Defects in the DNA Repair and Transcription Gene ERCC2 in the Cancer-prone Disorder Xeroderma Pigmentosum Group D

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ABSTRACT

Xeroderma pigmentosum (XP) is a sun-sensitive, cancer-prone genetic disorder characterized by a defect in nucleotide excision repair. The human nucleotide excision repair and transcription gene ERCC2 is able to restore survival to normal levels after exposure to UV light in XP complementation group D cells. No enhancement of UV survival is seen in groups C, E, F, or G. XP-CS-2 cells are complemented by ERCC2, confirming the reassignment to group D of this combined XP/Cockayne’s syndrome patient. Nucleotide sequence analysis of the ERCC2 cDNA from five XP group D cell strains [XP6BE(SV40), XP17PV, XP102LO, A31-27 (a HeLa/XP102LO hybrid), and XP-CS-2] revealed mutations predominantly affecting previously identified functional domains. The mutations include base substitutions resulting in amino acid substitutions, deletions due to splicing alterations, and defects in expression. XP6BE(SV40), XP17PV, XP102LO, and A31-27 all have one allele with an Arg683 to Trp substitution within the putative nuclear location signal. The genetic disorder trichothiodystrophy (which is not cancer-prone) can also result from mutations in the ERCC2 gene, some of which are the same as those found in XP-D. The various clinical presentations can be correlated with the particular mutations found in the ERCC2 locus.

INTRODUCTION

XP is a rare autosomal recessive disorder characterized by hypersensitivity to UV radiation and sunlight-induced cutaneous and ocular abnormalities, including skin cancers (1). Seven complementation groups have been identified for classical XP, and the genes conferring UV resistance to cells from five of these groups have been cloned (reviewed in Ref. 2; group D, Refs. 3–5). Specific defects in these genes in cells from XP patients have been demonstrated for the XPA, ERCC3/XPB, XPC, and ERCC2/XPD genes (reviewed in Ref. 2; ERCC2/XPD, Refs. 6 and 7).

Clinically, XP-D patients display great variability in the severity of their condition in terms of both tumor incidence and neurological abnormalities (8). At the cellular level, XP-D shows a high degree of UV sensitivity, with moderate to high levels of repair synthesis (8). Some XP-D patients also show clinical features of CS (6, 9–12). TTD (which is not cancer-prone) can also result from mutations in the ERCC2 gene, some of which are the same as those found in XP-D. The various clinical presentations can be correlated with the particular mutations found in the ERCC2 locus.

MATERIALS AND METHODS

Cell Strains and Culture Conditions. AA8 is a repair-competent CHO line (28) and is the parental line from which the UV-sensitive line UV5, a member of complementation group 2, was derived (29–31). Stock cultures of AA8 and UV5 and transformant lines containing the neo marker were maintained as described previously (3, 19). XP6BE(SV40), a SV40-transformed XP-D fibroblast cell line, and XP-CS-2 (also previously called XP-SC-8 and XP-H), an XP-D primary fibroblast cell strain, were obtained from the National Institute of General Medical Sciences Human Genetic Mutant Cell Repository (Camden, NJ; GM08207B and GM03248, respectively). A31-27 (32) is derived from HD2, a permanent HeLa/XP102LO hybrid cell line reported to retain the excision repair characteristics of XP-D cells (33), and was kindly provided by J. Arrand (Gray Laboratory, Middlesex, UK). XP17PV is an XP-D primary fibroblast cell strain (34). Human cells were grown in α-MEM (KC Biologicales, Lenexa, KS) with 15% fetal bovine serum (KC Biologicals), 1.5 μg/ml glutamine, 100 μg/ml of streptomycin, and 100 units/ml of penicillin. Human transformant strains containing the neo marker
were selected and maintained in normal medium with 0.6 or 0.8 mg/ml G418 (GIBCO-BRL, Grand Island, NY).

**ERCC2 cDNA Expression Plasmid Construction.** ERCC2 cDNA expression plasmids with and without a neo dominant selectable marker (pE-ER2 and p2E-ER2, respectively) were constructed using a modified pcD vector (Fig. 1).

**Differentially Toxicity Assays.** Cells were electroporated with p2E-ER2 (ERCC2 cDNA expression plasmid) or pcD2E (control vector plasmid) DNA and plated at 1.3–1.4 x 10^7 cells/well for UV5 cells and 2.5 x 10^7 cells/well for XP cells in 6-well microtiter trays. Cells were exposed to UV radiation 18 h after plating and then cultured for 3 days for UV5 and for 5–7 days for XP cells. In this assay, functional complementation is indicated by a specific enhancement of cell growth over the culture period in cells transformed with p2E-ER2 relative to cells transformed with pcD2E (Fig. 2). This assay, which relies on the transient expression of unintegrated transforming plasmid DNA, was conceived based on the methods described in Ref. 35.

**Transformant Isolation.** XP6BE(SV40) and A31-27 stable ERCC2 transformants were obtained by electroporation with p2E-ER2, followed by selection for neo resistance. Independent colonies were isolated and split into two cultures: one maintained as a stock and one tested for UV resistance. To test for UV resistance, cells were plated in 60-mm dishes, cultured to the 8–16-cell stage, irradiated at 4 J/m^2, and cultured further. Dishes were examined daily from 2 to 4 days after irradiation to determine whether the cultures were UV resistant.

**UV Survival Curves.** Isolated transformant colonies positive for UV resistance were tested for UV survival by a colony-forming assay as described in Ref. 19, with UV exposure as described in Ref. 29.

**FISH.** FISH analysis of human metaphase cells was carried out as described in Ref. 36 using a biotin-labeled human ERCC2-containing cosmid clone (1986; Ref. 37).

**Reverse Transcription and PCR Amplification.** Total RNA was isolated from ~1 x 10^6 cells using the RNA isolation kit (Stratagene, La Jolla, CA). RNA from XP102LO was kindly provided by J. Arrand. Reverse transcription reactions were carried out using Moloney murine leukemia virus RNAse H^− reverse transcriptase (GIBCO-BRL, Gaithersburg, MD) as described previously (38).

**PCR was used to amplify overlapping fragments of ERCC2 using primers determined from the cDNA sequence (Table 1). Gene-specific primers for PCR amplification and nucleotide sequence determination were synthesized on a 394 DNA/RNA synthesizer (Applied Biosystems, Inc., Foster City, CA). PCR reactions contained 3 μl of the reverse transcription reaction and 2.5 units AmpliTaq (Perkin Elmer Cetus, Norwalk, CT) in 50 μl total volume and were 1X PCR buffer (Perkin Elmer Cetus), 0.2 mM each dNTP, 0.2 μM each primer, and 4% DMSO. After 90 s at 93°C, reactions were cycled 35 times with 1-min denaturing at 93°C, 1-min annealing at 65°C, and 1-min extension at 72°C (7 min in the final round).

**Genomic DNA Preparation and PCR Amplification.** Genomic DNA was prepared from ~1 x 10^7 cells using the QIAamp Blood Kit (Qiagen, Chatsworth, CA). PCR amplification primer pairs are summarized in Table 1. Amplification conditions were as described above, except 0.1 μg of genomic DNA or 50 ng of HindIII-digested genomic DNA was used as template, and no DMSO was used.

**Cloning and DNA Preparation for Nucleotide Sequence Analysis.** For the 997-bp 3' cDNA fragment, a second round of PCR amplification was performed, and the products were purified by gel electrophoresis. These 997-bp PCR products were isolated from 3% NuSieve GTG agarose (FMC BioProducts, Rockland, ME) using Bioclean (United States Biochemicals, Cleveland, OH). All remaining PCR products were cloned into the pCRII vector using the TA cloning kit (Invitrogen, San Diego, CA) or into the pGEM-T vector using the pGEM-T vector system (Promega, Madison, WI). Double-stranded plasmid DNA was prepared by the mini alkaline lysis method using the QIAprep-8 system (Qiagen). Clones with appropriate size inserts were identified on agarose gels and confirmed by nucleotide sequence analysis. DNAs from appropriate clones of each fragment were then pooled for complete nucleotide sequence analysis (10–15 clones/pool for cDNA analysis and 5–8 clones/pool for genomic DNA analysis).

**Nucleotide Sequence Determination and Analysis.** The nucleotide sequence of the 997-bp 3' cDNA PCR-amplified fragment was determined using the fmol DNA sequencing system (Promega) and labeling with [α-35S]dithio-dATP (Amersham, Arlington Heights, IL). The nucleotide sequence of cloned fragments was determined on double-stranded templates using the Laddermaker dye sequencing kit (Takara; Pan Vera Co., Madison, WI) and labeling with [α-35S]dithio-dCTP (Amersham). The reaction products were analyzed in 6% acrylamide(38:2)-urea gels. All nucleotide and protein sequence analysis and comparison was done using the GeneWorks software for the Macintosh (IntelliGenetics, Mountain View, CA).

**RESULTS**

**ERCC2 cDNA Expression Plasmid Testing in CHO Cells.** Stable transformants of CHO UV5 cells, the mutant line used to clone the ERCC2 gene, were obtained with pE-ER2 (cotransformed with the neo-containing pcD2E vector) and p2E-ER2. Both plasmids gave quantitatively efficient correction of the UV sensitivity of CHO UV5 cells (Fig. 3A). Conditions for a differential cytotoxicity assay were also established in UV5 cells using p2E-ER2 and the control plasmid pcD2E (see "Materials and Methods"). In this assay, which is based on transient expression, UV-exposure 6 or 18 h postelectroporation gave indistinguishably high levels of complementation, as reflected by a significant growth differential (data not shown). Previous work with a minigenie under the native promoter had shown a significant growth differential when UV exposure was 18 h postelectroporation and little or no growth enhancement when UV exposure was 24 h or more postelectroporation.5

**Correction of XP-D Cells by ERCC2.** Having demonstrated the efficient function of pE-ER2 and p2E-ER2 in both stable and transient expression systems in UV5 cells, these plasmids were used to rapidly screen for correction of XP cell strains representing groups C through G using the differential cytotoxicity assay. Enhanced growth was observed for the XP-D cell strains XP6BE(SV40), XP-CS-2, and A31-27 transformed with p2E-ER2 relative to those same cell strains transformed with the control plasmid pcD2E (Fig. 2, B–D). No growth enhancement was observed for XP groups C, E, F, or G (Fig. 2A and data not shown).

The quantitative efficiency of the correction of XP-D cells by the ERCC2 cDNA expression plasmid p2E-ER2 was tested in clonogenic

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Fig. 2. Differential cytotoxicity assays to test for correction of the UV sensitivity of XP cells. Functional complementation is indicated by a specific enhancement of cell growth over a period of 5–7 days in cells transformed with p2E-ER2 relative to cells transformed with pcD2E. Results for XP2YO(SV40) (A, group F); XP6BE(SV40) (B, group D); XP-CS-2 (C, group D); and A31-27 (D, group D) fibroblasts transformed with the p2E-ER2 ERCC2 expression plasmid or the pcD2E control plasmid are shown.

Survival assays using stable transformants of the two immortal XP-D lines XP6BE(SV40) and A31-27 (Fig. 3, B and C). Substantial correction of the UV sensitivity was observed in all transformants tested from both cell lines. The transformant D6BE-2ER2-9 was as UV resistant as the HeLa control cell line. This transformant has also been found to be as UV resistant as GM0637, an SV40-immortalized repair-proficient fibroblast line (39). Thus, ERCC2 cDNA is able to specifically and efficiently correct the UV sensitivity of XP-D cells.

Nucleotide Sequence Analysis of ERCC2 in XP-D Cells. To conclusively demonstrate that a defect in ERCC2 is present in XP-D cells, the nucleotide sequence of ERCC2 in XP6BE(SV40), XP17PV, XP102LO, A31-27, and XP-CS-2 cells was analyzed using overlap-
Table 1 PCR amplification primers for ERCC2

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a Base numbers are cDNA position where coding is from 79 to 2361 (19).

b The final three fragments are amplified from genomic DNA. Base numbers are the corresponding cDNA base position as complete genomic sequence data is not available.

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ping cDNA fragments (see “Materials and Methods”). For XP6BE(SV40), A31-27, and XP-CS-2, FISH indicated the presence of two ERCC2 alleles (data not shown). XP17PV and XP102LO were not examined by FISH.

XP6BE(SV40). Patient XP6BE had xeroderma pigmentosum with neurological abnormalities (40). She had early acute sun sensitivity and freckles in sun-exposed areas by 2 years of age. She had normal growth but severe mental retardation. By age 20, she had had 25–50 tumors, including 2 malignant melanomas. UDS levels were 25–55% of normal. The SV40-transformed fibroblast line derived from this patient, XP6BE(SV40), retains the UV hypersensitivity characteristics of XP (41).

Nucleotide sequence analysis of ERCC2 from XP6BE(SV40) revealed two populations of mRNA (Figs. 4, A and B, and 5, B and C). At position 2125, a C to T transition, which would result in a nonconservative Arg683 to Trp substitution in the putative nuclear location signal, was identified (Figs. 4A and SB). The equivalent amino acid position is also arginine in the four homologues (Fig. 6A). A deletion of exon 3 (bases 184–261; amino acids 36–61), which includes the conserved ATP-binding domain (helicase domain I; Figs. 5C and 6B), was also identified (Fig. 4B). Although the two altered cDNA regions are not within a single PCR amplified region, it is assumed that these mutations are from different alleles. Nucleotide sequence analysis of the genomic DNA around exon 3 revealed a T to A transversion in the consensus GT of the splice donor site of intron 3 (Fig. 4I and 5B). Using a matrix to score potential splice site sequences (42), the original intron donor scored +47, whereas the mutated donor scored −2. The deletion of the previous exon is a common result of splice donor site mutations (discussed in detail in Ref. 43).

XP17PV. Patient XP17PV had xeroderma pigmentosum with mild neurological abnormalities. He was 33 years old at the time of biopsy (in 1988) and was still alive in 1993. The first tumor appeared at age 22 years. Despite this mild clinical phenotype, the UDS level was reported as <10% of normal (34).

Nucleotide sequence analysis of ERCC2 from XP17PV revealed two populations of mRNA (Figs. 4, A and B, and 5, B and D). One allele has a C to T transition at position 2125 (Fig. 4A), which is identical to one allele found in XP6BE(SV40) (Fig. 5B). The second XP17PV allele has a G to A transition at position 1925 (Fig. 4C), which results in a nonconservative Arg616 to Pro substitution (Fig. 5C). Using a matrix to score potential splice site sequences (42), the original intron donor scored +47, whereas the mutated donor scored −2. The deletion of the previous exon is a common result of splice donor site mutations (discussed in detail in Ref. 43).

XP102LO. Patient XP102LO had xeroderma pigmentosum, showed normal growth and development at age 10, and had no neurological complications at age 18 (44). She had early acute sun sensitivity and freckles in sun-exposed areas. UDS levels were 20–30% of normal (8, 44).

Fig. 3. UV survival curves for stable pC2E/pE2R2 (5-EER2-2) cotransformants and p2E2R2 (5-2ER2-2) transformants of UVS (A) and p2E-ER2 transformants of XP6BE(SV40) (B) and A31-27 (C), along with untransformed and repair proficient controls, are shown. All measurements represent single determinations using triplicate dishes. A: , AAS; , UV5; , 5-EER2-5; , 5-EER2-6; , 5-EER2-2; and , 5-EER2-6. B: , and ; HeLa; , and ; XP6BE(SV40); , and , D6BE-2ER2-2; , and , D6BE-2ER2-9; and , and , D6BE-2ER2-10. C: , and ; HeLa; , and , A31-27; , and , D31-2ER2-13; and , and , D31-2ER2-18.
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Fig. 4. Autoradiograms showing the mutations identified in the ERCC2 cDNAs are shown for alleles 1 (A) and 2 (B, C, and D–F, respectively) of XP6BE(SV40), XP17PV, and XP102LO, and for XP-CS-2 (G). cDNA data are from pools of allele-sorted clones, except for the XP-CS-2 data, which are from direct sequencing of the PCR product. Autoradiograms showing the mutations identified in the ERCC2 gene from genomic DNA are shown for XP-CS-2 (H) and for allele 2 of XP6BE(SV40) (I) and A31-27 (J). Genomic DNA data are from pools of unsorted clones, except for the XP-CS-2 data, which are from direct sequencing of the PCR product. For A31-27, two pools of four clones each are shown because both pools have biased representation of one allele or the other. All sequences are in the sense direction except for I, which is in the antisense direction.

Analysis of both A31-27 and XP102LO revealed the same two mutant ERCC2 alleles, indicating that the hybrid line and the primary fibroblast carry the same ERCC2 alleles. One allele has a C to T transition at position 2125 (XP102LO, Fig. 4A; A31-27, data not shown), which is identical to one allele found in both XP6BE(SV40) and XP17PV (Fig. 5B). In the second ERCC2 allele, three alterations were identified: (a) a silent A to C transversion at position 546; (b) a C to G transversion at position 1459, resulting in a conservative Leu461 to Val substitution in helicase domain III; and (c) a 45-base deletion (bases 2224–2268), resulting in a 15 amino acid deletion near the COOH terminus (amino acids 716–730) (XP102LO: Fig. 4, D, E, and F; A31-27: data not shown; Fig. 5E). The equivalent of amino acid position 461 is also Leu in the S. pombe, fish, and hamster homologues, but is Ile in the S. cerevisiae homologue (Fig. 6D). The 15-amino acid deletion includes a region with 8 of 10 positions identical in all 5 homologues (Fig. 6E). Genomic DNA analysis in A31-27 revealed a C to G transversion at base 2228 of the corresponding cDNA that creates a new splice donor site for the final intron, resulting in the 45-base deletion (Figs. 4J and 5G; Ref. 19). This analysis also revealed that the final ERCC2 intron junction originally reported as occurring between positions 2269 and 2270 (19) is actually between positions 2268 and 2269 (error also identified in Ref. 45). The new splice donor site created at 2223 scored +52, whereas before the mutation, this site scored +5. Although not altered, the donor at 2268, which scored +49, is apparently not used when the mutation creating the site at 2223 is present.

Position 1459 is within both the 483–2090 and the 1001–2397 cloned PCR-amplified fragments. Thus, the linkage of both the 546 alteration and the deletion to the 1459 alteration, and the nonlinkage
of the 2125 alteration, were determined by analysis of individual clones.

XP-CS-2. Patient XP-CS-2 showed symptoms of both CS and XP (9), including neurological symptoms associated with CS rather than with XP. He had early acute sun sensitivity, was freckled, and had skin cancer at age 2. He had mental and growth retardation, and he died at age 13 from cancer (6). UDS levels for XP-CS-2 cells are reported to be 30–52% of normal (8, 46).

In XP-CS-2, only one ERCC2 cDNA type was observed (Fig. 4G). It has a G to A transition at position 1883, which would result in a nonconservative Gly602 to Asp substitution in the DNA binding domain (helicase domain V; Figs. 4G and SF). The equivalent amino acid position is also glycine in the four homologues (Fig. 6C). To determine whether the single cDNA type was due to the presence of the same mutation in both alleles or the apparent lack of expression of one of the alleles, analysis of the genomic DNA at this position was done. This analysis of XP-CS-2 genomic DNA revealed both G and A at this position (Fig. 4H). Thus, the two alleles in XP-CS-2 are different, and only one appears to be expressed. A mutation in the 5' flanking region of ERCC2 would be the most likely cause of an expression problem. This region was not analyzed, so there is no information as to the causative mutation in the unexpressed allele.

DISCUSSION

In this report, we describe the ability of ERCC2 to specifically and efficiently correct the UV sensitivity of XP-D cells and to identify causative mutations in five cell strains deriving from four XP-D patients, including a combined XP/CS patient. The differential cytotoxicity assay using the ERCC2 cDNA expression vector with XP cells from groups C through G showed a specific enhancement of UV resistance only for XP-D cell strains. The patient XP-CS-2 was initially reported to comprise XP group H (46), but further complementation studies concluded that this patient is a member of group D (10–12). The correction of both XP6BE(SV4O) and XP-CS-2 by ERCC2 confirms the assignment of XP-CS-2 to XP-D.

Fig. 5. ERCC2 cDNA mutations and resulting ERCC2 protein alterations are diagrammed for alleles 1 (B) and 2 (C, D, and E, respectively) of XP6BE(SV4O), XP17PV, and XP102LO (and A31-27), and for XP-CS-2 (F). Numbers below cDNAs indicate the affected nucleotide positions where coding is from 79 to 2361, including the stop codon. The under- and overlined regions are the putative nuclear location signal and conserved helicase domains (see Fig. 5, legend). Arrows, position of the New splice donor (final Intron) and Consensus TLDAKGHGVL

1. Normal
2. ATP binding
3. Mg^2+ binding
4. DNA binding
5. NLS
6. 2125 C->T
7. 603 Arg->Trp
8. 194-261 deleted
9. as 36-61 deleted
10. 1925 G->A
11. 546 A->C
12. 1459 C->G
13. 2224-2268 deleted
14. 461 Leu->Val as 716-730 deleted
15. 1883 G->A
16. 602 Gly->Asp

Fig. 6. Alignment of the ERCC2 homologues from four species in the regions of the XP6BE(SV40) (A and B), XP17PV (A and C), XP102LO/A31-27 (A, D, and E), and XP-CS-2 (C) mutations is shown. Rad15 is from S. pombe, hamster ERCC2 is from Chinese hamster, fish ERCC2 is from X. niaculatus, and Rad3 is from S. cerevisiae (SwissProt Accession nos.: rad15, P26659; human ERCC2, P18074; Rad3, P06839. Genomic DNA sequence Genbank accession nos. hamster ERCC2, U04967 and U04968; fish ERCC2, 417986). The under- and overlined regions are the putative nuclear location signal and conserved helicase domains (see Fig. 5, legend). Arrows, position of the identified amino acid substitution, boxed amino acids, deletions in the corresponding cell strain.

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Partial correction of the UV sensitivity of XP-D cells by transformation with an ERCC2-containing cosmid clone was observed previously (5). In our study, stable ERCC2 cDNA transformants of the XP-D lines XP6BE(SV40) and A31-27 were used to measure the quantitative efficiency of the correction of UV sensitivity by ERCC2. On the basis of relative D10 values (dose corresponding to 10% survival), XP6BE(SV40) is ~9.6-fold sensitive, whereas the ERCC2 cDNA transformants of XP6BE(SV40) range from 1.0- to 1.8-fold sensitive. Thus, ERCC2 alone can efficiently restore UV resistance to XP-D cells. In further studies (39), ERCC2 antibody-reactive protein levels in transformants D6BE-2ER2-2 and D6BE-2ER2-9 were found to be elevated 4.8- and 17.6-fold, respectively, and the shuttle vector plasmid UV hypersensitivity and hypermutability were both corrected.

XP/CS. In cells from pure CS patients, the repair of actively transcribed regions of the DNA is deficient (47). Recently, both ERCC2/XPD and ERCC3/XPB have been identified as components of transcription factor IIH (reviewed in Ref. 2). The three known patients in XP-B all have combined clinical features of XP and CS (11, 40). These data support the hypothesis that mutations affecting the roles of the ERCC2/XPD and ERCC3/XPB proteins in transcription and/or transcription-coupled repair result in the clinical features of CS. The expressed allele in XP-CS-2 has a Gly602 to Asp change in the DNA-binding box (helicase domain V). Gly604 of Rad3, which is analogous to position 602 of ERCC2, is mutated to Arg in S. cerevisiae strain rad3-24 (48). This yeast mutant is viable but UV sensitive. Future studies to assess the effect of the XP-CS-2 mutation in the DNA-binding domain on the repair and transcription activities of ERCC2 may provide further insights.

XP. XP6BE(SV40), XP17PV, and XP102LO (and A31-27) all have one allele with the same mutation in the putative nuclear location signal. This same mutation is also found in XP1DU.6 Because this mutation is due to a C to T transition at a CpG site, it is likely due to deamination of 5-methylcytosine. The identification of this mutation in cell strains from all four XP patients we have studied, and not in any of the cell strains from XP/CS (2 patients) or TTD (15 patients) we have studied (6, 45), strongly suggests that it is related to the XP presentation in these patients. This mutation was not identified in either XP1NE or XP67MA (7); however, for both cell strains, a mutation was identified in only one allele. Given the partial nature of the analysis, it is uncertain whether either of these strains may carry the mutation in the putative nuclear location signal on the other allele. This mutation indicates that the region identified as the putative nuclear location signal is essential for the DNA repair function of ERCC2 protein and suggests that this region may indeed function as a nuclear location signal. If the product of this allele in fact fails to enter the nucleus, it would be necessary for the product of the second allele to provide the viability function (presumably transcription). However, mutation of the nuclear location signal may not completely eliminate nuclear location of the protein. If the mutation does not otherwise alter the biochemistry of ERCC2, then there could be a small amount of functional ERCC2 present in the nucleus of these XP cells. We speculate that this limited amount of functional protein in the nucleus may be sufficient for transcription but insufficient for DNA repair. Analysis of the cellular localization of ERCC2 protein in these cells may demonstrate whether nuclear localization is the role of this protein region.

In XP6BE(SV40), the second allele has a deletion of the ATP-binding domain. The Rad3-Arg48 mutation in the ATP-binding domain results in loss of the ATPase and helicase activities of Rad3, but not loss of ATP binding (49). Yeast cells with this mutation are viable but UV sensitive. In XP17PV, the second allele has an Arg618 to Pro substitution. This same mutation is found in one allele of TTD1BEL, a highly UV-sensitive TTD cell strain (45). In XP102LO (and A31-27), the second allele has both a Leu461 to Val substitution in helicase domain III and a deletion of amino acids 716–730. The Leu461 to Val substitution is also found in XP1NE, and an expression construct with the conservative Leu461 to Val mutation is unable to correct the UV sensitivity of mutant cells (7). The 45-base deletion results in the deletion of amino acids 716–730, which includes a 10-amino acid region with very high evolutionary conservation. Additional evidence that this region also plays an important role in repair comes from the finding of an Arg722 to Trp mutation in TTD1BEL (45). Thus, both the conservative Leu461 to Val substitution and the 716–730 deletion seen in allele 2 of XP102LO (and A31-27) are predicted to be functionally important. This allele is also found in several TTD patients.

Cancer. TTD patients are not cancer prone, whereas XP-D is cancer prone, and one of two XP-D/CS patients had cancers. For the purpose of this discussion, we are assuming that the phenotype is attributable to the ERCC2 mutations and not to any other factors. The allele with the mutation in the putative nuclear location signal is found in all the cell strains from the 4 XP-D patients we have examined, and is not found in the 2 XP-D/CS or the 15 TTD strains we have examined. Because the second allele for XP102LO and the second allele for XP17PV are also found in TTD patients, the other allele (with the mutation in the putative nuclear location signal in the XP-D patients) is likely to be responsible for the phenotypic differences. None of the mutations found in the two XP-D/CS strains has been seen in any of the TTD or XP-D cell strains we have examined. Thus, these mutations are likely responsible for the unique clinical presentations of these patients. We suggest, as have others (45, 50), that the TTD presentation is due to mutations that primarily alter the transcriptional role of ERCC2, whereas the XP-D presentation is due to mutations that primarily alter the repair role of ERCC2. Consistent with this suggestion, some TTD cell strains are not UV sensitive, and some show only a reduction in the rate of repair of (6-4)photoproducts (51).

Concluding Remarks. Mutations in the ERCC2 gene have been identified in cells from XP-D patients (this study and Ref. 7), XP-D/CS patients (this study and Ref. 6), and photosensitive TTD patients (Ref. 45). Continued analysis of the molecular defects in the ERCC2 gene in cells from additional patients with diverse clinical presentations, including nonphotosensitive TTD patients, and studies of the effects of these mutations on the biochemical properties of the ERCC2 protein, may provide insights into the roles of the ERCC2 protein in DNA repair, transcription-coupled repair, and transcription.

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Defects in the DNA Repair and Transcription Gene *ERCC2* in the Cancer-prone Disorder Xeroderma Pigmentosum Group D

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