, and resuspended in TE at a concentration of 0.6 μg/μl. The T4 Endonuclease V assay (van Hoffen *et al.*, 1995) was performed on an aliquot of photoreactivated DNA to ensure that all dimers had been photoreactivated subsequently to performing the UVR ABC Excinuclease assay.

The enzymatic reaction mixture was prepared as follows: 10× ABC buffer [0.1M Tris (pH 7.5), 25 mM $MgCl_2$, 0.2 M DTT, 0.02 M ATP (final concentration) was mixed with 75 mM KCl and 20 pmole each of subunits A and B of the enzyme (Visse *et al.*, 1992). Following an incubation period of 10 min at 37°C, 20 pmole of subunit C was added to the enzymatic mixture. Five μg of photoreactivated DNA was incubated with the enzymatic reaction mixture whilst mock treated (photoreactivated) DNA was mixed with 10× ABC buffer and 15 mM KCl. DNA samples were incubated for 60 min at 37°C. The reaction was stopped with the addition of 0.1 μg/ml Proteinase K, 0.1% SDS, 20 mM EDTA and further incubation at 50°C for 2 h. The DNA was ethanol precipitated and resuspended in TE (at a concentration of 0.3 μg/μl) prior to electrophoresis.

Electrophoresis, Southern transfer and hybridization Excinuclease and mock-treated samples were electrophoresed on a 0.6% alkaline agarose gel for 16 h at 20V in a buffer containing 30 mM NaOH and 1 mM EDTA. After electrophoresis, the DNA was transferred onto nylon (Hybond N+) membranes in transfer solution (0.4 N NaOH, 0.6 M NaCl) using a Possiblot pressure blotter (Stratagene) according to the manufacturer's instructions. Filters were hybridized for 48 h at 65°C in 5 ml hybridization mix (5× SSPE, 0.5% SDS, 10% Dextran Sulphate, 5× Denhardt's Solution, 100 μg/μl salmon sperm DNA). DNA probes were labeled with α-^{32}P-dCTP by random primer extension (Feinberg and Vogelstein, 1983). Filters were exposed to preflashed X-Ray film (Fuji) at −70°C in the presence of intensifying screens. Band intensities were quantitated using a Joyce-Loebl Chromoscan 3 densitometer. The number of UVR ABC Excinuclease sensitive sites (UNSS) per fragment was estimated from the densities of full length fragments using the Poisson equation as described by Bohr (1991).

DNA Probes

Double-stranded DNA fragments (i) a *Pst*I fragment [B_O (exons 5–11)] of the human ADA cDNA clone pLL subcloned into pUC 19 (Berkvens *et al.*, 1987); (ii) a 1.8 kb *Eco*RI fragment of the human DHFR gene (Yang *et al.*, 1984); (iii) a 2 kb *Hin*dIII fragment of the 754 locus cloned into pAT 153 (Hofker *et al.*, 1986) (Fig. 1).

Single-stranded DNA fragments (i) Fragment B_O subcloned into M13 SSEV-18 and -19 vectors (Biernat *et al.*, 1989b); (ii) a genomic *Eco*R1-*Hin*dIII fragment from intron V of the *DHFR* gene (Will and Dolnick, 1986) cloned into M13 SSEV-18 and -19 vectors (Fig. 2).

Preparation of radiolabeled single-stranded DNA Fifty μg of single-stranded DNA was heated to 65°C for 5 min and cooled very slowly to room temperature. This aids in the production of the stem-loop structure (see Biernat *et al.*, 1989a). Two hundred

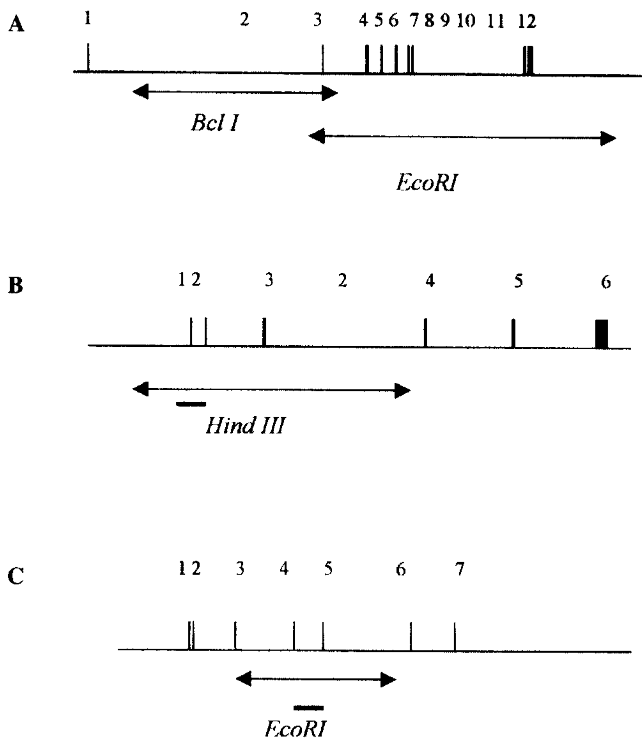

Fig. 1. Molecular organization of the human *ADA*, *DHFR*, and 754 genes. Filled boxes represent exons of the genes. The solid line indicates the genomic *DHFR* and 754 probes (Venema *et al.*, 1990b). **A.** *ADA* **B.** *DHFR* **C.** *754*

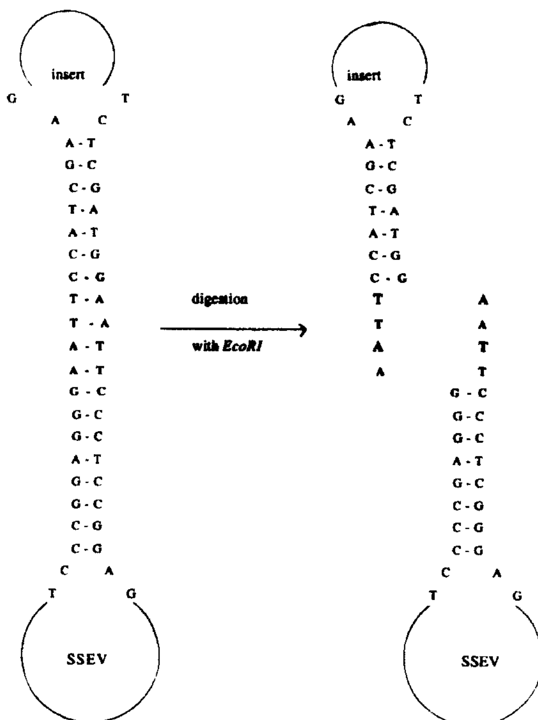

Fig. 2. Schematic diagram of the isolation of single-stranded DNA fragments from SSEV-18 and -19 vector clones (Venema *et al.*, 1990b).

units of *Eco*R1 were added and the sample was incubated at 37°C for 1 h. An additional 100 U were added and the digestion was carried out for a further hour.

The reaction was stopped by heating the mixture to 65°C or adding alkaline loading buffer (see above). The sample was then electrophoresed and the required fragment was excised from the gel and DNA was purified using the GENECLEAN II Kit (BIO101 Unc.) or by electro-elution in a biotrap Electro-elution BT 1000 (Schleicher and Schuell) apparatus as described by the manufacturers. The labeling process of single-stranded DNA involved the filling in of the 3′ recessed end of the stem-loop. Purified DNA (50–100 μg) was heated to 65°C and allowed to cool slowly to room temperature. The DNA was then labeled to a high specific activity with α-^{32}P-dCTP.

Results

Repair of 6-4 photoproducts from TTD1BI cells The induction and removal of 6-4 photoproducts from active and inactive genes in TTD group 3 cells was performed utilizing an assay system previously described by Thomas *et al.* (1989). *E. coli* UVR ABC Excinuclease is used to excise DNA at the sites of UV-induced DNA damage. As the enzyme recognizes most DNA adducts that create significant helical distortions (Sancar and Sancar, 1988), it was necessary to remove pyrimidine dimers from the UV-irradiated DNA. This was achieved by treating the DNA with photoreactivating enzyme (photolyase). The complete removal of pyrimidine dimers by photoreactivation was confirmed by the absence of nicking by T4 endonuclease V. Induction and repair of the remaining damage (mainly 6-4 photoproducts) was then examined as described above.

Confluent fibroblast cells were irradiated with 30 Jm^{-2} UV light to induce an average of one 6-4 photoproduct per 10 kb DNA. Following treatment with DNA photolyase and T4 endonuclease V, the DNA was analyzed and full-length restriction fragments in the enzyme-treated (+) lanes indicated the lack of pyrimidine dimers. Densitometric scans of the autoradiograph demonstrated that fewer than 0.045% pyrimidine dimers remained in the gene investigated. This demonstrates that the photoreactivation is more than 99% complete and establishes a base line from which to observe and quantify the 6-4 photoproducts.

The UVR ABC excinuclease was used to introduce single-strand breaks and incise the DNA at the site of non-pyrimidine dimers leading to the loss of gene-specific bands when the DNA was separated on alkaline agarose gels and analyzed by Southern blotting. In this assay, repair is observed when full-length restriction fragments in enzyme-treated DNA reappear. Resulting band intensities were quantified by densitometry and lesion frequencies were calculated using the Poisson expression. Induction and repair of 6-4 photoproducts were analyzed in the *DHFR*, *ADA*, and 754 genes and an average of 0.8–0.9 excinuclease sensitive sites (UNSS) was observed. van

Hoffen *et al.* (1995) have previously reported of an induction rate of approximately one 6-4 photoproduct per 10 kb after a fluence of 30 Jm^{-2}.

Figure 3 is a representative autoradiograph of repair in the *Eco*RI fragment of the *ADA* gene in normal MRC5 and TTD1BI cells. The autoradiographs were scanned by densitometry and the data are summarized in Table 1. Removal of 6-4 photoproducts from this fragment in normal cells was rapid at early times with almost complete repair (95%) by 8 h. In the TTD1BI cells, 43% of 6-4 photoproducts were removed by 4 h whereas 82% were removed by 8 h and complete repair was observed at 24 h. Thus, repair at early times in the TTD1BI cells was slower than that observed for non-UV sensitive cells. The rate of repair was also measured within the *DHFR* gene transcriptional unit. In MRC5 cells, 83% of 6-4 photoproducts were removed by 8 h post-irradiation and 100% repair was achieved by 24 h. In the TTD1BI cells, repair at both 4 and 8 h was reduced but full repair was achieved by 24 h.

The nylon membranes were reprobed with the 754 locus to determine the rate of repair in a nonexpressed region. Densitometric scans of autoradiographs demonstrated that repair was only 15% in TTD and normal cells and these rates were much slower than previously published rates of removal of 6-4 photoproducts from the overall genome of these cells (Broughton *et al.*, 1990).

Fig. 3. Autoradiograms measuring removal of UV-induced 6-4 photoproducts from the *ADA* gene in (A) MRC5 and (B) TTD1BI cells. Cells were irradiated with 30 Jm^{-2} and incubated for 4, 8, and 24 h. DNA was purified, restricted, treated with photolyase, followed by UVR ABC excinuclease (+) or not (−).

Removal of 6-4 photoproducts from the TS and NTS in TTD1BI cells Removal of 6-4 photoproducts from the TTD1BI fibroblast cells irradiated with 30 Jm^{-2} UV light was analyzed in the 3′-end of the *ADA* gene. The membranes used to study preferential repair of 6-4 photoproducts in normal and TTD1BI cells were reprobed with the single-stranded probes. The rate of repair in the defined sequences of the *ADA* gene was quantitated by densitometry. The initial number of 6-4 photoproducts induced in the TS was significantly higher than that in the NTS. This suggests that the induction of 6-4 photoproducts is not random and argues that data calculated based upon the Poisson expression might not be a true reflection of the repair patterns of transcriptionally active genes.

The analysis of 6-4 photoproduct repair in normal and TTD1BI cells is summarized in Table 2. In MRC5 cells, repair from the TS was rapid (72% by 4 h post-UV) and completed by 8 h. Repair in the NTS was generally slower than in the TS and both strands combined, but faster than in the 754 locus. The faster removal of 6-4 photoproducts from the TS was also observed in the TTD1BI cells while the slower rate of repair from the NTS was significant at 4 and 8 h post-UV. However, both strands were repaired faster than the 754 locus with complete repair at 24 h.

Table 1. Percentage of 6-4 photoproducts removed from defined DNA sequences and the overall genome in confluent normal (MRC5) and TTD1BI cells (± SEM).

Cell strain	Repair time (h)	*ADA Eco*RI	*DHFR Hin*dIII	*754 Eco*RI	Genome overall[1] (10 Jm^{-2})
MRC5	4	—	60	—	80
	8	95	83	22	85
	24	100	106	50	95
No. of experiments		2	2	1	
TTD1BI	4	43 ± 9	45 ± 6	15	50
	8	82 ± 11	55 ± 8	33	75
	24	103 ± 2	114 ± 10	61	90
No. of experiments		4	3	2	

[1] Broughton *et al.* (1990)

Table 2. Removal of 6-4 photoproducts from respective strands in the *ADA EcoRI* fragment in normal human (MRC5) and TTD1BI fibroblasts.

Strand probed	Repair time (h)	Mean % repair*	
		MRC5	TTD1BI
Both	4	63	43 (9)
	8	94	82 (11)
	24	100	103 (2)
TS	4	72	71 (5)
	8	100	92 (4)
	24	110	99 (1)
NTS	4	48	43 (2)
	8	73	62 (7)
	24	96	92 (3)
754	4	18	2
	8	22	33
	24	50	62

* (SEM) – Standard Errors of the Mean of 3 experiments.

Discussion

Removal of damage by the nucleotide excision repair pathway has been shown to occur heterogenously throughout the genome. This preferential repair of DNA adducts from the transcribed strand of an active gene has been attributed to a direct coupling of the excision repair system to the transcriptional apparatus (Weeda *et al.*, 1997; Coin *et al.*, 1999). Mutations that prevent this coupling have been shown to contribute to the phenotype of Xeroderma pigmentosum and sun-sensitive TTD (Weeda *et al.*, 1997; Coin *et al.*, 1998; de Boer *et al.*, 1998).

This study was initiated to elucidate a better understanding of the relationship between the cellular effects of UV light, the biochemical consequences and the associated clinical phenotype of the non-UV sensitive trichothiodystrophy cells. These cells presented a unique phenotype of UV resistance in the presence of efficient dimer removal from the genome overall, but deficient repair of 6-4 photoproducts.

In the overall genome of normal cells, 100% of 6-4 photoproducts were removed within 6–8 h after a UV fluence of 10 Jm^{-2} (Mitchell *et al.*, 1985; Broughton *et al.*, 1990). Studies on repair of 6-4 photoproducts at the gene level after a fluence of 30 Jm^{-2} in normal cells indicate that 6-4 photoproducts are also repaired preferentially in transcriptionally active genes despite the rapid kinetics of repair seen in the overall genome. Repair of 6-4 photoproducts was biphasic with a faster rate at early times. The repair of 6-4 photoproducts at the gene level was much faster than that previously observed for pyrimidine dimers, a phenomenon that is also present for overall genome repair. This difference is attributable to a different accessibility of repair proteins to 6-4 photoproducts or that repair enzymes are much more efficient at incising and removing these adducts when compared to pyrimidine dimers (Suquet *et al.*, 1995). Data presented here indicate that 6-4 photoproducts in normal cells are repaired more rapidly from transcriptionally active genes when compared to inactive sequences.

Further data presented from this study also indicate that the TTD1BI cells have retained the ability to perform preferential repair of 6-4 photoproducts at the 3′ end of the *ADA* gene but only at later times post-irradiation. This suggests that the defect at the gene level was consistent with the overall defect. Bohr (1991) showed that Chinese hamster cells were able to undergo fast repair of 6-4 photoproducts in the *DHFR* gene.

Induction of 6-4 photoproducts was consistently higher in the TS of the *ADA* gene. Repair of 6-4 photoproducts in the TS and NTS was indistinguishable between TTD1BI and MRC5 cells with rapid repair in the TS when compared to the NTS demonstrating strand specificity in the removal of these adducts from the 3′ end of the *ADA* gene. Repair of both individual strands was faster than that in the 754 locus. Mullenders *et al.* (1993) and May *et al.* (1993) have previously reported a lack of preferential repair of 6-4 photoproducts in the TS of the *HPRT* and *DHFR* genes in Chinese hamster cells. Thus, the strand bias reported here may be limited to human cells. Nevertheless, Link *et al.* (1992) showed some strand specificity of 6-4 photoproduct repair towards the TS in the hamster *DHFR* gene with a non-enzymatic method. Tijsterman *et al.* (1999) demonstrated that 6-4 photoproducts were preferentially repaired from the TS of the *Saccharomyces cerevisiae URA3* gene indicating that transcription-coupled repair predominates in the repair of 6-4 photoproducts from transcribed DNA.

As the repair rates of the TS and NTS of TTD1BI cells were comparable to normal cells, the above data obtained with double-stranded probes need to be reassessed. Strand specificity of 6-4 photoproduct repair observed in TTD1BI cells confirms that repair in the *ADA* gene was essentially normal while any defect in 6-4 photoproduct removal was only significant in the *DHFR* gene. This suggests an anomaly with TTD1BI in that the *DHFR* but not the *ADA* gene was affected and this is consistent with the TTD defect not being completely linked to the transcriptional status of a gene. The proficient repair of 6-4 photoproducts from active genes could contribute to the eventual UV survival of these TTD1BI cells.

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References

Berkvens, Th. M., Gerritsen, E. J. A., Oldenburg, M., van der Eb, A. J. and Meera Khan, P. (1987) Severe combined immunodeficiency due to a homozygous 3.2 kb deletion spanning the promoter and first exon of the adenosine deaminase gene. *Nucleic Acid. Res.* **15**, 9365–9378.

Biernat, J., Gobel, U. B. and Kostel, H. (1989a) Preparation of single-stranded insert DNA free of vector sequences. *Nucleic Acid. Res.* **15**, 9365–9378.

Biernat, J., Gobel, U. B. and Kostel, H. (1989b) New bacteriophage vectors for the large-scale production of single-stranded DNA. *J. Biochem. Biophys. Methods* **19**, 155–168.

Bohr, V. A. (1991) Gene specific damage and repair after treatment of cells with UV and chemotherapeutical agents. *Adv. Exp. Med. Biol.* **283**, 225–233.

Broughton, B. C., Lehmann, A. R., Harcourt, S. A., Arlett, C. F., Sarasin, A., Kleijer, W. J., Beemer, F. A., Nairn, R. and Mitchell, D. L. (1990) Relationships between pyrimidine dimers, 6-4 photoproducts, repair synthesis and cell survival: Studies using cells from patients with trichothiodystrophy. *Mutation Res.* **235**, 33–40.

Coin, F., Marinoni, J. C. and Egly, J. M. (1998) Mutations in XPD helicase prevent its interaction and regulation by p44, another subunit of TFIIH, resulting in Xeroderma pigmentosum and trichothiodystrophy phenotypes. *Pathol. Biol.* **46**, 679–680.

Coin, F., Bergmann, E., Tremeau-Bravard, A. and Egly, J. M. (1999) Mutations in XPB and XPD helicases found in xeroderma pigmentosum patients impair the transcription function of TFIIH. *EMBO J.* **18**, 1357–1366.

de Boer, J., de Wit, J., van Steeg, H., Berg, R. J., Morreau, H., Visser, P., Lehmann, A. R., Duran, M., Hoeijmakers, J. H. and Weeda, G. A. (1998) Mouse model for the basal transcription/ DNA repair syndrome trichothiodystrophy. *Mol. Cell* **1**, 981–990.

Eker, A. P. M., Kooiman, P., Hessels, J. K. C. and Yasui A. (1990) DNA photoreactivating enzyme from the cyanobacterium *Anacystis nidulans*. *J. Biol. Chem.* **265**, 8009–8015.

Eveno, E., Bourre, F., Quilliet, X., Chevallier-Lagente, O., Roza, L., Eker, A. P., Kleijer, W. J., Nikaido, O., Stefanini, M. and Hoeijmakers, J.H. (1995) Different removal of ultraviolet photoproducts in genetically related xeroderma pigmentosum and trichothiodystrophy diseases. *Cancer Res.* **55**, 4325–4332.

Feinberg, A. P. and Vogelstein B. (1983) A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.* **132**, 6.

Friedberg, E. C., Bardwell, A. J., Bardwell, L., Wang, Z. and Dianov, G. (1994) Transcription and nucleotide excision repair — reflections, considerations and recent biochemical insights. *Mutation Res.* **305**, 5–14.

Hofker, M. H., Van Ommen, G. J. B., Bakker, E., Burmeister, M. and Pearson, P. L. (1986) Development of additional RFLP probes near the locus for Duchenne muscular dystrophy by cosmid cloning of the DSX84(754) locus. *Human Genet.* **74**, 270–274.

Huschschta, L. I. and Holliday R. (1983) Limited and unlimited growth of SV40 transformed cells from human diploid MRC5 fibroblasts. *J. Cell. Sci.* **63**, 77–99.

Kantor, G. J. (1990) Characteristics of DNA excision repair in nondividing xeroderma pigmentosum cells, complementation group C. *Basic Life Sci.* **53**, 203–214.

Lehmann, A. R., Arlett, C. F., Broughton, B. C., Harcourt, S. A., Steingrimsdottir, H., Stefanini, M., Taylor, M. R., Natarajan, A. T., Green, S., King, M. D., Mackie, R. M. M., Stephenson, J. B. P. and Tolmie, J. L. (1988) TTD, a human DNA repair disorder with heterogeneity in the response to UV light. *Cancer Res.* **48**, 6090–6096.

Link, C. J. Jr. Robbins, J. H. and Bohr, V. A. (1992) Gene specific DNA repair of damage induced in familial Alzheimer disease cells by ultraviolet irradiation or by nitrogen mustard. *Mutation Res.* **336**, 115–121.

May, A., Nairn, R. S., Okumoto, D. S., Wassermann, K., Stevsner, T., Jones, J. C. and Bohr, V. A. (1993) Repair of individual DNA strands in the hamster dihydrofolate reductase gene after treatment with ultraviolet light, alkylating agents and cisplatin. *J. Biol. Chem.* **268**, 1650–1657.

Mitchell, D. L., Haipek, C. A. and Clarkson, J. M. (1985) (6-4) photoproducts are removed from the DNA of UV irradiated mammalian cells more efficiently than cyclobutane pyrimidine dimers. *Mutation Res.* **143**, 109–112.

Mullenders, L. H., Hazekamp-van Dokkum, A. M., Kalle, W. H., Vrieling, H., Zdzienicka, M. Z. and van Zeeland, A. A. (1993) UV-induced photolesions, their repair and mutations. *Mutation Res.* **299**, 271–276.

Petit Frere, C., Clingen, P. H., Arlett, C. F. and Green, M. H. (1996) Inhibition of RNA and DNA synthesis in UV-irradiated normal human fibroblasts is correlated with pyrimidine (6-4) pyrimidone photoproduct formation. *Mutation Res.* **5**, 87–94.

Price, V. H., Odom, R. B., Ward, W. H. and Jones, F. T. (1980) Trichothiodystrophy: Sulphur-deficient brittle hair as a marker for a neuroectodermal symptom complex. *Arch. Dermatol.* **116**, 1375–1384.

Sancar, A. and Sancar, G. B. (1988) DNA repair enzymes. *Annu. Rev. Biochem.* **57**, 29–67.

Suquet, C., Mitchell, D. L. and Smerdon, M. J. (1995) Repair of UV-induced (6-4) photoproducts in nucleosome core DNA. *J. Biol. Chem.* **270**, 16507–16509.

Thomas, D. C., Okumoto, D. S., Sancar, A. and Bohr, V. A. (1989) Preferential repair of (6-4) photoproducts in the dihydrofolate reductase gene of Chinese Hamster ovary cells. *J. Biol. Chem.* **264**, 18005–18010.

Tijsterman, M., de Pril, R., Tasseron-de Jong, J. G. and Brouwer, J. (1999) RNA polymerase II transcription suppresses nucleosomal modulation of UV induced (6-4) photoproduct and cyclobutane pyrimidine dimer repair in yeast. *Mol. Cell. Biol.* **19**, 934–940.

van Hoffen, A., Venema, J., Meschini, R., van Zeeland, A. A. and Mullenders, L. H. (1995) Transcription-coupled repair removes both cyclobutane pyrimidine dimers and 6-4 photoproducts with equal efficiency and in a sequential way from transcribed DNA in xeroderma pigmentosum group C fibroblasts. *EMBO J.* **14**, 360–367.

Venema, J., Mullenders, L. H., Natarajan, A. T., van Zeeland, A. A. and Mayne, L. V. (1990a) The genetic defect in Cockayne syndrome is associated with a defect in repair of UV-induced DNA damage in transcriptionally active DNA. *Proc. Natl. Acad. Sci. USA* **87**, 4707–4711.

Venema, J., van Hoffen, A., Natarajan, A. T., van Zeeland, A. A. and Mullenders, L. H. (1990b) The residual repair capacity of

xeroderma pigmentosum complementation group C fibroblasts is highly specific for transcriptionally active DNA. *Nucleic Acid Res.* **18**, 443–448.

Visse R., de Ruijter, M., Moolenaar, G. F. and van de Putte, P. (1992) Analysis of UVR ABC endonuclease reaction intermediates on cisplatin-damaged DNA using mobility shift gel electrophoresis. *J. Biol. Chem.* **267**, 6736–6742.

Vreeswijk, M. P., Overkamp, M. W., Westland, B. E., van Hees-Stuivenberg, S., Vrieling, H., Zdzienicka, M. Z., van Zeeland, A. A. and Mullenders, L. H. (1998) Enhanced UV-induced mutagenesis in the UV61 cell line, the Chinese hamster homologue of Cockayne's syndrome B, is associated with defective transcription coupled repair of cyclobutane pyrimidine dimers. *Mutation Res.* **409**, 49–56.

Weeda, G., Eveno, E., Donker, I., Vermeulen, W., Chevallier-Lagente, O., Taieb, A., Stary, A., Hoeijmakers, J. H., Mezzina, M. and Sarasin, A. (1997) A mutation in the XPB/ERCC3 DNA repair transcription gene, associated with trichothiodystrophy. *Am. J. Human Genet.* **60**, 320–329.

Will, C. L. and Dolnick, B. J. (1986) Fluorouracil augmentation of dihydrofolate reductase gene transcripts containing intervening sequences in methotrexate-resistance KB cells. *Mol. Pharmacol.* **29**, 643–648.

Yang, I. K., Masters, J. N. and Atardi, G. (1984) Human dihydrofolate reductase gene organization. *J. Mol. Biol.* **176**, 169–187.