DNA Repair and Ultraviolet Mutagenesis in Cells From a New Patient With Xeroderma Pigmentosum Group G and Cockayne Syndrome Resemble Xeroderma Pigmentosum Cells

Shin-Ichi Moriwaki, Miria Stefanini,† Alan R. Lehmann,‡ Jan H.J. Hoeijmakers,§ Jay H. Robbins,* Isabelle Rapin,¶ Elena Botta,† Bianca Tanganelli,† Wim Vermeulen,§ Bernard C. Broughton,‡ and Kenneth H. Kraemer

Laboratory of Molecular Carcinogenesis and *Dermatology Branch, National Cancer Institute, Bethesda, Maryland, U.S.A.; †Istituto di Genetica Biochimicaed Evoluzionistica, Consiglio Nazionale delle Ricerche, Pavia, Italy; ‡MRC Cell Mutation Unit, University of Sussex, Falmer, Brighton, England; §Department of Cell Biology and Genetics, Medical Genetics Center, Erasmus University, Rotterdam, The Netherlands; and ¶Department of Neurology, Albert Einstein College of Medicine, Bronx, New York, U.S.A.

Xeroderma pigmentosum (XP)/Cockayne syndrome (CS) complex is a combination of clinical features of two rare genetic disorders in one individual. A sun-sensitive boy (XP20BE) who had severe symptoms of CS, with dwarfism, microcephaly, retinal degeneration, and mental impairment, had XP-type pigmentation and died at 6 y with marked cachexia (weight 14.5 lb) without skin cancers. We evaluated his cultured cells for characteristic CS or XP DNA-repair abnormalities. The level of ultraviolet (UV)-induced unscheduled DNA synthesis was less than 5% of normal, characteristic of the excision-repair defect of XP. Cell fusion studies indicated that his cells were in XP complementation group G. His cells were hypersensitive to killing by UV, and their post-UV recovery of RNA synthesis was abnormally low, features of both CS and XP. Post-UV survival of plasmid pSP189 in his cells was markedly reduced, and post-UV plasmid mutation frequency was higher than with normal cells, as in both CS and XP. Sequence analysis of the mutated plasmid marker gene showed normal frequency of plasmids with multiple base substitutions, as in CS, and an abnormally increased frequency of G:C → A:T mutations, a feature of XP. Transfection of UV-treated pRSVcat with or without photoreactivation revealed that his cells, like XP cells, could not repair either cyclobutane pyrimidine dimers or nondimer photoproducts. These results indicate that the DNA-repair features of the XP20BE (XP-G/CS) cells are phenotypically more like XP cells than CS cells, whereas clinically the CS phenotype is more prominent than XP. ***Key words: shuttle vector plasmid/skin cancer/transfection/photoproducts. J Invest Dermatol 107: 647–653, 1996***

Xeroderma pigmentosum (XP) is a rare autosomal recessive disease characterized clinically by hypersensitivity to sunlight, abnormal pigmentation, neurologic abnormalities (in about 20% of the patients; Kraemer *et al*, 1987), and predisposition to skin cancers, especially on sun-exposed areas (Kraemer, 1993; Cleaver and Kraemer, 1995). Cultured cells derived from XP patients have a reduced DNA-repair capacity and are hypersensitive to killing by ultraviolet light (UV). Cells from patients with XP repair neither pyrimidine dimers nor nondimer photoproducts in DNA after UV exposure (Protić-Sabljić and Kraemer, 1985, 1986). Cell fusion analysis has identified seven complementation groups (A through G) of excision-repair–deficient cells, and there is also a "variant" form that is proficient in excision repair (Kraemer, 1993; Cleaver and Kraemer, 1995). XP group G is one of the rarest forms of XP, and only seven other patients have been reported (Cheesbrough and Kinmont, 1978; Keijzer *et al*, 1979; Arlett *et al*, 1980; Ichihashi *et al*, 1985; Norris *et al*, 1987; Jaeken *et al*, 1989; Vermeulen *et al*, 1993). The human gene responsible for XP group G was recently identified as *ERCC5* (Mudgett and MacInnes, 1990; O'Donovan and Wood, 1993; Nouspikel and Clarkson, 1994), whose encoded product functions as a structure-specific DNA endonuclease (O'Donovan and Wood, 1993).

Cockayne syndrome (CS) is another rare autosomal recessive disease causing acute sun sensitivity that has characteristic progressive neurologic symptoms in addition to short stature, microcephaly, pigmentary retinal degeneration, progressive sensorineural hearing loss, and calcification of basal ganglia. Although CS patients may have severe sunburn on minimal exposure, they do not develop the characteristic pigmentary abnormalities and skin cancers seen in XP (Cantani *et al*, 1987; Nance and Berry, 1992;

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Reprint requests to: Dr. Kenneth H. Kraemer, Laboratory of Molecular Carcinogenesis, National Cancer Institute, Building 37, Room 3E24, Bethesda, MD 20892.

Abbreviations: CS, Cockayne syndrome; XP, xeroderma pigmentosum; UDS, unscheduled DNA synthesis.

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Kraemer, 1993; Cleaver and Kraemer, 1995). Cultured cells from CS patients have XP-type hypersensitivity to killing by UV (Andrews *et al*, 1978; Marshall *et al*, 1980). Unlike XP, however, the level of DNA repair in CS cells measured by UV-induced unscheduled DNA synthesis (UDS) is normal. CS cells have defective repair of actively transcribing genes, whereas repair of total genomic DNA is normal (Venema *et al*, 1990). There are two CS complementation groups (CS-A and CS-B) (Lehmann, 1982; Stefanini *et al*, 1996), and the corresponding genes have been cloned (Troelstra *et al*, 1992; Henning *et al*, 1995). Recently, Barrett *et al* (1991) and Parris and Kraemer (1993) used shuttle vector plasmids to demonstrate that CS cells are deficient in repair of cyclobutane dimers but are proficient in repair of nondimer photoproducts.

XP/CS complex has been proposed as a clinical entity with features of the two rare genetic diseases, XP and CS (Robbins *et al*, 1974; Robbins, 1988). Patients show the neurologic abnormalities of CS plus the skin abnormalities of XP, including skin cancers. Cells from patients with the XP/CS complex are hypersensitive to killing by UV, but unlike the usual CS cells, have reduced UV-induced UDS (Kraemer, 1993; Cleaver and Kraemer, 1995). This study provides information about XP20BE, the eighth patient with the XP/CS complex and the third patient with this complex in XP complementation group G. The DNA-repair characteristics of the XP20BE (XP-G/CS) cells are more like XP than CS, whereas clinically the CS phenotype is more prominent than that of XP.

MATERIALS AND METHODS

Cells Lymphoblastoid cell lines were established by Epstein-Barr virus transformation of peripheral blood lymphocytes from the new patient with XP/CS (XP20BE = AG08802), a patient with XP group A (XP12BE = GM02250) (Robbins *et al*, 1974), and a normal individual (GM606). All of the cells were transformed at the Institute of Medical Research (Camden, NJ) and were cultured in RPMI 1640 medium (GIBCO BRL, Bethesda, MD), supplemented with 15% fetal bovine serum (S&S Media, Rockville, MD) and 2 mM L-glutamine (Gibco BRL), at 37°C in a 5% CO_2 atmosphere, as described previously (Moriwaki *et al*, 1994).

Primary fibroblast cultures were established at the Institute of Medical Research from skin biopsy specimens of the new patient with XP/CS (XP20BE = AG08803), his mother (AG8805), and his father (AG8807); patients with XP group A (XP12BE = GM05509) (Robbins *et al*, 1974), group C (XP21BE = GM09943), and group D (XPLA-BE = KR5959) (Kraemer *et al*, 1989); and four normal subjects (GM1652, GM2987, GM3377, and GM3651). Additional fibroblast strains used were XP11PV and XP25RO (XP group A), XP11BE = XP/CS-1 (XP group B) (Robbins *et al*, 1974), XP13PV and XP21RO (XP group C), XP3NE and XP/CS-2 = GM03248 (XP group D), XP2RO = GM02415 (XP group E), XP2YO = GM04313 (XP group F), XP2BI = GM03021 (XP group G), CS4BR and CS1PV (CS group B) (Stefanini *et al*, 1996), and C5RO, C3PV, and 1BR (normals). Cells were maintained in Dulbecco's modified minimum essential medium (GIBCO BRL) with 15% fetal bovine serum (S&S Media) and 2 mM L-glutamine (GIBCO BRL).

Post-UV Cell Survival, Measurement of UV-Induced UDS, Genetic Complementation Testing, and RNA Synthesis Inhibition UV treatment was performed with a germicidal lamp, and cell survival was measured in nonproliferating fibroblasts as described previously (Stefanini *et al*, 1992, 1993). Autoradiographic measurement of post-UV UDS and complementation group determination were performed as described previously (Vermeulen *et al*, 1991; Stefanini *et al*, 1992). Briefly, primary fibroblasts were washed with phosphate-buffered saline before UV irradiation, followed by incubation in a medium containing 10 μCi methyl [^{3}H]thymidine per ml for 3 h. After labeling, the cells were fixed with Bouin solution. The slides were dipped in nuclear track emulsion for autoradiography. The number of grains per interphase nucleus was scored for 100 nuclei in each sample.

Genetic complementation tests were performed by assessment of UV-induced UDS (10–30 J/m^2) of the patient's fibroblasts fused with representative XP strains of complementation groups A, B, C, D, E, F, and G (Vermeulen *et al*, 1991). Each cell line to be fused was treated with a different size of microscopic beads, which were incorporated into the cytoplasm as a marker. Forty-eight hours after fusion by polyethylene glycol or by β-propiolactone–inactivated Sendai virus, post-UV UDS was measured. The number of grains in 10–60 nuclei of heterokaryon cells containing both sizes of microscopic beads was scored.

Post-UV inhibition of RNA synthesis was performed as described previously (Lehmann *et al*, 1993). Fibroblasts were exposed to UV, and [^{3}H]uridine incorporation was measured after 24 h incubation.

Plasmid UV Treatment, Transfection, Chloramphenicol Acetyl Transferase (CAT), and Mutation Assay Assessment of post-UV DNA repair and mutagenesis by plasmid host cell reactivation was performed as described previously (Protić-Sabljić and Kraemer, 1985; Moriwaki *et al*, 1994). The pRSVcat and pSP189 plasmids were diluted to 31 μg per ml in sterile distilled water and were irradiated on ice using a germicidal lamp at a rate of 1.5 J/m^2/s, measured with an International Light (Newburyport, MA) IL770A Research Radiometer. Transfection was performed by use of diethylaminoethyl dextran (Pharmacia Fine Chemicals, Uppsala, Sweden).

For the mutagenesis assay, 3×10^7 lymphoblastoid cells were transfected with UV-treated or untreated pSP189. At the same time, nonirradiated pZ189K was co-transfected as an internal standard. Two days after the transfection, replicated plasmids were recovered from the human cells by alkaline lysis and used to transform an indicator strain of *Escherichia coli* by electroporation. The ratio of the total number of ampicillin-resistant colonies to the total number of kanamycin-resistant colonies reflects plasmid survival. The proportion of white or light blue colonies (representing inactivating mutations in the *supF* gene) to the total number of colonies is a measure of the mutation frequency. Mutant white or light blue colonies were subjected to sequence analysis using the SequiTherm Cycle Sequencing Kit (Epicenter Technologies, Madison, WI).

For the CAT assay, 3×10^7 lymphoblastoid cells were transfected with UV-treated or untreated pRSVcat, and CAT assays were performed according to the scintillation cocktail extraction procedure (Gözükara *et al*, 1994). Enzyme activity was determined after 1–6 h incubation and expressed as pmol/min/mg protein. Protein determination was performed with the Bio-Rad protein assay kit (Bio-Rad Laboratories, Hercules, CA).

The *E. coli* enzyme photolyase, a gift from A. Sancar (University of North Carolina), was used for dimer removal from UV-irradiated pRSVcat plasmid. UV-treated and untreated pRSVcat was mixed with photolyase and treated with 405 nm monochromatic visible light for 1 h, as described previously (Protić-Sabljić and Kraemer, 1985, 1986; Parris and Kraemer, 1993). The efficiency of dimer removal from pRSVcat by photoreactivation was determined by digestion of the DNA with T4 endonuclease V (a gift from S. Lloyd, Galveston, TX), which cleaves the plasmid at sites containing dimers. Plasmid DNA with or without photoreactivation was analyzed by agarose gel electrophoresis, and the proportion of form I (supercoiled monomer plasmid) was determined by a laser densitometer (Molecular Dynamics, Sunnyvale, CA). We found that T4 endonuclease V digestion of 250 J/m^2 UV-treated pSRVcat plus photoreactivation resulted in the retention of 88% of form I molecules, compared with 9% of form I molecules in the T4 endonuclease V–digested, UV-treated plasmid without photoreactivation. These results indicate that the photolyase treatment removed about 95% of the cyclobutane pyrimidine dimers, calculated as described (Protić-Sabljić and Kraemer, 1985, 1986).

Statistical Analysis Statistical comparisons of differences between specific mutations were performed by Fisher exact test, and 1-tailed p values are shown. For the studies of cell survival, plasmid survival, and plasmid mutation frequency, means were compared with Student t test and curves were plotted using the SlideWrite Plus computer program (Advanced Graphics, Carlsbad, CA).

RESULTS

Case Report The 6-y-old boy (XP20BE) was referred to the National Institutes of Health at age 18 mo because of marked sun sensitivity and developmental delay. He was born in December 1984 after a normal pregnancy, weighed 6 lb 10 oz, and had a normal Apgar score. His parents were clinically normal and unrelated. They reported that in the spring, minimal exposure of the patient to sunlight would be followed on the next day by progressive redness and swelling, especially of his face. The reaction became severe and persisted for about 1 wk. He had about nine separate episodes of severe sun reaction. The parents began protecting him with sunscreens and long-sleeved clothes at all times.

At age 5 mo, he weighed 16 lb and could roll over. His motor skills then ceased to develop. At 1 y of age, he had microcephaly and failure to thrive and was diagnosed as having CS.

He reached his maximum weight of 19 lb at age 18 mo. His skin showed multiple hyperpigmented 1–2–mm macules of varying color on sun-exposed surfaces, including his lips. These freckles were interspersed with hypopigmented macules and were associated with mild atrophy and telangiectasia **(Fig 1,** *left*). There were

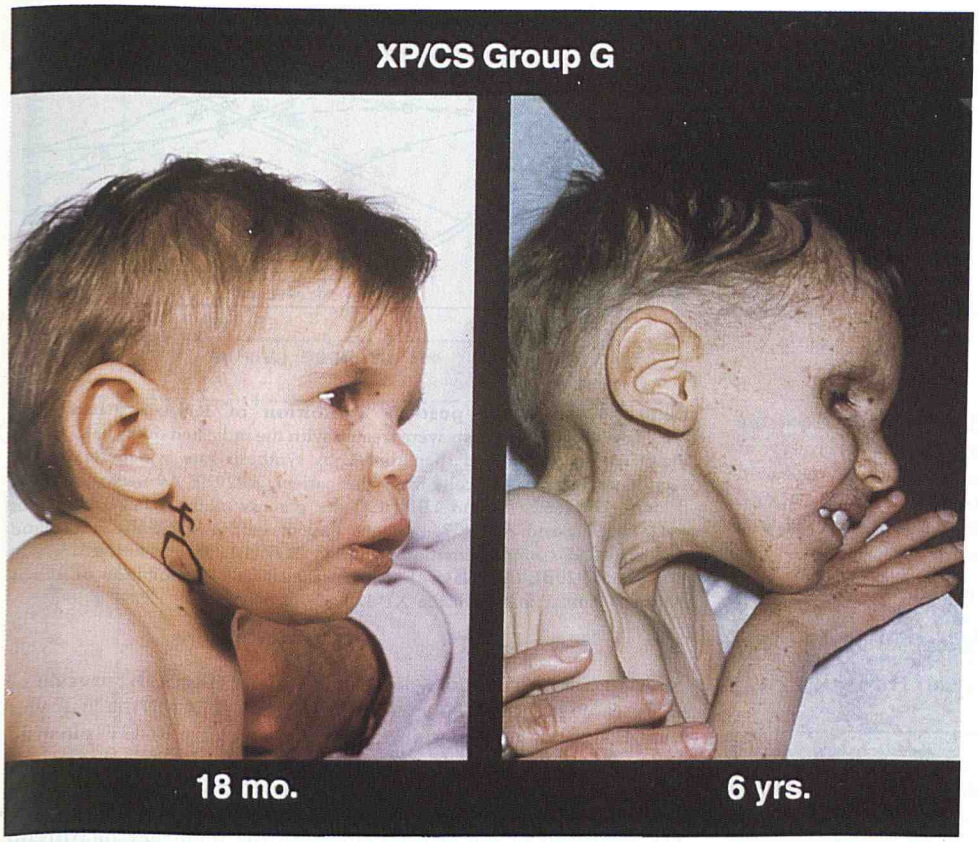

Figure 1. Patient XP20BE, with features of CS and XP. *Left*, Age 18 mo, showing interspersed areas of XP-like increased and decreased pigmentation on the cheek. *Right*, Age 6 y, showing typical CS cachexia, deep-set eyes, loss of subcutaneous tissue, and XP-like pigmentation of the face and arm.

no actinic keratoses or skin cancers. He was diagnosed as having the clinical features of XP. Skin biopsies and blood cultures were done for DNA-repair tests.

Neurologic examination showed microcephaly with markedly delayed motor and language skills. He had pigmentary degeneration of the retina and moderate to severe sensorineural hearing loss. Magnetic resonance imaging showed cerebellar hypoplasia and enlarged ventricles. Electrophysiologic studies showed slowed conduction and diffuse denervation.

He experienced progressive cachexia, weighing only 14.5 lb at age 6 y **(Fig 1,** *right***)**. He died of pneumonia in February 1991. Autopsy of the nervous system showed demyelination of the white matter, calcification of the brain, cerebellar degeneration, and a demyelinating peripheral neuropathy. These autopsy findings are typical of CS. A detailed report will be presented elsewhere.

Reduced Post-UV Survival Post-UV survival of nonproliferating XP20BE fibroblasts was compared with that of fibroblasts from patients in XP complementation groups A, C, and D; from one CS patient; and from one normal donor **(Fig 2)**. The post-UV survival of the XP20BE cells was extremely low and similar to that from cells in XP complementation group A.

Reduced UDS and Complementation Group G Assignment The level of post-UV UDS in XP20BE cells was compared with that of cells from two normal subjects, his parents, and from patients in XP complementation groups A, B, and C **(Table I)**. The level of UV-induced UDS in the XP20BE cells was less than 5% of normal, characteristic of a severe form of the nucleotide excision-repair defect of XP. The post-UV UDS of his parents' cells was normal, as is usually seen in obligate XP heterozygotes.

XP20BE fibroblasts were fused pairwise with representative fibroblasts from XP groups A, C, D, E, F, and G in Pavia **(Fig 3*A*)** and with fibroblasts from XP groups A, B, C, and G in Rotterdam **(Fig 3*B*)**. Binuclear heterokaryons showed normal or near-normal levels of post-UV UDS when the XP20BE cells were fused with cells from XP groups A, B, C, D, E, and F. In contrast, binuclear heterokaryons of XP20BE cells resulting from fusion with the XP-G cells showed no increase in UDS, indicating assignment of the XP20BE cells to XP complementation group G.

Reduced Post-UV RNA Synthesis Post-UV inhibition of RNA synthesis was assessed in the XP20BE cells in comparison with cells from his parents, from XP-C and XP-D patients, from a patient with CS, and from two normal donors **(Fig 4)**. CS cells typically show dose-related delayed recovery of RNA synthesis (Mayne and Lehmann, 1982; Lehmann *et al*, 1993). There was a

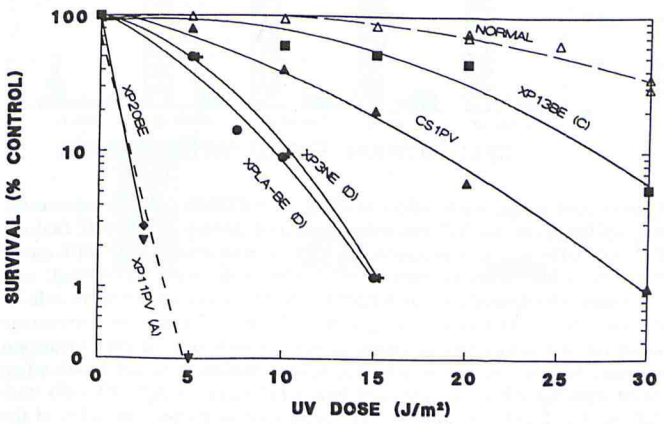

Figure 2. Reduced post-UV survival of XP20BE cells. Nonproliferating fibroblasts from the patient XP20BE (◆); patients in XP complementation groups A (XP11PV, ▼), C (XP13BE, ■), and D (XP3NE, +); XPLA-BE (●); CS complementation group B (CS1PV, ▲); and normal subject (C3PV, △) were exposed to different doses of UV, and cell survival was determined (see *Materials and Methods*). Data points for XP3NE are displaced for better visibility.

Table I. Markedly Reduced Post-UV Unscheduled DNA Synthesis in XP20BE Fibroblasts

Cells	Grains/Nucleus[a]	Normal
Normal (GM1652)	56 ± 2	100%
Normal (C5RO)	57 ± 2	102%
Mother (AG8805)	58 ± 1	105%
Father (AG8807)	55 ± 2	98%
XP20BE	1 ± 0.3	1.7%
XP-A (XP25RO)	1 ± 0.3	1.7%
XP-B (XP11BE)	3 ± 0.5	5%
XP-C (XP21BE)	16 ± 1	29%

[a] Mean ± SEM; UV dose = 16 J/m^2.

greater reduction in post-UV RNA synthesis in cells from the XP-D patient. The XP-C cell line showed normal recovery of post-UV RNA synthesis. The XP20BE cells showed reduced post-UV RNA synthesis to a similar extent as in the XP-D cells. The cells from the clinically normal obligate heterozygotes showed normal post-UV inhibition of RNA synthesis.

Increased Post-UV Plasmid Mutagenesis UV-treated pSP189 passed through normal, XP20BE, and XP-A lymphoblastoid cells showed a decrease in the relative number of ampicillin-resistant colonies in a dose-dependent manner **(Fig 5*A*)**. Survival of UV-treated pSP189 recovered from XP20BE cells was much lower than that from normal cells. The reduction of post-UV plasmid survival in XP20BE cells was similar to that in the XP-A cells.

Post-UV pSP189 mutation frequency was measured in the XP20BE, normal, and XP-A lymphoblastoid cell lines **(Fig 5*B*)**. UV treatment of the plasmid enhanced the frequency of mutant colonies recovered, in a dose-dependent manner, with all of the cells. The mutation frequency of the UV-treated plasmid recovered from the XP20BE cells was significantly greater than normal at 250 J/m^2 and 500 J/m^2. The XP-A cells showed greater plasmid hypermutability than the XP20BE cells. The frequency of spontaneous mutations in pSP189 was similar in XP20BE, normal, and XP-A cells (data not shown).

DNA sequence analysis was performed on 69 independent post-UV mutant plasmids recovered from the normal cells and on 80 post-UV mutant plasmids recovered from the XP20BE cells. The classes of mutations in the *supF* marker gene in UV-treated pSP189 are shown in **Table II**. With both cell lines, most mutant plasmids contained point mutations (83% and 79%, respectively), with very few frameshift mutations (one large deletion and one single-base insertion along with a base-substitution mutation with the normal line). Plasmids with tandem mutations (two base substitutions zero to two bases apart or three adjacent base substitutions), a feature of UV mutagenesis, were recovered from 9% of the mutants with both cell lines. Multiple base-substitution mutations (two substitutions three or more bases apart or three or more nonadjacent base substitutions in the approximately 150-bp marker gene) were found in plasmids recovered from both cell lines. As with CS cells (Parris and Kraemer, 1993), there were no significant differences in the frequencies of classes of mutations with both cell lines.

Analysis of the types of single and tandem base-substitution mutations showed a significant increase in G:C → A:T transition mutations in the plasmids recovered from the XP20BE cells **(Table III)**. Similar results were seen at all UV doses. This predominance of G:C → A:T transitions in post-UV plasmid mutagenesis is typical of XP cells (Cleaver and Kraemer, 1995) but is not seen with CS cells (Muriel *et al*, 1991; Parris and Kraemer, 1993).

Reduced Repair of All UV Photoproducts UV-treated pRSVcat plasmid with or without photoreactivation was used to measure the DNA-repair ability of normal, XP20BE, and XP-A lymphoblastoid cells **(Fig 6)**. This assay reflects the ability of the cells to repair the damaged plasmid DNA by assessing the recovery of transcriptional activity, measured indirectly as enzyme activity of

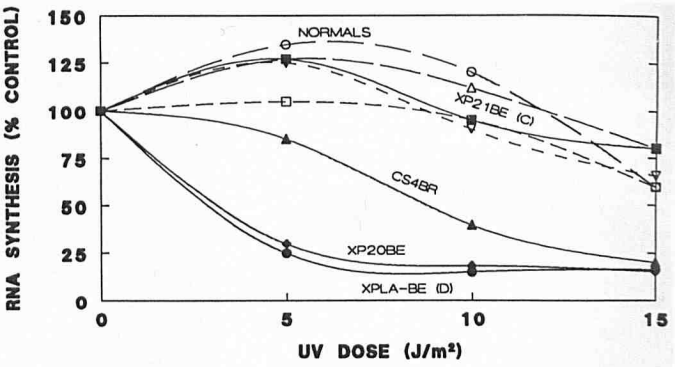

Figure 4. Abnormal post-UV inhibition of RNA synthesis in XP20BE cells. Fibroblasts were treated with the indicated doses of UV and incubated for 24 h, and then the RNA synthesis rate was determined. Results are shown for cells from the patient XP20BE (♦), two normal donors (GM1652, △; and 1BR, ○), the parents of the XP20BE patient (AG8805, ▽; and AG8807, □), one patient with CS (CS4BR, ▲), and patients in XP complementation groups C (XP21BE, ■) and D (XPLA-BE, ●). The XP20BE fibroblasts showed marked post-UV inhibition of RNA synthesis similar to that of the XP-D patient.

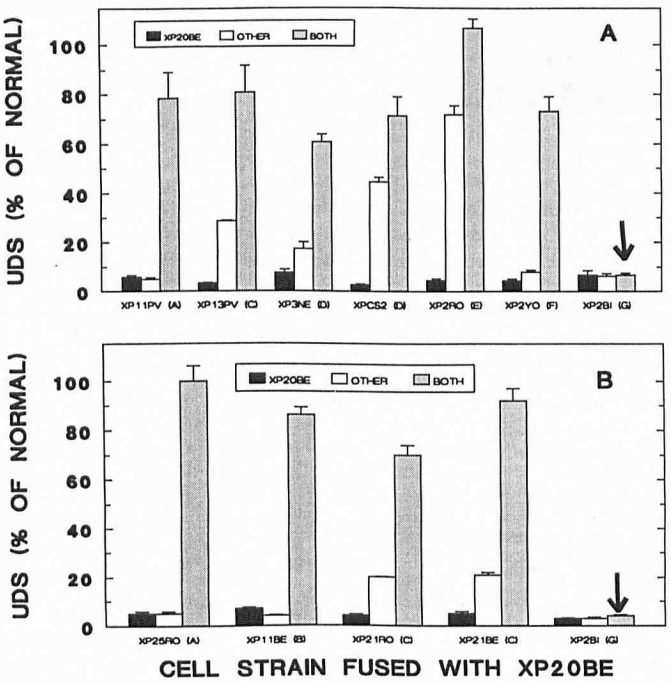

Figure 3. Complementation testing of XP20BE cells demonstrating assignment to XP complementation group G by cell fusion. Post-UV UDS was determined in binuclear homokaryons of XP20BE *(closed bars)*, binuclear homokaryons of the other cell strain *(open bars)*, and heterokaryons *(shaded bars)* of XP20BE cells fused to representative cells of different XP complementation groups. The *bars* indicate the percentage UDS for the indicated cell strain compared with that of the concurrent normal. The *arrow* indicates lack of complementation of XP20BE cells when fused with the XP-G (XP2BI) cell line. *(A)* Fusions of XP20BE cells with XP-A, -C, -D, -E, -F, and -G cells performed in Pavia. The UDS of the normal control was 45 grains/nucleus for fusions with XP-A (XP11PV), XP-C (XP13PV), and XP-D (XP3NE); 62 grains/nucleus for fusions with XP-D (XPCS2) and XP-G (XP2BI); and 85 grains/nucleus for fusions with XP-E (XP2RO) and XP-F (XP2YO). *(B)* Fusions of XP20BE cells with XP-A, -B, -C, and -G cells performed in Rotterdam. The UDS of the normal control was 41 grains/nucleus for fusions with XP-A (XP25RO), XP-C (XP21RO and XP21BE), and XP-G (XP2BI); and 98 grains/nucleus for fusions with XP-B (XP11BE). *Error bars*, mean ± SEM.

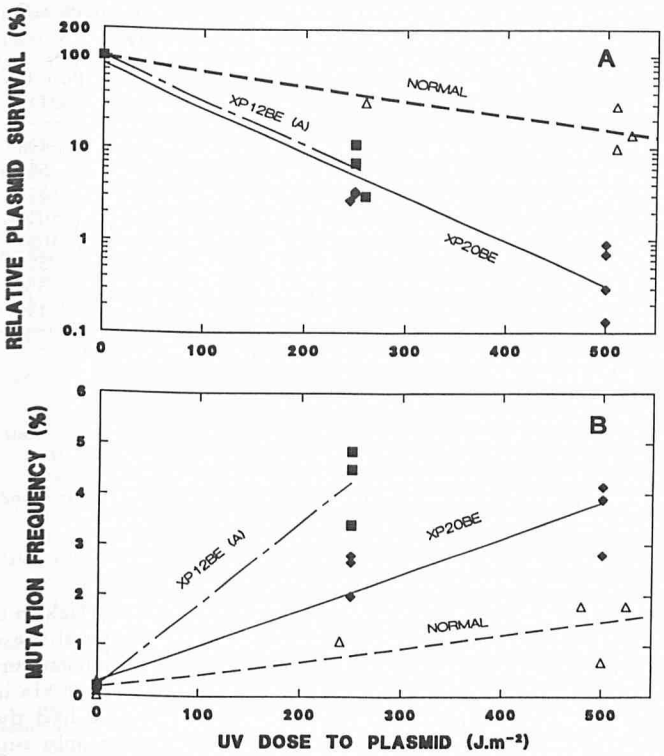

Figure 5. Reduced survival and increased mutation frequency of UV-treated plasmid replicated in XP20BE, XP-A, and normal lymphoblastoid cells. pSP189 was treated with 0–500 J/m^2 UV and transfected along with untreated internal standard plasmid (pZ189K) by diethylaminoethyl dextran into the XP20BE (♦), XP-A (XP12BE, ■), and normal donor (GM606, △) lymphoblastoid cells. After 2 d, the replicated plasmids were harvested and used to transform indicator bacteria to resistance to ampicillin or kanamycin. Each point represents the mean of two to three independent determinations in separate experiments. *(A)* Normalized relative plasmid survival plotted as a function of UV dose to the plasmid. *(B)* The bacterial colonies obtained were analyzed for plasmids with wild-type *supF* (dark blue colonies) or mutated *supF* gene (white or light blue colonies). The mutation frequencies of the plasmids are plotted as a function of UV dose to the plasmid.

the transfected gene. When the transfecting plasmid was irradiated with 250 J/m^2 and not photoreactivated, the CAT activity of the XP20BE and XP-A lines was lower than that of the normal line ($p < 0.01$). This reduced post-UV CAT activity is typical of both XP and CS cells (Barrett *et al*, 1991; Parris and Kraemer, 1993).

Reversal of cyclobutane dimers by photoreactivating the plasmid treated with 250 J/m^2 before transfection resulted in increased CAT activity in all lines. The level of CAT activity after 250 J/m^2 plus photoreactivation in the XP20BE or the XP-A cells, however, was significantly lower than that of the normal cells ($p < 10^{-4}$).

Table II. Normal Classes of Mutations in UV-Treated pSP189 Replicated in XP20BE Lymphoblastoid Cells

	Number of Plasmids With Base Changes (%)	
	Normal (GM606)[a]	XP20BE[b]
Independent plasmids sequenced	69 (100%)	80 (100%)
Point mutations		
Single	57 (83%)	63 (79%)
Tandem	6 (9%)	7 (9%)
Multiple	5 (7%)	10 (13%)
Deletions	1 (1%)	0

[a] UV dose to plasmid-1000 or 500 J/m^2.
[b] UV dose to plasmid-250 or 500 J/m^2.

Table III. High Frequency of Transition Mutations in UV-Treated pSP189 Replicated in XP20BE Lymphoblastoid Cells

	Number of Base Changes (%)	
Base Change	Normal (GM606)[a]	XP20BE (XP-G/CS)[b]
Transitions	26 (38%)	59 (76%)[c]
G:C → A:T	21 (30%)	55 (71%)[c]
A:T → G:C	5 (7%)	4 (5%)
Transversions	43 (62%)	19 (24%)[c]
G:C → T:A	14 (20%)	11 (14%)
G:C → C:G	10 (15%)	2 (3%)[d]
A:T → T:A	10 (15%)	4 (5%)
A:T → C:G	9 (13%)	2 (3%)[d]
Total	69 (100%)	78 (100%)

[a] UV dose to plasmid-1000 or 500 J/m^2.
[b] UV dose to plasmid-250 or 500 J/m^2.
[c] $p < 0.0001$.
[d] $p < 0.02$.

This finding is typical of XP cells, which are unable to repair nondimer photoproducts, but is different from CS cells, which have normal CAT expression of UV-treated plasmids that are treated by photoreactivation (Barrett *et al*, 1991; Parris and Kraemer, 1993).

DISCUSSION

XP and CS are clinically distinct disorders that have in common sun sensitivity, microcephaly, deafness, and progressive neurologic degeneration (Kraemer, 1993; Cleaver and Kraemer, 1995). Approximately 20% of XP patients have associated neurologic abnormalities of varying severity (Kraemer *et al*, 1987). The most severe form has been called the De Sanctis-Cacchione syndrome. These patients can be distinguished from those with CS by absence of retinal degeneration, decreased to absent deep tendon reflexes, primary neuronal degeneration, and absence of calcification of the basal ganglia and other brain structures. Patients with CS, in contrast, do not show XP-type skin freckling or skin cancer but do have increased or normal deep tendon reflexes, signs of primary

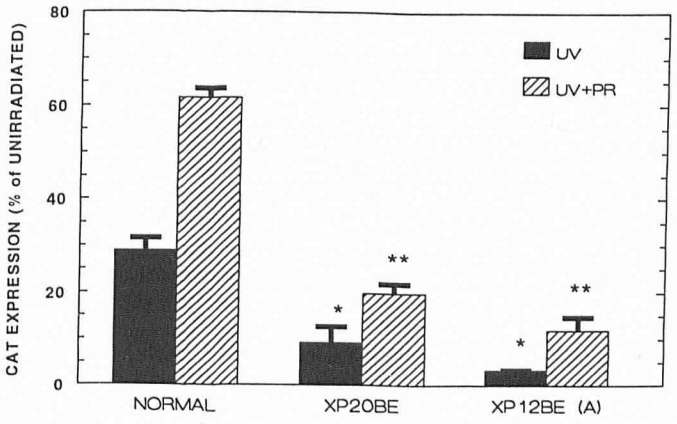

Figure 6. Reduced repair of cyclobutane dimer and nondimer photoproducts in XP20BE cells. Plasmid pRSVcat was treated with 250 J/m^2 UV alone (■) or with UV followed by photoreactivation (PR) (▨) to remove cyclobutane dimer photoproducts, and then transfected into XP20BE, XP-A, and normal lymphoblastoid cells. Each bar represents the mean ± SEM of triplicate determinations of CAT activity for each cell line. The mean specific CAT activity with the control unirradiated plasmid was 0.6 pmol/mg/min with the normal (GM606) line, 2 pmol/mg/min with the XP20BE line, and 7 pmol/mg/min with the XP-A (XP12BE) line. The post-UV CAT activity with the XP20BE and XP-A cells was significantly lower than that of the normal line with UV ($p < 0.01$) and with UV plus photoreactivation ($p < 10^{-4}$).

demyelination (tigroid leukodystrophy), and often, calcification of the brain (Cantani *et al*, 1987; Nance and Berry, 1992; Kraemer, 1993; Cleaver and Kraemer, 1995). The neurologic abnormalities of XP result from a primary neuronal degeneration (Robbins *et al*, 1974, 1991); those of CS result from abnormal myelination (Cantani *et al*, 1987; Nance and Berry, 1992). The term "XP neurologic disease" (Robbins *et al*, 1991) has been applied to the neurologic signs and symptoms described in XP-A patients and in certain XP-D patients (Robbins *et al*, 1974, 1991). The term "XP/CS complex" has been proposed (Robbins *et al*, 1974; Robbins, 1988) for patients who have pigmentary cutaneous features of XP together with one or more of the characteristic features of CS (e.g., primary demyelination, pigmentary retinal degeneration, calcification of the basal ganglia).

The patient reported here, XP20BE, had severe signs of CS including acute sun sensitivity, retinal degeneration, cachectic dwarfism, deafness, mental impairment, microcephaly, demyelination, and calcification of the brain. In addition, he showed the XP type of pigmentation on his sun-exposed skin, which led to the clinical diagnosis of XP even though there was no evidence of skin cancer. Clinically, CS was predominant, but he also had pigmentary changes of XP. He therefore fulfills the clinical diagnosis of the XP/CS complex.

The XP20BE cells derived from our patient showed DNA-repair abnormalities common to XP and CS, including cellular and plasmid UV hypersensitivity. The XP20BE cells had very low UV-induced UDS **(Table I)**, a feature of XP, and were found to be in the rare XP group G by genetic complementation study **(Fig 3*A,B*)**. The XP20BE cells showed delayed post-UV recovery of RNA synthesis **(Fig 4)**, an abnormality seen in CS cells and also in XP-D **(Fig 4)**, XP-A (XP4LO), and XP-G (XP2BI) cells (Mayne and Lehmann, 1982); in the two other XP-G/CS cell lines studied (Jaeken *et al*, 1989); and in XP-B/CS (Vermeulen *et al*, 1994a). We found a significantly elevated frequency of transition mutations, especially G:C → A:T base changes **(Table III)**, in UV-treated plasmids recovered from XP20BE cells compared with the normal line, a feature seen in XP cells (Cleaver and Kraemer, 1995) but not in CS cells (Muriel *et al*, 1991; Parris and Kraemer, 1993). The frequency of UV-treated plasmids with multiple base substitutions recovered from the XP20BE cells was normal, however **(Table II)**, which is a feature of CS cells (Parris and Kraemer, 1993). The activity of CAT enzyme using UV-irradiated pRSVcat was very low in XP20BE cells and in XP-A cells compared with a normal line **(Fig 6)**. A similar abnormality was described for the other two XP-G/CS cell lines (Jaeken *et al*, 1989). This finding indicates the presence of a DNA-repair deficiency in actively transcribing genes in XP20BE cells, as in XP and CS cells (Barrett *et al*, 1991). The low post-UV UDS implies that the XP20BE cells also have a DNA-repair deficiency in the entire genome. After photoreactivation of UV-irradiated pRSVcat, the CAT activity in the XP20BE line was still very low, as in the XP-A line, and did not increase to the level of a normal line **(Fig 6)**. This result indicates that the XP20BE cells are defective in the repair of both cyclobutane dimers and nondimer photoproducts, a characteristic of XP cells that distinguishes them from CS cells, which are able to repair nondimer photoproducts (Barrett *et al*, 1991; Parris and Kraemer, 1993).

Cell fusion studies indicated that XP20BE, who is the eighth XP/CS patient assigned to a complementation group, is in XP complementation group G **(Fig 3*A,B*)** and represents the third XP-G/CS patient **(Table IV)**. The rare XP-G complementation group so far has been diagnosed in only seven other patients (Cheesbrough and Kinmont, 1978; Keijzer *et al*, 1979; Arlett *et al*, 1980; Ichihashi *et al*, 1985; Norris *et al*, 1987; Jaeken *et al*, 1989; Vermeulen *et al*, 1993). Clinical symptoms of XP were variable in these seven XP-G patients, including mild or severe disease and with different rates of post-UV UDS (<5% to 25% of normal).

The seven other XP/CS patients described in the literature who have been assigned to complementation groups fall into three different XP complementation groups **(Table IV)**: group B, three patients (Robbins *et al*, 1974; Scott *et al*, 1993; Vermeulen *et al*, 1994a); group D, two patients (Moshell *et al*, 1983; Vermeulen *et al*, 1991; Broughton *et al*, 1995); and group G, two patients (Jaeken *et al*, 1989; Vermeulen *et al*, 1993). The clinical presentations of these patients were variable, with predominance of XP with skin cancers in some patients and predominance of CS features of cachexia in others. All three XP-B patients that have been reported had the XP/CS complex. They lived beyond 30 y of age, but only one developed skin cancer **(Table IV)**. Two patients with the XP-B defect had trichothiodystrophy, a disorder with sun sensitivity, brittle sulfur-deficient hair, and mental retardation without XP or skin cancer (Vermeulen *et al*, 1994b). XP complementation group D shows marked clinical heterogeneity. More than 30 patients with the XP-D defect have been described (Cleaver and Kraemer, 1995). Most had clinical XP, and many had XP-type progressive neurologic degeneration. Two XP-D patients had the XP/CS complex and one had skin cancer; both died before age 15. More than a dozen patients with the XP-D cellular defect have been reported as having trichothiodystrophy without clinical features of XP or skin cancer (Stefanini *et al*, 1992, 1993). Like XP20BE, the other two XP-G/CS patients had severe CS and died in early childhood (Jaeken *et al*, 1989; Vermeulen *et al*, 1993) **(Table IV)**.

Table IV. Varied Clinical and Laboratory Features of Eight Patients With the XP/CS Complex[a]

Patient	Complementation Group	Age/Sex	Skin Cancer	Post-UV UDS
XP11BE[b]	B	33 y[c]/F	Yes (18 y)[d]	4%
XPCS1BA[e,f]	B	38 y/M	No	5%
XPCS2BA[e,f]	B	41 y/M	No	5%
XPCS2[g]	D	13 y[c]/M	Yes (2 y)[d]	30%
XP8BR[g]	D	2.5 y[c]/M	No	30%
XPCS1LV[h]	G	6.5 y[c]/F	No	5%
XPCS2LV[h]	G	1.7 y[c]/M	No	5%
XP20BE[i]	G	6.1 y[c]/M	No	1%

[a] All patients had sun sensitivity, neurologic abnormalities, and retinopathy.
[b] Robbins *et al*, 1974.
[c] Age at death.
[d] Age at first skin cancer.
[e] Brothers.
[f] Scott *et al*, 1993; Vermeulen *et al*, 1994a.
[g] Moshell *et al*, 1983; Vermeulen *et al*, 1991; Broughton *et al*, 1995.
[h] Jaeken *et al*, 1989; Vermeulen *et al*, 1993.
[i] This report.

The genes responsible for XP-B (Weeda *et al*, 1990), XP-D (Weber *et al*, 1990; Flejter *et al*, 1992), and XP-G (Mudgett and MacInnes, 1990; O'Donovan and Wood, 1993) have been cloned and located on different chromosomes (2, 19, and 13, respectively). There is evidence that the gene products of XP-B, XP-D, and XP-G interact with several other proteins to form a DNA-repair complex (Aboussekhra *et al*, 1995; Bootsma *et al*, 1995; Mu *et al*, 1995). The XP-B and XP-D gene products are thought to function as helicases, which unwind DNA, and the XP-G product is a UV endonuclease that incises the DNA 3′ to the photoproduct (O'Donovan and Wood, 1993). The XP-B and XP-D gene products are also part of the basal transcription factor TFIIH (Bootsma *et al*, 1995). It has been postulated that these genes play a dual role, acting in either DNA repair or transcription, and that patients with XP represent defects primarily in the repair function, whereas those with symptoms of CS represent "transcription defective disorders" (Bootsma *et al*, 1995). Thus, patients with the XP/CS complex would hypothetically have defects in a gene involving both transcription and repair activities. This postulate might explain the existence of patients with XP/CS complex in XP complementation groups B and D, but to date no transcription activity has been reported in the XP-G gene product. Analysis of the mutations in the XP-G gene (Nouspikel and Clarkson, 1994) has been published for one XP-G patient, XP125LO (Norris *et al*, 1987), who at age 12 y had mild XP with no skin cancers or neurologic abnormalities. XP125LO had a paternally derived nonsense mutation and a maternally derived missense mutation. Analysis of the XP-G gene in XP20BE may provide insights into the origin of the XP-G/CS

complex and explain our findings that the XP20BE cells are more like XP cells than CS cells in DNA-repair tests, whereas clinically the CS phenotype is more prominent than the XP phenotype.

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