Carcinogenesis vol.15 no.8 pp.1493–1498, 1994

ACCELERATED PAPER

Correction by the *ERCC2* gene of UV sensitivity and repair deficiency phenotype in a subset of trichothiodystrophy cells

Mauro Mezzina[5], Eric Eveno, Odile Chevallier-Lagente, Annie Benoit, Madeleine Carreau, Wim Vermeulen[1], Jan H.J.Hoeijmakers[1], Miria Stefanini[2], Alan R.Lehmann[3], Christine A.Weber[4] and Alain Sarasin

Laboratory of Molecular Genetics, UPR42 CNRS IFC H01, BP no. 8, 94801 Villejuif, France, [1]Department of Cell Biology and Genetics, Erasmus University, PO Box 1738, Rotterdam, The Netherlands, [2]Consiglio Nazionale delle Ricerche, Istituto di Genetica Biochimica ed Evoluzionistica, Via Abbiategrasso 207, 27100 Pavia, Italy, [3]MRC Cell Mutation Unit, University of Sussex, Falmer, Brighton BN1 9RR, UK and [4]Biology and Biotechnology Research Program, Lawrence Livermore National Laboratory, PO Box 5507, Livermore, CA 94550, USA

[5]To whom correspondence should be addressed

Trichothiodystrophy (TTD) is a rare genetic disease with heterogeneous clinical features associated with specific deficiencies in nucleotide excision repair. Patients have brittle hair due to a reduced content of cysteine-rich matrix proteins. About 50% of the cases reported in the literature are photosensitive. In these patients an altered cellular response to UV, due to a specific deficiency in nucleotide excision repair, has been observed. The majority of repair-defective TTD patients have been assigned by complementation analysis to group D of xeroderma pigmentosum (XP). Recently, the human excision repair gene *ERCC2* has been shown to correct the UV sensitivity of XP-D fibroblasts. In this work we describe the effect of *ERCC2* on the DNA repair deficient phenotype of XP-D and on two repair-defective TTD cell strains (TTD1VI and TTD2VI) assigned by complementation analysis to group D of XP. *ERCC2* cDNA, cloned into a mammalian expression vector, was introduced into TTD and XP fibroblasts via DNA-mediated transfection or microneedle injection. UV sensitivity and cellular DNA repair properties, including unscheduled DNA synthesis and reactivation of a UV-irradiated plasmid containing the chloramphenicol acetyltransferase reporter gene (pRSVCat), were corrected to wild-type levels in both TTD and XP-D cells. These data show that a functional *ERCC2* gene is sufficient to re-establish a wild-type DNA repair phenotype in TTD1VI and TTD2VI cells, confirming the genetic relationship between TTD and XP-D. Furthermore, our findings suggest that mutations at the *ERCC2* locus are responsible for causing a similar phenotype in TTD and XP-D cells in response to UV irradiation, but produce quite different clinical symptoms.

Introduction

The study of the molecular mechanisms of UV-induced DNA repair in mammalian cells has recently been advanced by the identification and molecular cloning of an increasing number of genes involved in this process. This has been possible thanks to the generation and characterization of rodent mutant cell lines with abnormal responses to DNA damaging agents (1). In addition, human mutants with DNA repair defects are represented by patients suffering from different inherited syndromes, such as xeroderma pigmentosum (XP*), Cockayne's syndrome (CS) and trichothiodystrophy (TTD). These diseases are characterized by a very heterogeneous pattern of clinical symptoms. XP and CS patients present solar-induced skin changes and progressive neurological and ocular degenerations. A large proportion of XP patients develop skin cancer, which is not the case for any of the CS patients described (2). The typical abnormality in TTD is brittle hair, linked to reduced sulphur and cystine contents. Most TTD patients present peculiar faces, ichthyosis, growth and mental retardation. About 50% of all patients described are sun-sensitive, however, like CS, TTD is not associated with skin cancer (3). In all XP, CS and photosensitive TTD patients the common phenotypes are photosensitivity, cellular UV sensitivity and some kind of deficiency in removal of UV-induced photoproducts. Genetic complementation analysis has revealed seven excision-deficient groups for XP, two for CS and three for TTD. Several patients exhibit the pathological manifestations of both XP and CS, three patients are XP-B/CS, one is XP-D/CS and two are XP-G/CS (4). Therefore, two CS groups do not involve XP, while at least three XP groups involve CS. All XP cells are sensitive to the killing effect of UV irradiation and have reduced levels of nucleotide excision repair synthesis [unscheduled DNA synthesis (UDS)], while CS cells with a similar UV sensitivity have normal levels of UDS. This phenotype is a result of a specific defect in repairing UV photoproducts in actively transcribed genes (5). The factor correcting UV survival and defective recovery of RNA synthesis in CS group B has recently been identified as the *ERCC6* gene product (6).

Analysis of the cellular responses to UV in TTD patients indicates that clinical photosensitivity is usually correlated with cellular photosensitivity and cells from patients without sun sensitivity have a normal response to UV and a normal capacity to complement the DNA repair defect in TTD hypersensitive cells. Somatic cell fusion and UDS analysis (7–9) revealed that UV sensitivity in TTD is due to three different genetic defects and that cells from most of the UV-sensitive individuals failed to complement XP-D cells. This complementation data suggested that the DNA repair deficient phenotype of XP-D and TTD results from mutations in the same gene. Generally, the phenotype of these TTD cells is similar to that of XP cells: reduced UV survival, DNA excision repair deficiency and increased mutability (10,11). However, the abnormality in the cellular responses of TTD cells is not homogeneous: UDS levels vary from as low as 10% to as high as 50% of normal. The sensitivity to killing after UV irradiation also varies significantly between different cell lines, some being more sensitive than XP-D cells whereas other lines are UV sensitive only at doses higher than 10 J/m^2 (9).

ERCC2 is the human gene which corrects the UV sensitivity

*Abbreviations: XP, xeroderma pigmentosum; CS, Cockayne's syndrome; TTD, trichothiodystrophy; UDS, unscheduled DNA synthesis; NER, nucleotide excision repair; CAT, chloramphenicol acetyltransferase.

of CHO cells belonging to complementation group 2 (12–13). The purification of the *ERCC2* gene product demonstrated that it has helicase activity (14), as does the product of the homologous yeast *RAD3* gene (52% amino acid identity with *ERCC2*) (15). This activity is an essential function in DNA processing (16). *ERCC2* is tightly associated with the basic transcription factor II (BTFII) complex, (17) which has been shown to contain also the ERCC3 protein (18). This implies that some genes involved in the nucleotide excision repair (NER) pathway are also involved in the transcription machinery. The involvement of *ERCC2* in the XP-D phenotype has been previously shown (19,20). Therefore, it was of primary interest to introduce *ERCC2* into TTD cells to verify the genetic complementation data based on somatic cell fusion. In this work we carried out DNA-mediated transfer experiments of *ERCC2* cDNA into both TTD (TTD1VI and TTD2VI) and XP-D (XP6BESV and XPCS2VI) cells and the resulting phenotypic changes were studied with respect to several DNA repair parameters. Our data show that the wild-type *ERCC2* gene corrects the repair deficient phenotypes of both TTD and XP-D cells, resulting in the complete restoration of wild-type DNA repair properties.

Materials and methods

Cell lines, growth conditions and SV40 transformation

SV40 immortalized XP6BESV fibroblasts (XP-D, GM08207) were obtained from the Human Genetic Mutant Cell Repository (Cadmen, NY). XP12ROSV (XP-A) fibroblasts were obtained from Dr D.Bootsma (Rotterdam). Diploid XP1BR (XP-D) and SV40-transformed MRC5V1 (wild-type) fibroblasts were from Dr C.Arlett (Brighton). The XP-CS2VI fibroblast line corresponds to a new biopsy performed in our laboratory from a XP-D/CS patient. Another cell line from the same patient is known as XP-CS2 (21). This cell line was originally assigned to XP group H, but has been recently shown to belong to XP group D (22). These cells were SV40-transformed in our laboratory (see below) and were designated XP-CS2VILas. XP4PASV (XP-C) was established in our laboratory (23). TTD1VI and TTD2VI were obtained from skin biopsies of unrelated French TTD patients (24,25). All cell lines were cultivated in MEM supplemented with 15% fetal calf serum, antibiotics (penicillin and streptomycin) and fungizone. The two diploid TTD strains were SV40-transformed with plasmid pLASwt harbouring SV40 large T antigen sequences but lacking the SV40 origin of replication. This plasmid has been shown to be an efficient tool for generating immortalized human cell lines (23). Cells were transfected by the standard calcium phosphate precipitation method (see below). About 40 divisions after transfection, cells acquired a transformed phenotype in terms of morphology, increased division rates, efficiency to express exogenous sequences [such as chloramphenicol acetyltransferase (CAT) or β-galactosidase reporter genes] and cloning efficiency. After TTD1VI and TTD2VI had undergone the first crisis, subcloning experiments were performed to select cells with the best cloning efficiency. Viable clones giving rise to stable cell populations could be obtained with TTD2VILas subcloned cells. TTD1VILas was used in transient expression experiments.

Plasmids, DNA-mediated gene transfers and selection for stable transfectants

The expression plasmid p2E-ER2 contains the *ERCC2* cDNA under the control of the SV40 early promoter. The plasmid pcD2E, used as a negative control, was obtained by removal of the 2.4 kb *Eco*RI fragment containing the *ERCC2* cDNA. Other plasmids containing NER genes were: pSLM-XPAC, pBES7-XPCC, pCD1-ERCC3 and pSLM-ERCC6. Calcium phosphate transfections were carried out essentially as previously described (26). Cells (5×10^5) were seeded in 10 cm Petri dishes 1 day before transfection. Plasmid DNA (10–30 μg) solutions were adjusted to 0.125 M $CaCl_2$. One volume (0.250 ml) of solution was mixed with one volume of 2× HBS buffer (25 mM HEPES, 1.5 mM sodium phosphate, 0.28 M NaCl, pH 7.1) and after 30 min at room temperature the precipitated DNA samples were added to cell cultures. After a 6–8 h incubation at 37°C, cells were subjected to 15% glycerol shock for 2 min and then incubated further for 48 h prior to selection for G418 resistance. Subconfluent cells were re-fed with fresh medium containing 500 μg/ml geneticin sulphate (G418, GIBCO-BRL) and, 2–3 weeks later, G418-resistant clones were picked and expanded to generate mass cultures. UV-resistant clones were selected after assessment of viabilility following UV irradiation. Briefly, 5×10^4–10^5 cells were seeded in six-well tissue culture plates and, 24 h later, were irradiated at 5 and 10 J/m^2. Cells were stained 8–10 days later. Clones with increased UV resistance were analysed quantitatively for UV survival (see below). Microneedle injections of p2E-ER2 DNA were carried out in di- or multinucleated fibroblasts obtained after Sendai virus-mediated cell fusion (27), according to the microinjection procedure described previously (28).

Analysis of phenotypic complementation

UV survival. The colony-forming ability was determined after irradiation of an appropriate number of cells seeded in triplicate dishes for each dose to produce at least 50 clones per dish, allowing for plating efficiency. For instance, for UV-sensitive cells, 10^2–3×10^3 cells were seeded for doses from 0 to 6 J/m^2 and 10^4–3×10^5 cells for higher doses (up to 20 J/m^2). For UV-resistant cells, 10^2–3×10^3 cells were seeded for doses from 0 to 20 J/m^2.

Reactivation of UV-irradiated plasmid carrying the chloramphenicol acetyltranferase (CAT) reporter gene. Ten micrograms of pRSVCat plasmid (29) was irradiated at the UV doses indicated in Figure 3. DNA samples were transfected or co-transfected with p2E-ER2 or pcD2E plasmids using the calcium phosphate method described above. CAT activity was measured in transfected cells using a previously described method (30). The ability to reactivate the UV-irradiated plasmid was defined as the ratio of CAT activity in extracts from cells transfected with UV-irradiated to that in cells transfected with non-irradiated pRSVCat plasmid.

Unscheduled DNA synthesis (UDS). Analysis of repair synthesis was carried out in untransformed fibroblasts, essentially as already described (25,27).

Results

The plasmid p2E-ER2 was introduced into TTD cells using the calcium phosphate procedure and the *ERCC2* cDNA was expressed in stable and transient conditions. In order to obtain stable transformants it was necessary to use SV40-immortalized fibroblast lines, owing to the limited lifespan of diploid human fibroblasts in culture. TTD1VI and TTD2VI cells were immortalized as described in Materials and methods and designated TTDIVILas and TTD2VILasCl1. These transformed cells were transfected with p2E-ER2 or pcD2E. The latter, which lacks the *ERCC2* cDNA, was used as a negative control. pcD2E was also used to transfect MRC5V1 cells to obtain G418r wild-type cells. After selection in G418, several hundred G418r clones were obtained with TTD2VILasCl1 cells transfected with p2E-ER2 and 50 G418r viable clones gave rise to stable cell populations, designated TTD2-ER1, TTD2-ER2,..., TTD2-ER50. Several G418r clones which arose from TTD2VILasCl1 or MRC5V1 cells transfected with pcD2E yielded stable cell populations, designated TTD2pcD2E and MRC5pcD2E respectively. TTD2-ER cells were then scored for increased UV resistance as described in Materials and methods. Relative survival for TTD2VILasCl1 or TTD2pcD2E and MRC5V1 or MRC5pcD2E cells are shown in Figure 1. It appeared that SV40 transformation of TTD2VI cells did not affect UV survival, since they had a similar sensitivity (more than 10 times that of normal cells at 9 J/m^2) as their untransformed counterpart (24,25). Similarly, plasmid pcD2E did not affect the survival of wild-type MRC5V1 cells. Analysis of TTD2-ER clones revealed that 35% appeared to have been corrected to normal or near normal levels of UV resistance. For example, TTD2-ER5 fell within the same range as that observed for wild-type MRC5V1 cells. Another 35% of clones retained their UV-sensitive phenotype, as demonstrated by TTD2-ER26 cells (Figure 1A). The remaining clones displayed an intermediate level of survival. The presence of *ERCC2* plasmid sequences integrated into the genome was verified by Southern hybridization, which revealed the presence of multiple copies of the expected 2.4 kb fragment size after digestion of TTD2-ER5 genomic DNA with *Eco*R1 (data not shown). The non-complemented clones, and those displaying intermediate levels of survival, could be explained by the integration of incomplete copies of the *ERCC2* cDNA or by insufficient expression of *ERCC2* to compensate for the effect of mutations in homozygous alleles.

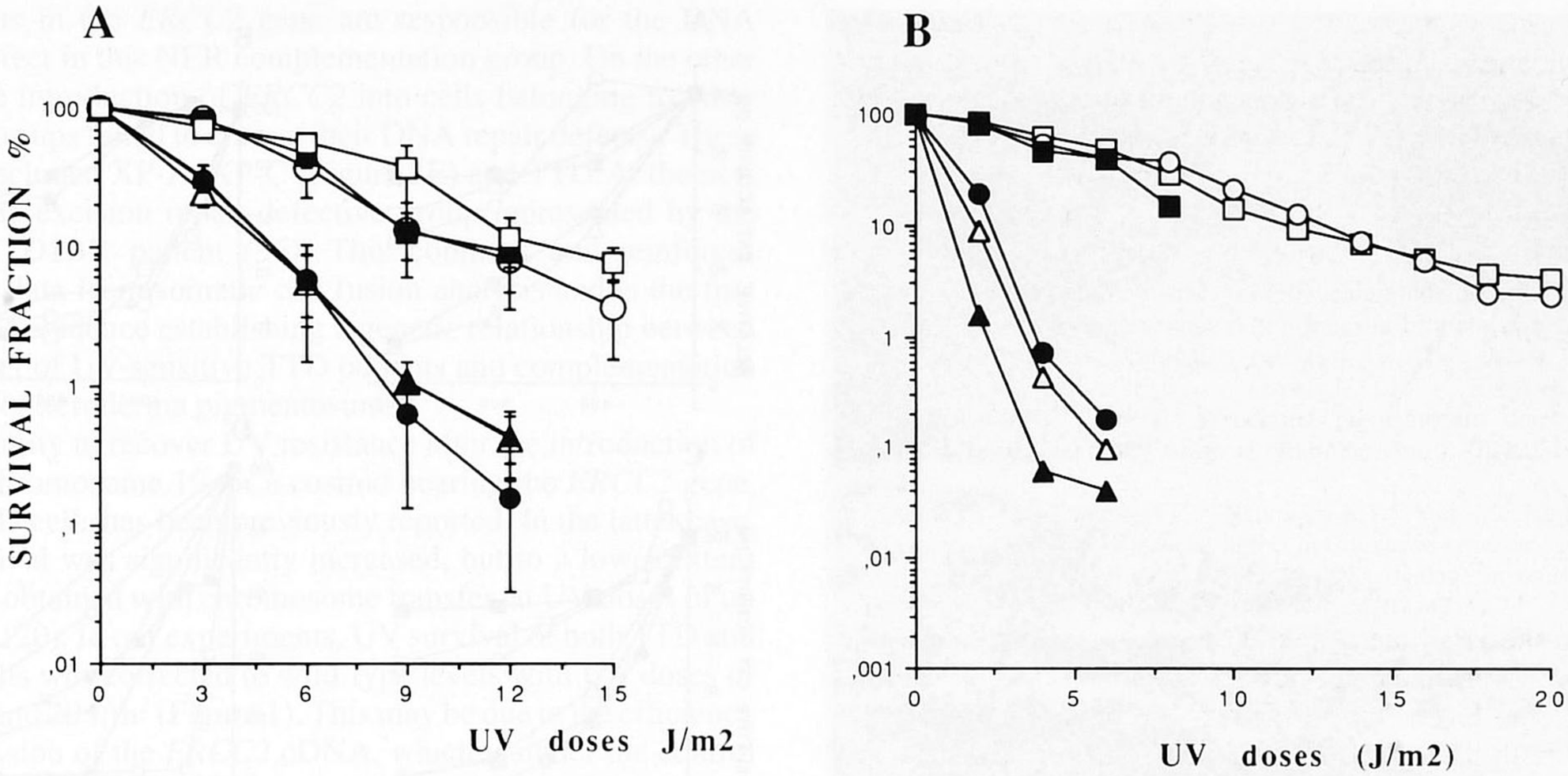
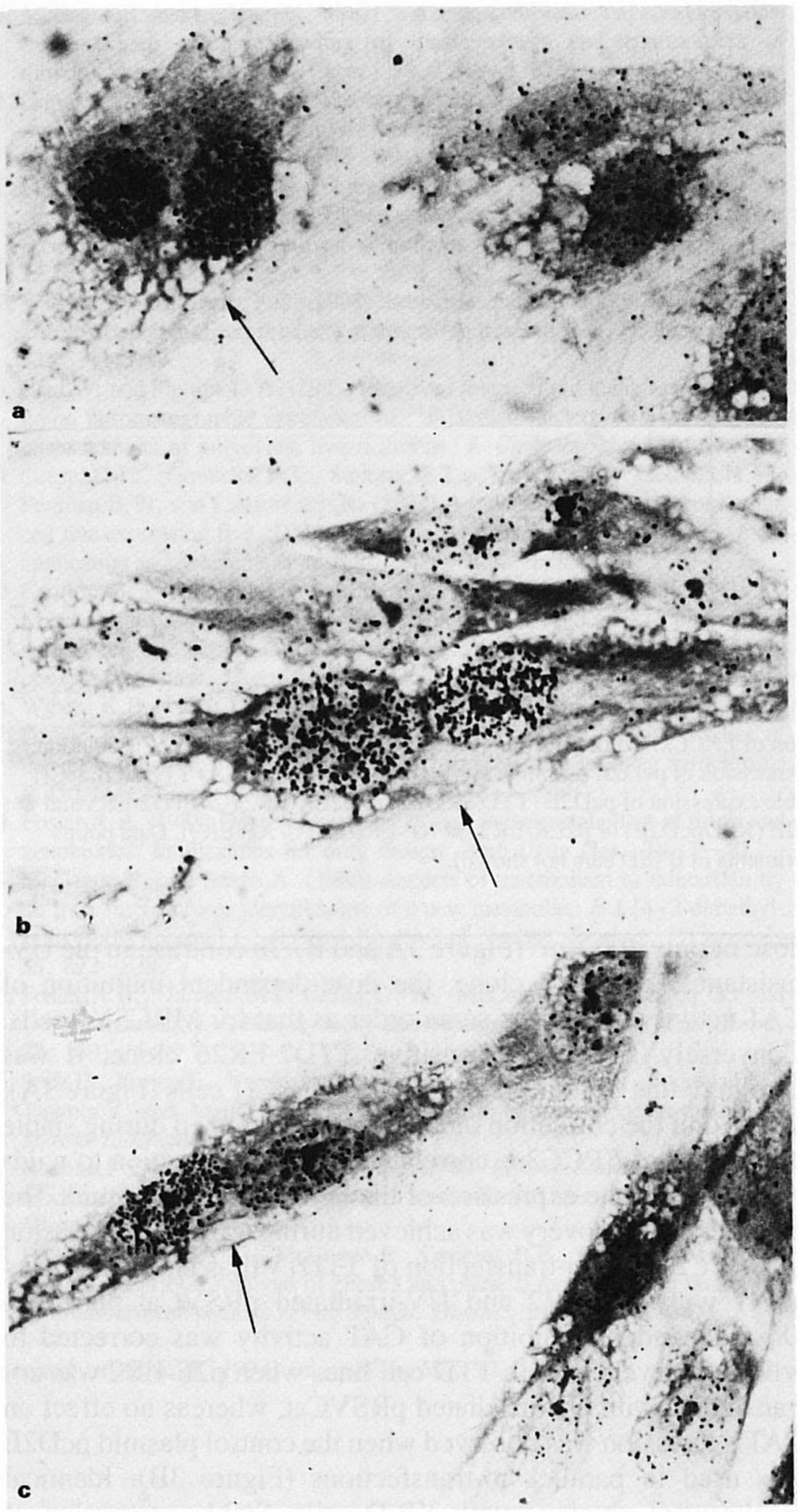


Fig. 1. Correction of UV survival of TTD (**A**) and XP-D (**B**) cells after stable expression of *ERCC2*. Colony-forming ability was determined after UV irradiation with the indicated doses for normal human MRC5V1 cells before (□) or after stable expression of pcD2E plasmid (■ MRC5pcD2E) and for (**A**) TTD2VILasCl1 cells, before (Δ this curve is undistinguishable from that of TTD2pcD2E) and after stable expression of pcD2E (TTD2pcD2E) or p2ER-ER2 (○ TTD2-ER5 and ● TTD2-ER26) and for (**B**) XP6BESV cells before (Δ) or after stable expression of pcD2E (XPDpcD2E) or p2ER-ER2 (● XP-ER8 and ○ XP-ER9). Data points were averaged over six experiments in A (with indicated SD bars) and over three experiments in B (SD bars not shown).

SV40-immortalized XP-D fibroblasts (XP6BESV) were also used in parallel experiments as positive controls for phenotypic complementation with *ERCC2*. Among 52 G418-resistant clones obtained and analysed, 43% were found to be fully corrected for UV survival. For example, XP-ER9 survival was indistinguishable from that of wild-type cells (MRC5V1 or MRC5pcD2E) after exposure to UV doses of up to 20 J/m^2. The survival of a non-complemented clone (XP-ER8) was almost identical to those of XP6BESV or pcD2E-transformed (XP6BEpcD2E) cells (Figure 1B).

The correction of UDS after microinjection of *ERCC2* cDNA into diploid fibroblasts was the second approach used. TTD1VI, TTD2VI and XP1BR cells were microinjected with plasmid p2E-ER2 18 h before irradiation and then processed for UDS as described in Materials and methods. Results showed that UDS was recovered in microinjected multinucleated cells for both TTD (TTD1VI and TTD2VI) and XP1BR cells, compared to uninjected cells (Figure 2A). Quantitative analysis of UDS indicated that the recovery was complete, since the level of UDS observed in the injected cells was similar to that in control cells (Figure 2B). Microinjection of the *XPAC*, *ERCC3* or *ERCC6* repair genes had no effect on the UDS levels of these three cell lines (data not shown).

The ability to reactivate UV-irradiated pRSVCat plasmid DNA was the third approach used to study the correction of DNA repair deficiency by *ERCC2*, in both stable and transient expression assays. This approach is based on the ability of a given cell line to repair lesions present in UV-irradiated plasmid DNA and to restore its ability to express the reporter gene. This technique has been extensively employed for monitoring excision repair of DNA in mammalian cells (31–35). A typical dose-dependent CAT inactivation profile in excision proficient MRC5V1 cells is presented in Figure 3. We observed a 60% reduction in CAT activity at 1200 J/m^2 compared to cells transfected with unirradiated plasmid. Stronger CAT inactivation was observed in both excision-deficient TTD1VILas and TTD2VILasCl1 cells, in which activity was reduced to 30–50% for a UV dose of only 400 J/m^2 (Figure 3A and B). In contrast, in the UV-resistant TTD2-ER5 clone, the dose-dependent inhibition of CAT activity was of the same order as that for MRC5V1 cells. Conversely, in the UV-sensitive TTD2-ER26 clone, it was similar to that of the parental TTD2VILasCl1 cells (Figure 3A). Therefore, the correction of UV survival obtained during stable expression of *ERCC2* is correlated with the correction to wild-type levels of the expression of the UV-irradiated plasmid. The same level of recovery was achieved during transient expression of *ERCC2* after co-transfection of TTD1VILas and TTD2VILasCl1 with p2E-ER2 and UV-irradiated pRSVCat plasmids. Dose-dependent inhibition of CAT activity was corrected to wild-type levels in both TTD cell lines when p2E-ER2 was co-transfected with UV-irradiated pRSVCat, whereas no effect on CAT expression was observed when the control plasmid pcD2E was used in parallel co-transfections (Figure 3B). Identical results were obtained with XP-D cells. Stable expression of *ERCC2* in UV-resistant XP-ER9 cells also promotes full correction of the XP-D deficiency in reactivating UV-damaged plasmids. In contrast, CAT inactivation in UV-sensititve XP-ER8 cells was similar to that of parental XP6BESV cells (Figure 3C). Similar results were obtained after co-transfections with p2E-ER2 or pcD2E and UV-irradiated pRSVCat plasmid into two XP-D cell lines, XP6BESV and XP-CS2VILas. The dose-dependent inhibition of CAT activity in both cells was corrected to near normal levels when p2E-ER2 was used (Figure 3D). Moreover, co-transfections with pcD2E and p2E-ER2 plasmids were also carried out with XP-A (XP12ROSV) and XP-C (XP4PASV) cell lines, as negative controls. In XP-C cells, the dose-dependent inhibition of CAT activity was similar to that observed for XP-D cells. For XP-A cells, CAT activity was inhibited to roughly the same extent for doses below 500 J/m^2 and at a much greater extent than in XP-D cells at doses equal to or higher than 500 J/m^2. With both XP-A and XP-C cells, no effect of the p2E-ER2 plasmid was observed as compared to the control, pcD2E (Figure 3E). Finally, no significant effects on TTD2VILas and XP6BESV cells were observed when other



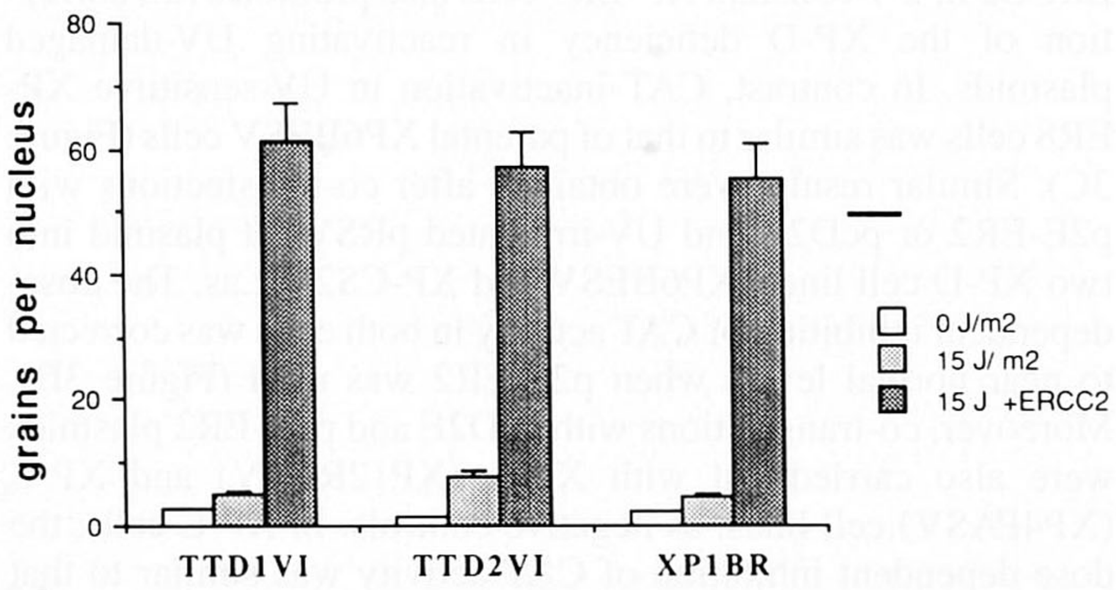


Fig. 2. (**Top**) Correction of UDS levels after microneedle injection of p2E-ER2 plasmid into fused bi- and multinucleated XP1BR (**a**), TTD1VI (**b**) and TTD2VI (**c**) cells. Arrows indicate microinjected heterokaryons. (**Bottom**) Quantification of level of correction. The repair capacity is expressed as mean number of autoradiographic grains per nucleus. Bars indicate standard error of the mean. The bold horizontal line corresponds to the UDS level of normal (AS198) cells at 15 J/m^2.

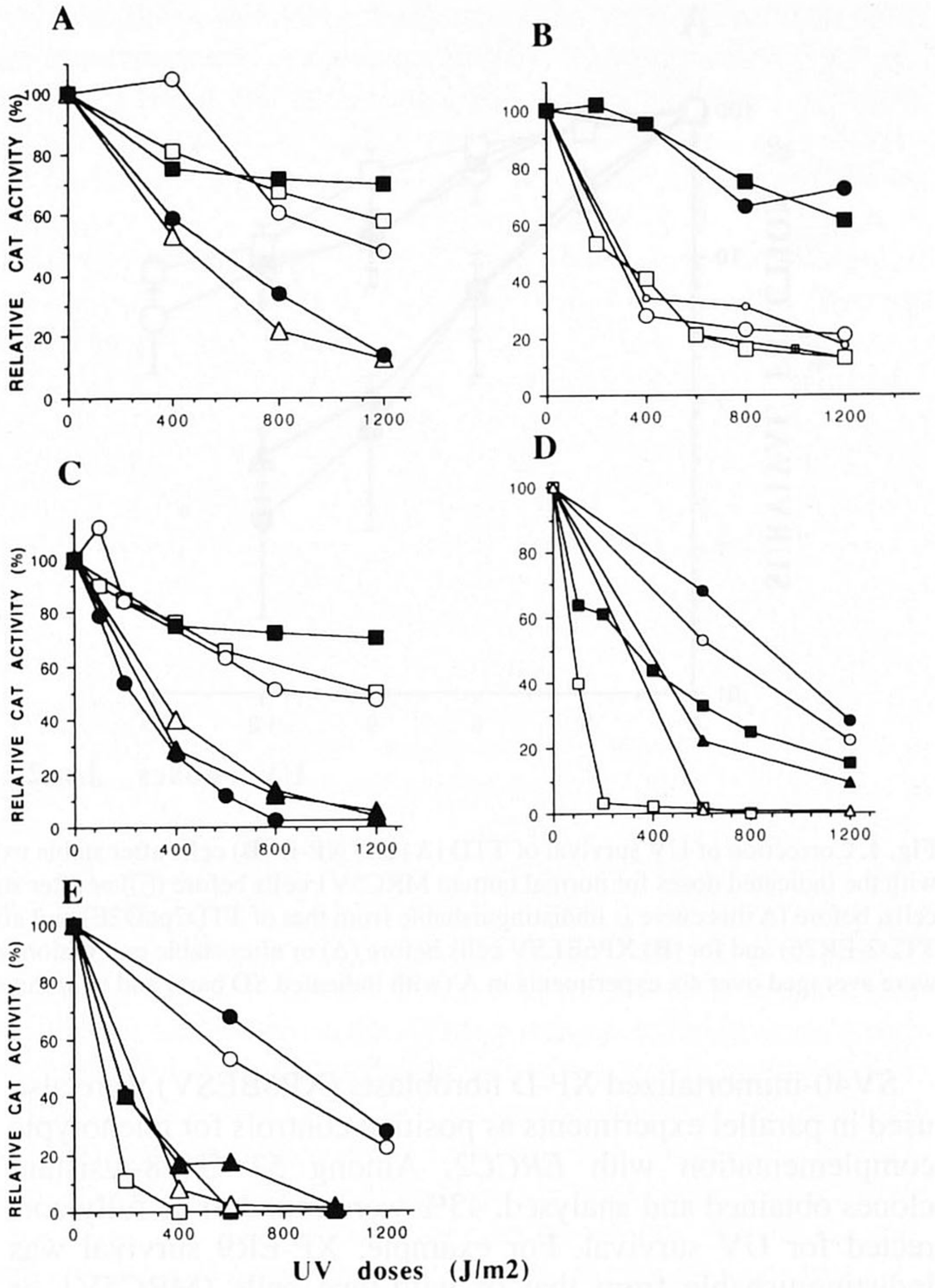


Fig. 3. Reactivation of UV-irradiated pRSVCat transfected into TTD and XP fibroblasts after stable or transient expression of *ERCC2*. UV-irradiated pRSVCat was transfected in cells stably expressing ERCC2 (**A** and **C**) or co-transfected with p2E-ER2 or pcD2E (**B**, **D** and **E**). **A**: MRC5V1 (—□—), MRC5pcD2E (—■—), TTD2VILasC11 (—△—), TTD2-ER5 (—○—) and TTD2-ER26 (—●—). **B**: TTD1VILas alone (—○—), TTD1VILas co-transfected with pcD2E (—○—) or with p2E-ER2 (—●—); TTD2VILas alone (—■—), TTD2VILas co-transfected with pcD2E (—□—) or p2E-ER2 (—■—). **C**: MRC5VI (—□—), MRC5pcD2E (—■—), XP6BESV (—△—), XP6BEpcD2E (—▲—), XP-ER8 (—●—) and XP-ER9 (—○—). **D**: MRC5V1 (—○— and —●—), XP6BESV (—□— and —■—) and XPCS2VILas (—△— and —▲—) cells co-transfected with pcD2E (open symbols) or with p2E-ER2 (closed symbols). **E**: MRC5V1 (—○— and —●—) , XP12ROSV (—□— and —■—) and XP4PASV (—△— and —▲—) cells co-transfected with pcD2E (open symbols) or with p2E-ER2 (closed symbols). CAT activity in cell extracts was expressed as percent of the activity in cells transfected with unirradiated pRSVCat. Data were averaged over four experiments in Figure 3A–D and two experiments for Figure 3E. SD was evaluated as about 15% for each experiment.

repair genes were used in co-transfections, such as *XPAC*, *XPCC*, *ERCC3* or *ERCC6* (data not shown). Therefore, the correction of the repair defect by *ERCC2* is specific for the genetic complementation group of XP-D/TTD cells.

Discussion

The experiments described in this paper demonstrate that the *ERCC2* cDNA can restore a wild-type DNA repair phenotype to TTD and XP-D cells in terms of UV survival (Figure 1), repair synthesis (Figure 2) and reactivation of UV-irradiated plasmids (Figure 3). The use of different techniques to introduce DNA into TTD and XP-D cells (calcium phosphate-mediated transfection and microneedle injections directly into single cells) and different analyses of DNA repair have demonstrated that functional

alterations in the *ERCC2* gene are responsible for the DNA repair defect in this NER complementation group. On the other hand, the introduction of *ERCC2* into cells belonging to other genetic groups failed to correct their DNA repair defects.. These groups included XP-A, XP-C (Figure 3E) and TTD-A, the new nucleotide excision repair-defective group represented by the single TTD1BR patient (36). This confirms and reinforces previous data from somatic cell fusion analysis and is the first molecular evidence establishing a genetic relationship between this subset of UV-sensitive TTD patients and complementation group D of xeroderma pigmentosum.

The ability to recover UV resistance after the introduction of human chromosome 19, or a cosmid bearing the *ERCC2* gene, into XP-D cells has been previously reported. In the latter case, UV survival was significantly increased, but to a lower extent than that obtained with chromosome transfer, at UV doses of up to 4 J/m^2 (20). In our experiments, UV survival of both TTD and XP-D cells was corrected to wild-type levels with UV doses of up to 15 and 20 J/m^2 (Figure 1). This may be due to the efficiency of expression of the *ERCC2* cDNA, which is under the control of the strong SV40 early promoter in the p2E-ER2 plasmid.

In UV survival experiments, TTD cells appeared to be less UV sensitive than XP-D cells: D_0 for TTD cells was 2.5 J/m^2, whereas it was 1 J/m^2 for XP-D cells (Figure 1). Similarly, the defect in reactivating the UV-damaged pRSVCat plasmid appeared to be less dramatic in TTD cells compared to both XP-D lines used (Figure 3). The levels of reduced UDS, however, did not correlate with UV survival in TTD and XP-D cells, since they were similar: 10–40% of normal for TTD cells and 15–40% for XP6BESV and all other XP-D cells (our unpublished results and refs 37,38), indicating the absence of a perfect correlation between cloning efficiency after UV irradiation and DNA repair measurements. This might be due to differing levels of repair efficiency of different UV lesions in genes essential for cell survival.

Therefore, XP-D and TTD/XP-D patients represent two classes of excision repair deficiencies in humans with a molecular defect in the same *ERCC2* gene. Partial sequence analysis of XP6BESV, XP-CS-2 (GM03248), TTD1VI, TTD2VI and TTD3VI has identified mutations in the *ERCC2* gene (C.A.Weber, in preparation). All the mutations result in a single amino acid substitution within previously proposed functional domains of ERCC2, except for a base change creating a new splice donor site in TTD1VI that results in a 45 bp deletion. Similar mutations were also recently identified in four other cell lines from British TTD/XPD patients presenting reduced levels of excision repair (39). The complete sequence analysis in these and other cell lines will permit us to compare the mutations and may allow the identification of classes of mutations responsible either for the XP or the TTD repair-deficient phenotypes.

Acknowledgements

This work was supported by grants from the La Fondation de France Association pour la Recherche sur le Cancer (Villejuif, France), the Ligue Nationale Française contre le Cancer, the Association Française contre les Myopathies (Paris, France) and the Commission of the European Communities, contrat PL 920131 (Brussels, Belgium). Works performed by C.A.W. at Lawrence Livermore National Laboratory and M.S. at the CNR Institute in Pavia were under the auspices of the US Department of Energy under contract W-7405-ENG-48 and the Associazione Italiana per la Ricerca sul Cancro respectively. We are grateful to Drs K.Tanaka (Osaka, Japan), R.Legerski (Houston, TX), G.Weeda and C.Troelstra (Rotterdam, The Netherlands) for providing plasmids containing some DNA repair cDNAs. We are also very grateful to Drs Errol C.Friedberg and Karla Henning (Dallas, TX) for helpful discussions and criticisms of the manuscript.

References

1. Thompson,L.H. (1988) Use of Chinese hamster ovary cell mutants to study human DNA repair genes. In Friedberg,E.C. and Hanawalt,P.C (eds), *DNA Repair. A Laboratory Manual of Research Procedures*. Marcel Dekker, New York, NY, Vol. 3, pp. 115–132.
2. Friedberg,E.C. and Henning,K.A. (1993) The conundrum of xeroderma pigmentosum—a rare disease with frequent complexities. *Mutat. Res.*, **289,** 47–53.
3. Itin,P.H. and Pitthelkow,M.R. (1990) Continuing medical education. Trichothiodystrophy: review of sulfur-deficient brittle hair syndromes and association with the ectodermal dysplasias. *J. Am. Acad. Derm.*, **22,** 705–717.
4. Vermeulen,W., Jaeken,J., Jaspers,N.G.J., Bootsma,D. and Hoeijmakers,J.H.J. (1993) Xeroderma pigmentosum complementation group G associated with Cockayne syndrome. *Am. J. Hum. Gen.*, **53**, 185–192.
5. Venema,J., Mullenders,H.F., Natarajan,A.T. Van Zeeland,A.A. and Mayne,L.V. (1990) 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.
6. Troelstra,C., van Gool,A., de Wit,J., Vermeulen,W., Bootsma,D. and Hoeijmakers,J.H.J. (1992) *ERCC6*, a member of subfamily of putative helicases, is involved in Cockayne's syndrome and preferential repair of active genes. *Cell*, **71,** 939–953.
7. Stefanini,M., Lagomarsini,P., Arlett,C.F., Marinoni,S., Borrone,C., Crovato,F., Trevisan,G., Cordone,G. and Nuzzo,F. (1986) Xeroderma pigmentosum (complementation group D) mutation is present in patients affected by trichothiodystrophy with photosensitivity. *Hum. Genet.*, **74**, 107–112.
8. Stefanini,M., Giliani,S., Nardo,T., Marinoni,S., Nazzaro,V., Rizzo,R. and Trevisan,G. (1992). DNA repair investigations in nine Italian patients affected by trichothiodystrophy. *Mutat. Res.*, **273,** 119–125
9. Stefanini,M., Lagomarsino,P., Gillani,S., Nardo,T., Botta,E., Persico,A., Kleijer,W.J., Lehmann,A.R. and Sarasin,A. (1993) Genetic heterogeneity of the excision repair defect associated with trichothiodystrophy. *Carcinogenesis*, **14**, 1101–1105.
10. Lehmann,A.R., Arlett,C.F.,Broughton,B.C., Harcourt, S.A., Steingrimsdottir,H., Stefanini,M., Taylor,A.M.R., Natarajan,A.T., Green,S., King,M.D., MacKie,R., Stephenson,J.B.P.and Tolmie,J.L.(1988) Trichothiodystrophy, a human DNA repair disorder with heterogeneity in the cellular response to ultraviolet light. *Cancer Res.*, **48,** 6090–6096.
11. Madzak,C., Armier,J., Stary,A., Daya-Grojean,L. and Sarasin,A. (1993) UV-induced mutations in a shuttle vector replicated in repair deficient trichothiodystrophy cells differ with those in genetically-related cancer prone xeroderma pigmentosum. *Carcinogenesis*, **14**, 1255–1260.
12. Weber,C.A., Salazar,E.P., Stewart,S.A. and Thompson,L.H. (1988) Molecular cloning and biological characterization of a human gene, *ERCC2*, that corrects the nucleotide excision repair defect in CHO UV5 cells. *Mol. Cell. Biol.*, **8,** 1137–1146.
13. Weber,C.A., Salazar,E.P., Stewart,S.A. and Thompson,L.H. (1990) *ERCC2*: cDNA cloning and molecular characterization of a human nucleotide excision repair gene with high homology to yeast RAD3. *EMBO J.*, **9,** 1437–1447.
14. Sung,P., Bailly,V., Weber,C., Thompson,L.H., Prakash,L. and Prakash,S. (1993) Human xeroderma pigmentosum group D gene encodes a DNA helicase. *Nature* , **365**, 852–855.
15. Harosh,I., Naumovski,L. and Friedberg,E.C. (1989) Purification and characterization of Rad3 ATPase/DNA helicase from *Saccharomyces cerevisiae. J. Biol. Chem.*, **264,** 20532–20539.
16. Thömmes,P. and Hübscher,H. (1992) Eukaryotic DNA helicases: essential enzymes for DNA transactions. *Chromosoma* , **101,** 467–473.
17. Schaeffer,L., Moncollin,V., Roy,R., Staub,A., Mezzina,M., Sarasin,A., Weeda,G., Hoeijmakers,J.H.J. and Egly, J.M. (1994) The *ERCC2*/DNA repair protein is associated with the class II BTF2/TFIIH transcription factor. *EMBO J.*, **13**, 2388–2392.
18. Schaeffer,L., Roy,R., Humbert,S., Moncollin,V., Vermeulen,W., Hoeijmakers,J.H.J., Chambon,P. and Egly,J.M. (1993) DNA repair helicase: a component of BTF2 (TFIIH) basic transcription factor. *Science*, **260**, 58–63.
19. Lehmann,A.R., Hoeijmakers,J.H.J., van Zeeland, A.A., Backendorf,C.M.P., Bridges,B.A., Collins,A., Fuchs,R.P.P., Margison,G.P., Montesano,R., Moustacchi,E., Natarajan,A.T., Radman,M., Sarasin,A., Seeberg,E., Smith,C.A., Stefanini, M., Thompson,L.H., van der Schans,G.P., Weber,C.A. and Zdzienicka,M.Z. (1992) Workshop on DNA repair. *Mutat. Res.*, **273,** 1–28
20. Flejter,W.L., McDaniel,L.D., JohnsD., Friedberg,E.C. and Schultz, R.A. (1992) Correction of xeroderma pigmentosum complementation group D mutant cell phenotypes by chromosome and gene transfer: involvement of the human ERCC2 DNA repair gene. *Proc. Natl Acad. Sci. USA*, **89,** 261–265.

21. Moshell,A.N., Ganges,M.A., Lutzner,M.A., Coon,H.G., Barant,W., Dupuy,J.-M. and Robbins,J.H. (1983) A new patient with both xeroderma pigmentosum and Cockayne syndrome establishes a new xeroderma pigmentosum complementation group H. In Friedberg,E.C. and Bridges,B.A. (eds), *Cellular Response to DNA Damage*. New York, NY, pp. 209–213.
22. Johnson,R.T., Elliott,G.C., Squires,S. and Joysey,V.C. (1989) Lack of complementation between xeroderma pigmentosum complementation groups D and H. *Hum. Genet.*, **81,** 203–210.
23. Daya-Grosjean,L., James,M.R., Drougard,C. and Sarasin,A. (1987) An immortalized xeroderma pigmentosum, group C, cell line which replicates SV40 shuttle vectors. *Mutat. Res.*, **183**, 185–196.
24. 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) Relationship between pyrimidine dimers, 6–4 photoproducts repair synthesis and cell survival: studies using cells from patients with trichothiodystrophy *Mutat. Res.*, **235**, 33–40.
25. Sarasin,A., Blanchet-Bardon,C., Renault,G., Lehmann,A., Arlett,C. and Dumez,Y. (1992) Prenatal diagnosis in a subset of trichothiodystrophy patients defective in DNA repair. *Br. J. Derm.*, **5**, 485–481.
26. Graham,F.L. and Van der Eb,A.J. (1973) A new technique for the assay of infectivity of human adenovirus 5 DNA. *Virology*, **52,** 456–467.
27. Vermeulen,W., Osseweijer,P., de Jonge,A.J.R. and Hoeijmakers,J.H.J. (1986) Transient correction of excision repair defects in fibroblasts of 9 xeroderma pigmentosum complementation groups by microinjection of crude human cell extracts. *Mutat. Res.*, **165**, 199–206.
28. Hoeijmakers,J.H.J. (1988) Use of microneedle injection to study DNA repair in mammalian cells DNA repair. In Friedberg,E.C. and Hanawalt,P.C. (eds), *A Laboratory Manual of Research Procedures.* Marcel Dekker, New York, NY, Vol. 3, pp. 133–150.
29. Gorman,C.M., Merlino,G.T., Willingham,M.C., Pastan,I. and Howard,B.H. (1982) The Rous sarcoma virus long terminal repeat is a strong promoter when introduced into a variety of eukaryotic cells by DNA-mediated transfection. *Proc. Natl Acad. Sci. USA*, **79,** 6777–6781.
30. Sleigh,M.J. (1986) A nonchromatographic assay for expression of the chloramphenicol acetyltransferase gene in eucaryotic cells. *Anal. Biochem.*, **156,** 251–256.
31. Protic-Sabljic, M. and Kraemer, K.H. (1985) One pyrimidine dimer inactivates expression of a transfected gene in xeroderma pigmentosum cells. *Proc. Natl Acad. Sci. USA*, **82,** 6622–6626.
32. Lehmann,A.R. and Oomen,A. (1985) Effect of DNA damage on the expression of the chloramphenicol acetyltransferase gene after transfection into diploid human fibroblast. *Nucleic Acids Res.*, **13**, 2087–2095.
33. Klocker,H., Schneider,R., Burtscher,H.J., Auer, B., Hirsch-Kauffmann,M. and Schweiger,M. (1985) Transient expression of a plasmid gene, a tool to study DNA repair in human cells: defect of DNA repair in Cockayne syndrome; one thymine cyclobutane dimer is sufficient to block transcription. *Eur. J. Cell Biol.*, **39,** 346–351.
34. Lambert,C., Couto,L.B., Weiss,W.A., Schultz,R.A., Thompson,L.H. and Friedberg,E.C. (1988) A yeast DNA repair gene partially complements defective excision repair in mammalian cells. *EMBO J.*, **10,** 3245–3253.
35. Athas,W.F., Hedayati,M.A., Matanoski,G.M., Farmer,E.R. and Grossman,L. (1991) Development and field-test validation of an assay for DNA repair in circulating human lymphocytes. *Cancer Res.*, **51,** 5786–5793.
36. Stefanini,M., Vermeulen,W., Giliani,S., Nardo,T., Mezzina,M., Sarasin,A., Harper,J.I., Arlett,C.F., Hoeijmakers,J.H.J. and Lehmann,A.R. (1993) A new nucleotide-excision-repair gene associated with the disorder trichothiodystrophy. *Am. J. Hum. Genet.* , **53**, 817–821.
37. Stefanini,M., Lagomarsini,P. and Nuzzo,F. (1989) Genetic analysis in trichothiodystrophy repair deficient cells confirms the occurrence of xeroderma pigmentosum group D mutation in unrelated patients. In Lambert,M.W. and Laval,J. (eds), *DNA Repair Mechanisms and Their Biological Implication in Mammalian Cells.* Plenum Press, New York, NY, pp. 523–533
38. Johnson,R.T. and Squires,S. (1992) The XPD complementation group. Insights into xeroderma pigmentosum, Cockayne's syndrome and trichothiodystrophy. *Mutat. Res.*, **273,** 97–118.
39. Broughton,B.C., Steingrimsdottir,H., Weber,C.A. and Lehmann,A.R. (1994) Mutations in xeroderma pigmentosum group D DNA repair gene in patients with trichothiodystrophy. *Nature Genet.*, **7**, 189–194.

Received on April 19, 1994; accepted on May 10, 1994