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Clinical Heterogeneity within Xeroderma Pigmentosum Associated with Mutations in the DNA Repair and Transcription Gene *ERCC3*

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Summary

The human DNA excision repair gene *ERCC3* specifically corrects the nucleotide excision repair (NER) defect of xeroderma pigmentosum (XP) complementation group B. In addition to its function in NER, the ERCC3 DNA helicase was recently identified as one of the components of the human BTF2/TFIIH transcription factor complex, which is required for initiation of transcription of class II genes. To date, a single patient (XP11BE) has been assigned to this XP group B (XP-B), with the remarkable conjunction of two autosomal recessive DNA repair deficiency disorders: XP and Cockayne syndrome (CS). The intriguing involvement of the ERCC3 protein in the vital process of transcription may provide an explanation for the rarity, severity, and wide spectrum of clinical features in this complementation group. Here we report the identification of two new XP-B patients: XPCS1BA and XPCS2BA (siblings), by microneedle injection of the cloned *ERCC3* repair gene as well as by cell hybridization. Molecular analysis of the *ERCC3* gene in both patients revealed a single base substitution causing a missense mutation in a region that is completely conserved in yeast, *Drosophila,* mouse, and human *ERCC3.* As in patient XP11BE, the expression of only one allele (paternal) is detected. The mutation causes a virtually complete inactivation of the NER function of the protein. Despite this severe NER defect, both patients display a late onset of neurologic impairment, mild cutaneous symptoms, and a striking absence of skin tumors even at an age of >40 years. Analysis of the frequency of *hprt*$^-$ mutant T-lymphocytes in blood samples suggests a relatively low in vivo mutation frequency in these patients. Factors in addition to NER deficiency may be required for the development of cutaneous tumors.

Introduction

Rare human hereditary syndromes with a predisposition toward the development of cancer—such as Bloom syndrome, Fanconi anemia, ataxia telangiectasia, and xeroderma pigmentosum (XP)—are believed to be based on a defective DNA repair mechanism or on an impaired cellular response to damage inflicted to DNA. XP is the prototype of these disorders, as it is the most intensively studied at the genetic, biochemical, and molecular level. Patients suffering from this autosomal recessive disease are extremely sensitive to the UV spectrum of solar irradiation (Cleaver and Kraemer 1989). This hallmark of the disease is reflected by an atrophic skin with (*a*) hyper- and hypopigmented spots on sun-exposed areas and (*b*) a >1,000-fold increased risk of developing skin cancer. Some patients also exhibit early and severe neurological degeneration. A striking heterogeneity is observed among XP individuals, varying from patients with only mild skin lesions to those with severe developmental disorders such as dwarfism, microcephaly, and neurologic (ataxia and mental retardation) abnormalities (Robbins et al. 1991). This heterogeneity is only partly explained by the involvement of multiple distinct genes as revealed by the identification of seven XP complementation groups

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(XP-A through XP-G) (Vermeulen et al. 1991). The primary defect in most XP patients resides in a disturbed nucleotide excision repair (NER) pathway (Cleaver 1968) (for a recent review on NER, see Hoeijmakers 1993). A subpopulation of XP patients, designated "XP-variants," appears to have a defect in the poorly defined postreplication repair system (Lehmann et al. 1975). NER consists of two subpathways: (1) preferential repair of the transcribed strand of transcriptionally active genes and (2) the slower and less efficient overall genome repair (Bohr 1991; Hanawalt 1991). Information available to date indicates that both NER subpathways are impaired in the various forms of XP, with the notable exception of XP group C, in which only the overall genome repair system appears to be affected (Venema et al. 1990*b*). Defective NER can be monitored biochemically by measuring the UV-induced DNA repair synthesis referred to as "unscheduled DNA synthesis" (UDS). Absence or reduced levels of UDS are found in XP groups A–G. Recently, it was demonstrated that another human genetic syndrome associated with UV sensitivity, Cockayne syndrome (CS), is also based on a defect in NER. Here only the preferential repair of active genes is disturbed, whereas the overall repair (responsible for the bulk of UDS) is normal (Venema et al. 1990*a*). Consequently, UDS levels in CS fall within the normal range. In addition to a UV-sensitive skin (without pigmentation abnormalities), neurological dysfunction due to demyelination of neurons and calcification of basal ganglia is obligate for the diagnosis of CS. General developmental impairment is often also seen (Nance and Berry 1992). Although CS is based on a partially deficient NER, no significant increased frequency of skin cancer is observed. Clinical and genetic heterogeneity is apparent in CS (Lehmann 1982). A class of extremely rare individuals with a conjunction of features of both syndromes ("XP-CS" complex) has been recognized. Three XP complementation groups are implicated (XP-B, XP-D, and XP-G). The genes responsible for the defect in XP-B, XP-D, and XP-G have recently been cloned; they are, respectively *ERCC3* (*XPBC*), *ERCC2* (*XPDC*), and *ERCC5* (*XPGC*) (Weeda et al. 1990; Flejter et al. 1992; Lehmann et al. 1992; O'Donovan and Wood 1993; Scherly et al. 1993). The *ERCC3* gene product was identified as one of the components of the human BTF2/TFIIH transcription factor, which is required for initiation of transcription of class II genes (Schaeffer et al. 1993).

Until now XP-B has consisted of a single patient (XP11BE), who displayed severe features of XP as well as CS and who developed numerous skin tumors at an early age. Mutational analysis of the *ERCC3* gene of this patient revealed that only one allele was detectably expressed and that it contained a splice-acceptor mutation in the last exon, causing a C-terminal frameshift at the protein level (Weeda et al. 1990). The few remaining fibroblasts of this patient grow poorly. Recently we received fibroblasts from two brothers, designated "XPCS1BA" and "XPCS2BA," who have combined XP and CS symptoms (Scott et al., in press). In the present paper we report both the assignment of these two patients to XP-B and the identification, at the molecular level, of the mutation in *ERCC3* in these patients.

Material and Methods

Cell Strains, Culture Conditions, and Cell Hybridization

Human fibroblast cultures C5RO (control), XP25RO (XP-A), XP11BE (XP-B), XPCS2 (XP-D), XPCS1LV (XP-G), XPCS1BA, and XPCS2BA (assigned to XP-B; present paper) were grown in Ham's F10 medium supplemented with 11% FCS and antibiotics (100 U penicillin/ml and 100 μg streptomycin/ml). Lymphoblastoid cells were grown in RPMI medium supplemented with 10% FCS and antibiotics.

Three days prior to cell hybridization, each fusion partner was labeled with latex beads of different sizes by adding a suspension of beads to normal culture medium (Vermeulen et al. 1991). After trypsinization, 0.5×10^6 cells of each were mixed in a 1:1 ratio, washed extensively to remove free beads, and fused with ß-propiolactone-inactivated Sendai virus. Fused cells were seeded onto coverslips, incubated for 2 d under normal culture conditions, and assayed for UV-induced UDS, which was measured by autoradiography as described below. Homopolykaryons, to be used for the microinjection experiments, were generated as described above, using only one cell strain and omitting the addition of latex beads.

Microneedle Injection

Plasmid cDNA (100 μg/ml) in PBS was injected into one of the nuclei of XP homopolykaryons by using a glass microcapillary, as described elsewhere (Van Duin et al. 1989). For each experiment at least 50 homopolykaryons were injected. Coverslips with injected cells were cultured for 24 h to allow expression of the injected cDNA before they were assayed for their repair capacity by means of UV-induced UDS.

UV-induced UDS

The UDS assay (Vermeulen et al. 1986) was performed 2 d after fusion and 1 d after injection. In brief,

cells grown on coverslips were washed with PBS, irradiated with 15 J/m^2 UV-C (Philips TUV lamp), immediately incubated for 2 h in culture medium containing 10 μCi (*methyl*-^{3}H) thymidine (50 Ci/mmol; Amersham)/ml, washed with PBS, and fixed. Coverslips with radioactively labeled cells were mounted onto slides and dipped in a photosensitive emulsion (Ilford K2). After exposure (2–7 d) slides were developed and stained. Repair capacity was quantitated by counting autoradiographic grains above the nuclei of ≥50 cells. UDS levels are expressed as the percentage of UDS of normal cells on an identically treated coverslip culture in the same experiment.

In Vivo Mutant Frequency at the hprt *Locus*

Mutant frequency estimations were undertaken by published procedures (Cole et al. 1988, 1992). In brief, for each determination, a mass culture of mononuclear cells (≈10^7 cells) was incubated overnight in RPMI 1640 medium supplemented with 10% human AB serum, before being cloned in 96-well microtiter trays in the presence of mitogen (phytohemagglutinin), lethally irradiated lymphoblastoid feeder cells, and the T-cell growth factor interleukin-2 (IL-2). For *hprt* mutant selection, the cells were plated at high density (1×10^4/well) in the presence of the purine analogue 6-thioguanine (6TG), in which nonmutants are unable to grow. To determine cloning efficiency in the absence of selection, a low cell density (3 cells/well) was used. The plates were scored for negative (no colony) wells after 15–20 d incubation, and the mutant frequency was calculated from the zero term of the Poisson distribution. Under the conditions used in our experiments, mononuclear cell cloning efficiencies of ≈50% in the absence of selection are routinely obtained; thus a high percentage of the T-lymphocytes in the sample are capable of colony formation in vitro. Therefore the mutant frequency should reflect the in vivo situation.

RNA Isolation, DNA Amplification, and Mutation Detection

RNA was isolated by the LiCl/urea method (Auffray and Rougeon 1980). The RNA was used for preparing cDNA with *ERCC3*-specific primers (as described by Weeda et al. 1990). Amplification was performed by 32 cycles of, consecutively, 2 min denaturing at 95°C, 2 min annealing (the temperature was dependent on the primers used), and 3 min extension at 70°C (Saiki et al. 1985). Amplified DNA was spotted onto Hybond filters (Amersham) and was UV-cross-linked and hybridized to wild-type and mutant ^{32}P-labeled primers. The amplified DNA was purified and digested with the appropriate restriction enzyme and subsequently was cloned into a M13mp18 vector for sequence analysis by the dideoxy-chain termination method (Sanger et al. 1977) using T7 DNA polymerase (Pharmacia). Oligonucleotide primers for cDNA, DNA amplification, and DNA sequencing were synthesized in an Applied Biosystem DNA synthesizer. The primers for mutation detection in cells of XP patients XPCS1BA and XPCS2BA were p68, 5′-CCCAAGACTTCTTGGTGG-3′; p69, 5′-CCCAAGACTCCTTGGTGG-3′.

cDNA Constructs

The wild-type *ERCC3* cDNA expression plasmid (pSVH3) and the mutant containing the XP11BE mutation (pSVH3M), used for microinjection into XP-B fibroblasts, have been described elsewhere (Weeda et al. 1990). The *ERCC3* gene harboring the mutation present in the two brothers was constructed as follows: the 5′ part of PSVH3 was replaced by a 0.7-kb *Eco*RI/*Sac*I DNA fragment that was synthesized by means of PCR-amplified *ERCC3* cDNA derived from mRNA isolated from cells of patient XPCS1BA, yielding pSVH3M2.

Results

UV-induced UDS

A detailed description of the clinical features of XP patients XPCS1BA and XPCS2BA (two brothers of unrelated parents) has been presented elsewhere (Scott et al., in press). The clinical pattern is most consistent with the "XP-CS complex" phenotype seen in a very limited number of patients with NER deficiency. The features of these patients, together with those of four other XP-CS complex patients, are summarized in table 1. The repair capacity of the fibroblasts from the two individuals (XPCS1BA and XPCS2BA), compared with the level found in normal repair-competent fibroblasts, is severely reduced, in contrast to classical CS, which exhibit normal levels of UDS. This is consistent with the high sensitivity of their fibroblasts to the cytotoxic effects of UV-C, as demonstrated by a colony-survival assay (Scott et al., in press) and our own unpublished observations. The level of residual UDS is in the same range as is usually found in XP-A, -B, and -G (i.e., <10% of normal) and is well below the 30%–50% residual UDS displayed by XPCS2 (XP-D). These findings make XP-B and -G likely candidates in the relatively mildly affected individuals.

Microneedle Injection of ERCC3 *cDNA*

Microneedle injection of the *ERCC3* cDNA specifically corrects the NER defect in XP-B cells, but not in

Table 1

Features of XP-CS-Complex Patients

Feature	CS[a]	XP[b]	XPCS2[c]	XPCS1/2LV[d]	XP11BE[e]	XPCS1/2BA[f]
Cutaneous:						
Photodermatitis	+	++	++	++	++	++
Pigmentation abnormalities	−	++	++	−	++	+
Skin cancer	−	>1,000 times	+(2 years)	−	+(18 years)	−(>40 years)
Neurologic:						
Primary defect	Demyelination	Degeneration	Demyelination	Unknown	Demyelination	Demyelination
Ganglia calcification	+	−	Unknown	+	+	−
Psychomotor retardation	+	−(+)[g]	+	+	+	+/−[h]
Hearing impairment	+	−(+)[g]	+	+	+	+/−[h]
Reflexes	Hyper	Hypo	Hyper	Unknown	Unknown	Hyper
Retinal pigmentation	+	−	+	+	+	+
Developmental:						
Dysmorphic dwarfism	+	−(+)[g]	+	+	+	−[i]
Immature sexual development	+	−(+)[g]	+	+	+	+
Microcephaly	+	−(+)[g]	+	+	+	−
Biochemical/cellular:						
UDS (% of control)	100	1–50	30–50	<5	5–10	5–10
UV sensitivity (in situ)[j]	Moderate	Severe/moderate	Unknown	Severe	Severe	Severe

NOTE.—Status of features is as follows: + = presence of symptom; ++ = severe phenotype; and − = absence of feature.

[a] Nance and Berry (1992).

[b] Robbins et al. (1991).

[c] XP-D (Vermeulen et al. 1991).

[d] XP-G (Vermeulen et al. 1993).

[e] XP-B (Robbins et al. 1974).

[f] XP-B (present study).

[g] Absent in classical XP; present in XP with neuropathology.

[h] Present, but relatively late onset.

[i] Weight and height not as low as in CS, but at the 3d percentile.

[j] Colony survival after UV irradiation, compared with control cells.

XP cells of other complementation groups (Weeda et al. 1990). To examine whether the *ERCC3* cDNA was able to correct the repair defect in cells of patients XPCS1BA and XPCS2BA, the cDNA in a mammalian expression vector was introduced into the nuclei of homopolykaryons of both patients. The results, shown in figure 1 and quantified in table 2, demonstrate that injected XPCS1BA and XPCS2BA fibroblasts were able to achieve normal UDS levels. The nuclear labeling was UV dependent, and no correction was obtained using an inactive *ERCC3* gene containing the XP11BE mutation. These data clearly demonstrate that the repair defect is corrected by the *ERCC3* gene and that both patients belong to XP-B.

Complementation by Cell Hybridization

The complementation assignment via the microneedle injection was verified by using the classical cell hybridization technique. When XPCS1BA cells were fused to XP-A fibroblasts (fig. 2*A* and table 3), clear complementation was observed up to the normal level of UDS, found in NER-proficient cells. In contrast, no complementation occurred after fusion with either XP-B cells (fig. 2*B* and table 3) or cells from the sib XPCS2BA. These data confirm the assignment of both patients to XP-B, by microneedle injection.

Determination of the ERCC3 *Mutation*

Analysis by Southern and northern blot hybridization made it clear that, in the cells of both patients, the *ERCC3* gene is not detectably rearranged and is normally expressed; the RNA has the expected size of 2.8 kb (data not shown). This suggests that the presence of a gross alteration in the gene is rather unlikely. Sequence analysis on PCR-amplified mRNA of patient XPCS1BA revealed a single base substitution (T→C transversion; see fig. 3) in the 5′ part of the cDNA, resulting, at the protein level, in a phenylalanine (F)–to–

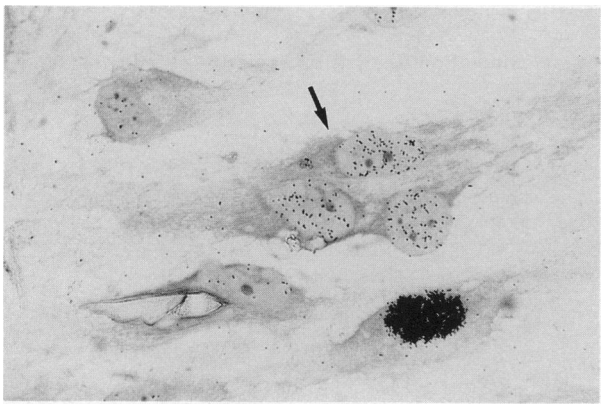

Figure 1 Effect of microinjection of *ERCC3* cDNA on UV-induced UDS in XPCS1BA fibroblasts. Shown is a micrograph of a XPCS1BA homopolykaryon (*arrow*) microinjected with wild-type *ERCC3* cDNA in one of the nuclei and subjected to the UDS procedure. The injected cell has a considerably larger number of grains above its nuclei than do the noninjected, surrounding mononuclear cells. In fact, the number of nuclear grains above the injected cell reaches that of repair-proficient fibroblasts assayed in parallel. The cell containing the heavily labeled nucleus was in S-phase during ^{3}H-TdR incubation.

serine (S) amino acid substitution at position 99. No other changes were observed in the remainder of the *ERCC3* cDNA. To determine whether this was the inherited mutation, the relevant part of the *ERCC3* cDNA of the two patients and their parents and of HeLa cDNA was amplified and subjected to dot blot analysis. The filters were hybridized with ^{32}P-labeled wild-type-specific (p68) and mutant-specific (p69) oligonucleotide probes. HeLa control DNA (fig. 4) hybridized only to the wild-type *ERCC3* oligonucleotide probe, whereas the cDNA of both patients hybridized only with the mutant-specific probe. PCR-amplified cDNA from the father, however, exhibited clear hybridization with both probes. This indicates that the father is heterozygous for the mutation. Since only the paternal allele of *ERCC3* can be detected, the maternal allele of the patient must contain another small mutation causing complete either absence or a greatly reduced level of *ERCC3* transcript.

A cDNA construct carrying the XPCS1BA mutation (PSVH3M2) was microinjected into XPCS2BA fibroblasts. As demonstrated in table 2, no significant increase of UDS is observed with this mutant cDNA. These experiments indicate that the XPCS1BA mutation completely inactivates the ERCC3 repair function, as was previously also shown for the XP11BE mutation (table 2).

In Vivo Mutant Frequency at the hprt$^-$ *Locus*

The frequency of 6-thioguanine-resistant T-lymphocytes in blood samples from the two patients was compared with similar information from our database on normal control subjects and other excision-defective patients, over the relevant age range. We have pub-

Table 2

***ERCC3* cDNA Microinjections into XPCS1BA and 2BA Fibroblasts**

Cell Strain	cDNA Injected	UV Irradiation (15 J/m^2)	UDS (mean ± SEM)	
			Grains per Nucleus	% of Control Value
XPCS1BA	pSV3H[a]	+	120 ± 8	80 ± 6
XPCS1BA	...[b]	+	10 ± 2	6 ± 1
XPCS2BA	pSV3H[a]	+	138 ± 10	93 ± 7
XPCS2BA	...[b]	+	12 ± 2	7 ± 1
XPCS2BA	pSV3H[a]	−	2 ± 1	1 ± 1
XPCS2BA	pSV3HM[c]	+	13 ± 2	7 ± 1
XPCS2BA	pSV3HM2[d]	+	10 ± 2	6 ± 1
C5RO	...	+	150 ± 11	100 ± 8

[a] *ERCC3* cDNA (wild-type).
[b] Uninjected polykaryon.
[c] *ERCC3* cDNA containing the XP11BE mutation.
[d] *ERCC3* cDNA containing the XPCS1BA mutation.

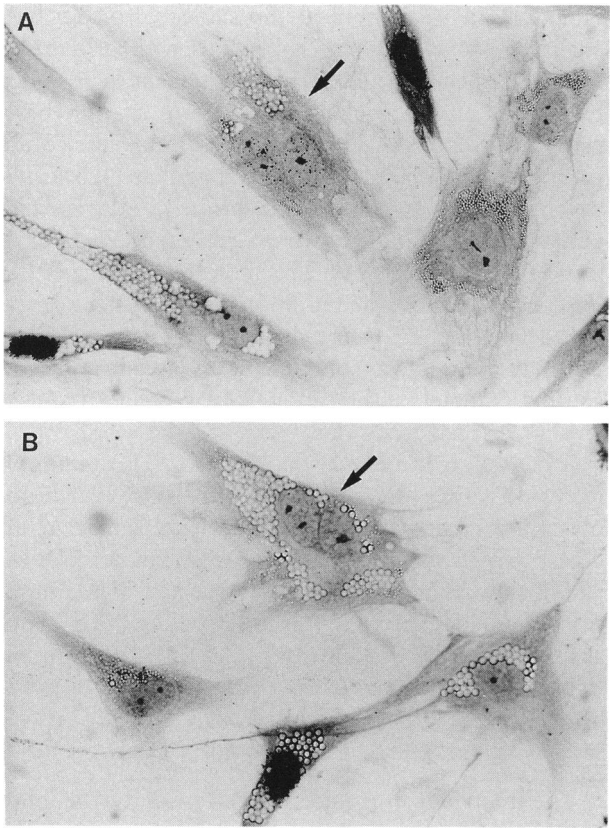

Figure 2 Cell hybridization of XPCS1BA with XP-A and XP-B fibroblasts. *A*, Monokaryons (expressing no detectable UDS): XP25RO, containing small (0.8 μm) latex beads, and XPCS1BA, containing large (2.0 μm) latex beads. The heterodikaryon (*arrow*) generated by Sendai virus–stimulated fusion of XP25RO(A) and XPCS1BA fibroblasts, identified by the presence of two types of beads, reaches a UDS level of NER-competent fibroblasts, which are visualized as autoradiographic grains above the nuclei of the hybrid cell. *B*, Fusion between XP11BE (0.8-μm beads) and XPCS1BA (2.0-μm beads). No increase in UV-induced UDS is observed above the nuclei of the heterodikaryon (*arrow*), compared with the monokaryons.

lished (Cole et al. 1992) additional results on older XP patients, consisting of three XP variants and an unusual XP-C excision-defective patient 65 years of age.

As shown in figure 5, the excision-defective XP patients as a group have an elevated mutant frequency compared with normal, nonsmoking subjects. Statistical analysis of these data has confirmed the conclusion of Cole et al. (1992) that the increase in *hprt* mutants in XPs is highly significant. However, the mutant frequencies of both XPCS1BA and XPCS2BA are at the top of the range for normal donors. When there is matching for age, a formal statistical analysis that tests for the inclusion/exclusion of the two patients' mutant frequency in the normal or XP populations shows that they are not significantly different from normal donors, nor can they be discriminated from the population of Xps (we are indebted to Dr. M. H. L. Green of the MRC Cell Mutation Unit for undertaking this analysis).

Table 3

Complementation Analysis by Somatic Cell Hybridization

Fused Cells (complement group) and Type of Dikaryon	UDS[a] (mean ± SEM) Grain per Nucleus	% of Control Value
XP25RO (A) × XPCS1BA:		
XPCS1BA	3 ± 1	7 ± 1
XP25RO	1 ± 1	2 ± 1
Heterodikaryon	37 ± 2	90 ± 5
XP11BE (B) × XPCS1BA:		
XPCS1BA	3 ± 1	7 ± 1
XP11BE	3 ± 1	7 ± 1
Heterodikaryon	3 ± 1	7 ± 1
XPCS1BA × XPCS2BA:		
XPCS1BA	3 ± 1	7 ± 1
XPCS2BA	3 ± 1	7 ± 1
Heterodikaryon	3 ± 1	7 ± 1
C5RO (control)	41 ± 3	100 ± 7

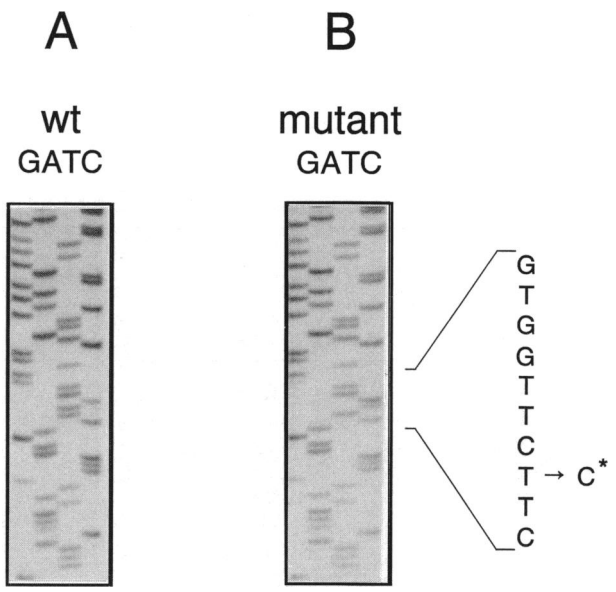

Figure 3 Nucleotide sequence analysis of the XPCS1BA mutation. Nucleotide sequence of a part of the *ERCC3* cDNA of a wild-type allele (wt), from HeLa (*A*) and from patient XPCS1BA (mutant) (*B*).

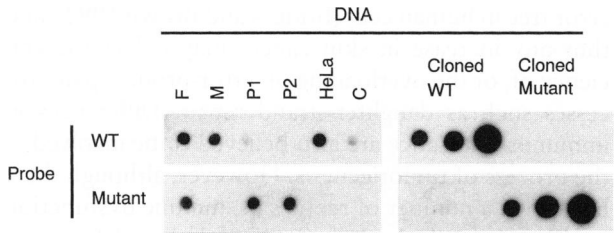

Figure 4 Dot blot analysis of the *ERCC3* in the XPCSBA family. Amplified DNA from HeLa, XPCS1BA (P1), XPCS2BA (P2), XPCSH5BA (F [father]), and XPCSH4BA (M [mother]) (in lane C, no cDNA was added) was spotted onto a nylon filter and hybridized with a labeled wild-type (Cloned WT) probe and with a mutant oligonucleotide (Cloned Mutant) probe (see experimental procedures). As a control for the specificity of hybridization of the mutant and wild-type *ERCC3*-specific oligonucleotide, plasmid DNA from clones PSVH3 (i.e., wild-type) and PSVH3M2 (i.e., XPCS1BA and 2BA mutant) was spotted in serial dilutions.

Two other XP patients, XP125LO and XP7NE, also clearly fall within the normal range. We have shown elsewhere (Cole et al. 1988) that smoking can increase the frequency of mutants recovered from normal donors. Among the population of XP patients discussed here, only one, XP125LO from XP-G (who is indicated by a distinctive symbol in fig. 5), had any history of smoking. We note that he only commenced this habit during the period in which we began our study of this mutant frequency.

Discussion

Cultured fibroblasts of two brothers with relatively mild clinical symptoms of XP and CS and with a virtually complete deficiency of NER were assigned to the extremely rare XP-B. The assignment was performed in two ways: by microinjection of the cloned *ERCC3* repair gene and by the classical somatic cell hybridization technique. Microinjection of NER genes has several advantages compared with the cell-fusion method: (1) It is a direct assay that scores for a positive result (i.e., induction of UDS). Assignment by cell fusion, on the other hand, is based on the absence of complementation (i.e., no correction of UDS). (2) Multiple copies of the gene can be injected into a nucleus, circumventing problems due to low levels of expression. (3) Only a limited number of cells are required, which may be important when only poorly growing cells are available. (4) Microinjection of a cloned NER gene excludes the possibility of intragenic complementation, which may complicate cell hybridization results. (5) Finally, microneedle injection permits assessment of NER genes for which no corresponding human complementation group has been identified thus far. This holds for genes for which only rodent mutants are known, such as the *ERCC1* gene (Van Duin et al. 1989), and for which complementation analysis by cell hybridization is complicated by the occurrence of poor interspecies cell fusion and instability of the resulting hybrids (Thompson et al. 1985). In addition, candidate repair genes for which no mammalian mutants are known can be tested using direct nuclear injection. This may be relevant for mammalian genes that have been isolated on the basis of sequence homology with cloned DNA repair genes of lower species, e.g., *Saccharomyces cerevisiae* or *S. pombe* (Koken et al. 1991). These considerations make the microinjection technique a useful additional method for complementation analysis, particularly in view of the fact that the number of cloned mammalian NER genes is rapidly increasing. At present, at least seven NER genes have been isolated: *ERCC1* (Westerveld et al. 1984); five genes involved in XP—namely, *XPAC* (Tanaka et al. 1989), *ERCC3* (*XPBC*) (Weeda et al. 1990), *XPCC* (Legerski and Peterson 1992), *ERCC2* (*XPDC*) (Weber cited in Lehmann et al. 1992; Flejter et al. 1992), and *ERCC5* (*XPGC*) (O'Donovan and Wood 1993; Scherly et al. 1993)—and, finally, one gene, *ERCC6* (*CSBC*), mutated in CS complementation group B (Troelstra et al. 1992).

The results presented here define the causative mutation in the *ERCC3* gene, responsible for the phenotype

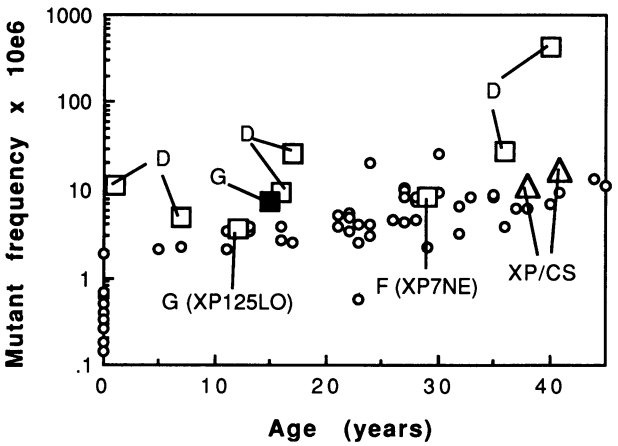

Figure 5 *hprt* mutant frequency in circulating T-lymphocytes in normal, nonsmoking subjects and in XP patients. Frequency of *hprt* mutants is set against the age of the donors, who are normal, nonsmoking donors (○), an XP nonsmoker (□), an XP smoker (■), and XPCS1BA and XPCS2BA (△).

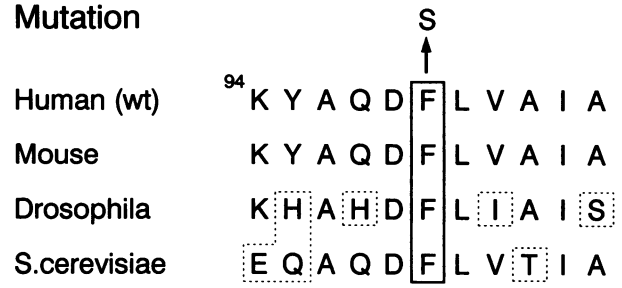

Figure 6 Amino acid sequence conservation of the mutated region in *ERCC3*. XPCS1BA and 2BA mutations in a conserved region of the ERCC3 protein are shown. There is homology between the amino acid sequences of *ERCC3*, around position 99 of human, mouse, *Drosophila*, and *Saccharomyces cerevisiae*. Homologous residues are boxed with a dashed line, and the conserved phenylalanine is boxed with a solid line. The amino acid substitution into a serine is indicated by an arrow.

of the XPCS1BA and XPCS2BA patients. A single-base-pair substitution resulting in an amino acid change of a phenylalanine to a serine at position 99 is found in the only allele that is detectably expressed in these patients. This phenylalanine resides in a region to which, as yet, no specific functional domain has been assigned. However, as shown in figure 6, this residue has not changed in the long evolutionary distance that separates yeast from man, and it is part of a protein segment that is very strongly conserved during eukaryotic evolution. This indicates that this portion of the protein has an important function that may not tolerate much change.

A comparison of the original XP-B case (XP11BE) with the two patients described here reveals a remarkable clinical heterogeneity. DNA repair studies with fibroblasts indicate the same low level (<10% of the normal level) of UDS, the absence of cyclobutane pyrimidine dimer removal, and a low UV-C survival in colony-forming assays, in all three patients (Scott et al., in press). However, despite a common severe deficiency in NER as measured in fibroblasts, the two newly identified XP-B cases are much less severely affected (Scott et al., in press) than is the original patient (Robbins et al. 1974). The original XP-B patient presented numerous cutaneous malignancies before she was 18 years old, while the two new patients were free of skin cancer after they were 40 years old, despite the fact that apparently they took few precautions to protect themselves from sunlight (Scott et al., in press).

The absence of skin cancers in these patients suggests that processes in addition to defects in NER may contribute to carcinogenesis. NER is considered to be error free in human cells (Bridges and Brown 1992), and thus any increase in skin cancer may reflect the efficiency of, or the overloading of, error-prone repair processes such as daughter-strand repair. Differences in immunosurveillance are also believed to be involved in the process of tumorigenesis. However, although there have been a number of reports of immune dysfunction in XP patients (Cole et al. 1992), including deficiencies of natural killer-cell activity in various XP, CS, and TTD patients (Lehmann and Bridges 1990; Mariani et al. 1992), no consistent pattern providing an adequate explanation for the discrepancies in tumor predisposition has emerged from these studies.

Since there is ample evidence indicating that mutation is an important early initiation step in carcinogenesis, it was of interest to see whether the new XP patients have a significantly increased frequency of *hprt* mutations in circulating T-lymphocytes. We have previously shown that XP patients with residual repair synthesis measured in fibroblasts that ranges from 15% to approximately that in wild-type cells have, as a group, a highly significantly increased frequency of this mutation in T-cells (Cole et al. 1992). Since there is no evidence that cells from XP donors have an elevated *spontaneous* mutant frequency, whereas they are clearly hypermutable by UV, it was postulated that the raised in vivo frequency is also induced, possibly by exposure of the lymphocytes to sunlight as they pass through the skin (see Cole et al. 1992, and references therein). Figure 5 shows that the *hprt* mutant frequency in the two new XP/CS cases is clearly not greatly elevated compared with that in normal subjects, despite both the almost total deficiency in NER and the clear hypersensitivity of their lymphocytes to the lethal effects of both UV-C and UV-B (J. Cole and C. F. Arlett, unpublished data). All XP patients whom we had studied previously had, with two exceptions (XP125LO [XP-G] and XP7NE [XP-F]) been shown to have an elevated in vivo *hprt* mutant frequency in T-cells (Cole et al. 1992). XP7NE has no cellular hypersensitivity to the lethal effects of UV-C, in either T-cells or fibroblasts (Arlett et al. 1992). In addition, we have recently shown that in T-lymphocytes there is no hypersensitivity to UV-B, for either XP7NE or XP125LO (J. Cole and C. F. Arlett, unpublished data). These individuals are free of skin cancer. It is plausible that their normal mutant frequency reflects the absence of target-cell hypersensitivity to wavelength of UV irradiation relevant to the in vivo situation. However, T-lymphocytes from the XP/CS patients described here *are* hypersensitive to both UV-C and UV-B (data not shown); thus their normal

mutant frequency and lack of skin cancer set them apart from the other XP patients with elevated mutant frequencies. Possible explanations for the absence of skin cancer in the two new XP/CS patients include (1) an increased efficiency of alternative repair pathways (such as recombination repair), which may compensate for the reduced NER; (2) modified immune surveillance; or (3) the nature of the mutation in the *ERCC3* gene. It is worth noting that the *ERCC3* gene has additional functions in the cell, beyond its role in excision repair. The work of Gulyas and Donahue (1992), Mounkes et al. (1992), and Park et al. (1992) indicates an additional essential function of the *ERCC3* gene in yeast and *Drosophila*. Schaeffer et al. (1993) identified the *ERCC3* gene product as one of the components of the human transcription factor BTF2/TFIIH required for a late step in the initiation of transcription of class II genes. These findings shed new light onto the clinical features of XP and XP/CS, which were difficult to reconcile solely on the basis of deficient NER (Bootsma and Hoeijmakers 1993). Partial dysfunction of *ERCC3* may have a subtle effect on gene expression, resulting in retarded growth, immature sexual development, and demyelination of neurons. A part of the symptomatology of these patients and, probably, of CS in general might be due to a control function, of the involved genes, in transcription. The dual involvement of *ERCC3* could also provide an explanation for the rarity and pronounced clinical heterogeneity of XP-B, perhaps including the predisposition to cancer.

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