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Functional complementation of xeroderma pigmentosum complementation group E by replication protein A in an *in vitro* system

(DNA repair/damage recognition/excision nuclease)

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ABSTRACT **Xeroderma pigmentosum (XP) is caused by a defect in nucleotide excision repair. Patients in the complementation group E (XP-E) have the mildest form of the disease and the highest level of residual repair activity. About 20% of the cell strains derived from XP-E patients lack a damaged DNA-binding protein (DDB) activity that binds to ultraviolet-induced (6-4) photoproducts with high affinity. We report here that cell-free extracts prepared from XP-E cell strains that either lacked or contained DDB activity were severely defective in excising DNA damage including (6-4) photoproducts. However, this excision activity defect was not restored by addition of purified DDB that, in fact, inhibited removal of (6-4) photoproducts by the human excision nuclease reconstituted from purified proteins. Extensive purification of correcting activity from HeLa cells revealed that the corrrecting activity is inseparable from the human replication/repair protein A [RPA (also known as human single stranded DNA binding protein, HSSB)]. Indeed, supplementing XP-E extracts with recombinant human RPA purified from *Escherichia coli* restored excision activity. However, no mutation was found in the genes encoding the three subunits of RPA in an XP-E (DDB$^-$) cell line. It is concluded that RPA functionally complements XP-E extracts *in vitro*, but it is not genetically altered in XP-E patients.**

Nucleotide excision repair is a DNA repair system that eliminates the large majority of modified nucleotides from DNA by dual incisions on both sides of the lesion in the damaged strand (1–4). In humans, defects in excision repair cause the disease xeroderma pigmentosum (XP), and genetic analyses of cell lines from XP patients defective in repair have identified seven complementation groups, XP-A–XP-G (5). In recent years, with the exception of XP-E, all of the XP genes have been cloned and characterized in some detail (1).

Patients belonging to the XP-E complementation group manifest a mild form of the disease, having 40–50% residual excision repair activity as measured by *in vivo* repair assays (5). The nature of the repair defect in XP-E cells is not known. However, cell strains from 2 out of 13 unrelated XP-E individuals lacked a damage-specific DNA-binding (DDB) activity in nuclear extracts (6–8) and therefore were termed as DDB$^-$ XP-E cells. The DDB activity is associated with a heterodimer of two polypeptides with molecular masses of 127,000 Da and 48,000 Da (9) and the genes for both subunits have been cloned and sequenced (10–12). Studies with DNA substrates containing single ultraviolet photoproduct or a psoralen-thymine adduct demonstrated that DDB bound with high affinity to (6-4) photoproducts but not to the *cis,syn*-thymine cyclobutane dimer or to the psoralen-thymine monoadduct (13); however, all these lesions are repaired by the human excision nuclease system (14, 15). A separate study found that microinjection of purified DDB corrected the ultraviolet-repair defect of XP-E (DDB$^-$) strains but not that of XP-E (DDB$^+$) cell lines (16). Thus, at present, the relationships among DDB, the biochemical defect in XP-E, and the cause of repair defect in XP-E cell strains remain to be clarified.

To answer these questions, we have taken a biochemical approach. Using an *in vitro* excision assay that measures the release of DNA damage in 24-32 nucleotide-long oligomers (14, 15, 17), we tested cell-free extracts (CFEs) from DDB$^+$ and DDB$^-$ XP-E cells and found that both types of cells were defective in excision activity. Attempts to correct the repair defect with purified DDB using CFEs from either type of XP-E cell lines failed, raising some questions regarding the role of DDB in nucleotide excision repair. To resolve this, we supplemented a reconstituted excision nulcease system of highly purified proteins with DDB and found that DDB inhibited excision under XP-A or RPA-limiting conditions but had no effect under optimal reaction conditions. Surprisingly, the protein purified for its XP-E complementing activity in the excision assay turned out to be the replication protein A [RPA (also known as HSSB)]. Indeed, a recombinant RPA purified from *Escherichia coli* also restored the excision activity of XP-E mutant extracts.

MATERIALS AND METHODS

Cultured Cells and CFEs. HeLa S3 and mutant cell strains were from the Lineberger Cancer Center (University of North Carolina). Mutant human strains used in this study were: XP-E 2RO (DDB$^-$), XP-E 82TO (DDB$^-$), XP-E 93TO (DDB$^+$), XP-A 20S, XP-C 9BE, and XP-G 2BI. The XPG mutant Chinese hamster ovary cell line UV135 (18) was a kind gift of L. H. Thompson (Lawrence Livermore National Laboratory CA). CFEs were prepared by the method of Manley *et al.* (19) with minor modifications as described (20).

Proteins. DDB was purified as described (9). The DDB used in this study contained stoichiometric amounts of both subunits. Human RPA was purified from HeLa cells (21, 22), or from an *E. coli* strain containing a plasmid expressing all of the three RPA subunits (23) kindly provided by M. S. Wold (University of Iowa-Iowa City). Yeast RPA was a kind gift of S. Matson (University of North Carolina).

Excision Assay. The excision assay measures the removal of the DNA lesion in 24- to 32-nt long oligomers (14, 17). Two substrates were used: (*i*) a 140-bp duplex with a cholesterol derivative substituted for a nucleotide at position 70 of one strand and ^{32}P label at the sixth phosphodiester bond 5′ to the cholesterol (21), and (*ii*) a 177-bp duplex with a centrally

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Abbreviations: XP, xeroderma pigmentosum; XP-A, etc., XP complementation group A, etc.; DDB, damage-specific DNA binding; CFE, cell-free extract; RPA, replication protein A.

located (6-4) photoproduct and ^{32}P label at the 20th phosphodiester bond 5′ to the lesion. Both substrates were assembled from six partially complementary oligomers as described previously (21, 24, 25). The oligonucleotide with the cholesterol substitution was purchased from Midland Certified Reagent (Midland, TX) and the oligonucleotide with the (6-4) photoproduct was prepared by the method of Smith and Taylor (26). The excision assay reaction mixture (25 μl) contained 35 mM Hepes (pH 7.9), 50 mM KCl, 40 mM NaCl, 5.6 mM $MgCl_2$, 2 mM ATP, 20 μM of each dNTP, 0.4 mM EDTA, 1 mM dithiothreitol, 3.5% (vol/vol) glycerol, 100 μg BSA, 1 nM DNA substrate, 50 μg of CFE (complementation assays with extracts from two different strains contained 25 μg from each cell type), and DDB or RPA as indicated. To detect residual excision in XP-G CFE, it was necessary to use 75 μg of protein. Incubation was for 60 min at 30°C and the reaction products were then analyzed on 10% denaturing polyacrylamide gels. Excision assay with the reconstituted excision nuclease was conducted as described (21, 22) using either ≈1 ng (limiting) or 25 ng of XPA (optimal) and the indicated amounts of DDB per 25 μl reaction mixture.

Gel Retardation Assay. Binding of DDB to substrate and control DNAs by electrophoretic mobility-shift assay was conducted as described (13).

Western Blot Analysis. Monoclonal antibodies against the 70-kDa subunit of RPA were a kind gift of B. Stillman (Cold Spring Harbor Laboratory) or were purchased from Oncogene Science. CFEs were separated by SDS/PAGE (10% polyacrylamide), and the proteins were transferred to nitrocellulose membranes and probed with antibodies following the manufacturer's protocol (Oncogene Science).

Sequencing of RPA cDNAs From an XP-E Cell Line. Total RNA was isolated from 3×10^7 XP-E 2RO cells, and 1.5 μg was used for first strand cDNA synthesis using reverse transcriptase Super Script II (BRL) and a poly(dT) primer. Gene amplifications by PCR with Taq polymerase (Boehringer Mannheim) were carried out with specific primers for each of the subunits which were designed based on published sequences (27–29) and placed outside the open reading frame and introduced restriction sites for subcloning: RPA 70, TATGAATTCTTGGCGGTGGAGCC and TAGGATCCAGGTGCTCATGACCGCTT; RPA34, CTGGATCCGGAGAATCGTGACCAAGATG and AAGGATCCAGACATATGCAGAGCTGGA; RPA14, CAGGATCCGCAGTCTTGGACCATAATC and AATGGTACCATATATTGGAGTAGCA.

The cDNA of RPA 70 was cloned into the *Eco*RI-*Bam*HI digested pUC19. The RPA 34 cDNA was cloned into *Bam*HI-*Kpn*I digested pUC18, and the RPA 14 cDNA was cloned into *Bam*HI-*Kpn*I digested pUC18. In case of RPA 34 cDNA, the unique *Kpn*I restriction site at the 3′ end was used for subcloning. Ten independent clones of each subunit were sequenced at the University of North Carolina (Chapel Hill) Automated DNA Sequencing Facility using a Model 373A DNA Sequencer (Applied Biosystems) and the Taq Dye Deoxy™ Terminator Cycle Sequencing Kit (Applied Biosystems). By sequencing 10 independent clones, we aimed at having a high probability of detecting a mutation that may be present in only one copy of the gene. XP is a recessive disease and cells from patients are expected to have separate mutations in each copy of the gene. Furthermore, multiple independent sequences allowed us to eliminate errors that may have been introduced during cDNA cloning and PCR amplification.

RESULTS

Purification of an XP-E Complementing Activity. Recently, we reconstituted human excision nuclease with six highly purified polypeptides or polypeptide complexes which contained all of the known XP gene products (21, 22). The six factors were XPA (recombinant), TFIIH (that contains seven polypeptides including XPB and XPD), XPC/HHR23B, XPG, XPF/ERCC1, and RPA (recombinant). Because the combination of these six factors gave an excision signal comparable with that obtained with CFE under optimal conditions, we reasoned that one of our reconstitution factors may contain the XPE protein as a contaminant. However, none of the factors purified from HeLa cells restored excision activity to an XP-E CFE (21). Therefore, we decided to test purification fractions at early stages of our purification scheme (21). An XP-E CFE complementing activity was found in fractions 12-13 of the third MonoQ column. This activity was further fractionated using Phenyl Superose HR5/5 chromatography (Fig. 1*A*). On this column, the XP-E-complementing activity was not separable from RPA as demonstrated by immunoblotting using anti-RPA p70 antibodies (Fig. 1*B*). Furthermore, subsequent single-stranded DNA cellulose column retained RPA and the XP-E complementing activity (data not shown).

The CFE used in these complementations was from XP-E 2RO, which is DDB^- (6, 8), so we also asked whether the active fractions complemented DDB^+ XP-E CFE as well. Both DDB^- and DDB^+ XP-E CFE were complemented by the fraction that contains RPA (Fig. 2) to the same level as that obtained on mixing XP-E CFE with CFE from a different complementation group (Fig. 2, compare lanes 2 and 3 and lanes 5 and 6).

Complementation of the XP-E Defect by Recombinant RPA. Since XP-E complementing activity was inseparable from RPA on all columns tested, RPA itself could have been responsible for the complementation. Therefore, human RPA expressed and purified from *E. coli* was tested (Fig. 3). Indeed, 250–400 ng of recombinant RPA is as active in restoring

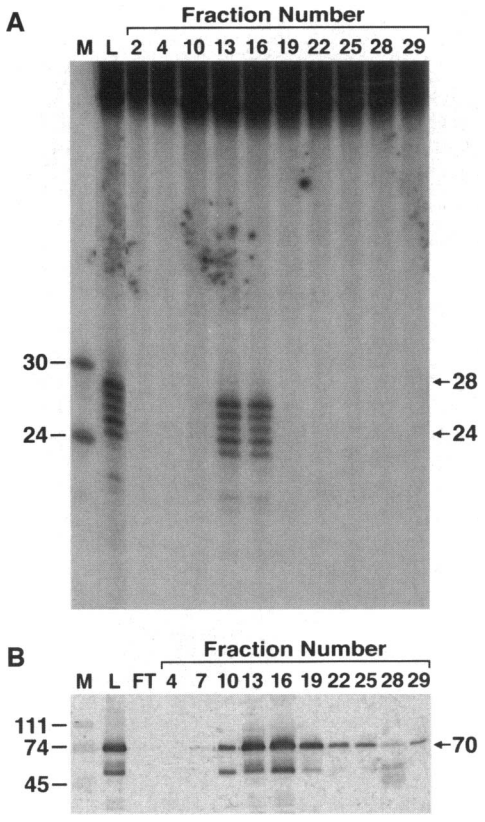

FIG. 1. Purification of XP-E complementing activity. Fractions of the phenyl superose column were tested for excision activity *(A)* and the RPA 70 subunit *(B)* by Western blot analysis. The material loaded onto the column was fractions 12-13 of the third MonoQ column as described by Mu *et al.* (21). The positions of the excision products (24–28) and of the RPA 70 subunit are indicated by arrows. The 50-kDa protein in *B* is a proteolytic product of the p70 subunit (30, 31).

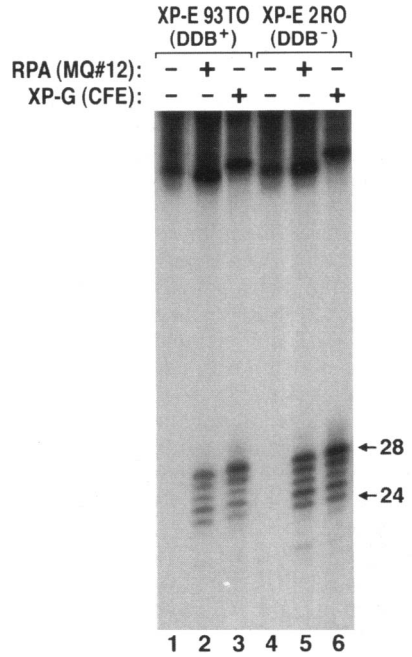

FIG. 2. Restoration of excision activity to XP-E 93TO (DDB$^+$) and XP-E 2RO (DDB$^-$) CFE by partially purified RPA. To the CFEs (50 μg), protein (1 μl) from the MonoQ peak fraction (MQ #12) containing RPA was added as indicated.

excision function to an XP-E CFE as the material purified from HeLa cells that contained ≈200 ng of RPA. Thus, RPA appears to be capable of restoring the XP-E defect.

Specificity of RPA Complementation to XP-E. RPA is required for both excision (21) and repair DNA synthesis (32, 33) and it stimulates repair synthesis even when added to extracts from normal cells (32). Therefore, we considered the possibility that XP-E may have been complemented by RPA due to nonspecific stimulation. In fact, 50 μg of XP-E CFE that contains about 100 ng of RPA (30) does have residual excision activity in the excision assay (ref. 13; see also Fig. 4, lane 1). Therefore, the effect of RPA on CFE from three other XP cell lines, XP-A (totally defective), XP-C (leaky), and XP-G (leaky), was tested (Fig. 4). RPA does not restore activity to the XP-A extract and has no stimulatory effect on the residual activity in XP-C extract but it does stimulate the residual activity in the XP-G extract. However, the stimulation of the activity in XP-G extract is similar to that we observe with normal CFE (2- to 3-fold) and is not comparable with the level of excision activity conferred to XP-E extract by RPA. Full restoration of activity (>25-fold stimulation of residual activity) is seen only with XP-E CFE (Fig. 4, lane 3). Yeast RPA, which has similar biochemical properties to human RPA (31, 34), has a slight inhibitory effect (Fig. 4, lane 2) on the residual excision activity in the XP-E CFE. Similarly, the addition of human RPA p70 subunit (23) only, has no effect on excision activity of the XP-E extracts (data not shown).

Effect of DDB on Excision Activity of XP-E CFE. Even though the excision defect of XP-E CFE can be complemented by RPA, there is no evidence for an RPA defect in XP-E cells. On the contrary, it is well-established that some XP-E cell lines are lacking a DDB activity in nuclear extracts (6–8). The protein responsible for this activity has been purified (8) and it binds with high affinity and specificity to ultraviolet-induced (6-4) photoproducts but not to *cis*, *syn*-cyclobutane dimers (13), nor to the cholesterol substrate used in our complementation assays (data not shown). Therefore, to find out if there is a causal relationship between the lack of DDB activity and defective repair of UV damage in XP-E cells, we constructed a substrate containing a single (6-4) photoproduct for the excision assay. Fig. 5*A* shows that the purified DDB binds this substrate with high specificity in agreement with previous results (13). As seen in this figure, 20 fmol of DDB in the reaction mixture binds stoichiometric amount (≈80%) of the substrate (lane 3) with only modest nonspecific binding (lane 6). Thus, to investigate the effect of DDB on repair, the excision assay was conducted with the substrate DNA preincubated with DDB to form DDB-DNA complex followed by addition of CFE (Fig. 5*B*). DDB did not restore the excision

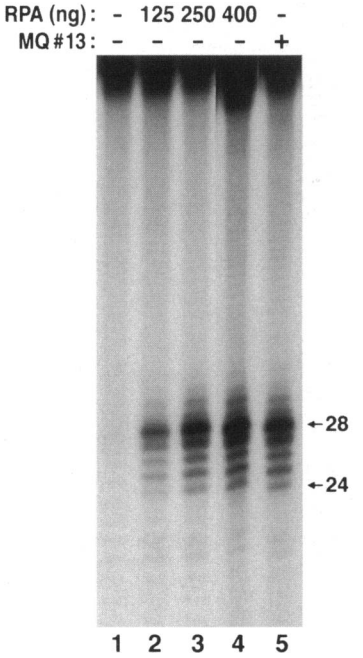

FIG. 3. Complementation of XPE defect by recombinant RPA. The indicated amounts of RPA overexpressed in and purified from *E. coli* were added to XP-E 2RO (DDB$^-$) CFE. As a control, a 1-μl aliquot of MonoQ fraction 13 (MQ #13) containing about 200 ng of RPA was added to the same CFE (lane 5).

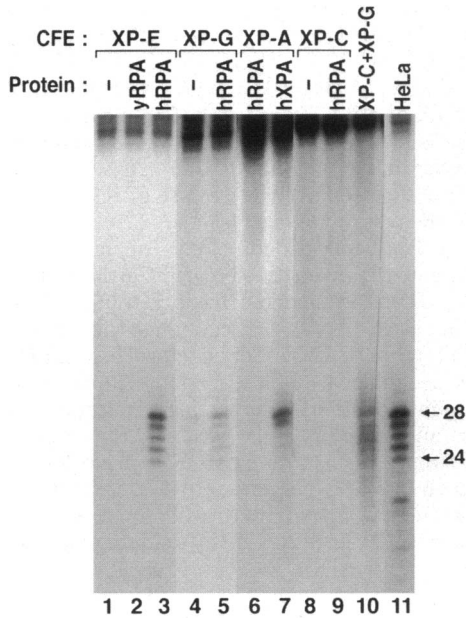

FIG. 4. Complementation specificity of human RPA. To CFE from the indicated XP cells, 400 ng of recombinant human RPA (hRPA) or 400 ng of yeast RPA (yRPA) was added and excision reaction was carried out. Lanes 10 and 11 show complementation by two mutant CFEs and excision with HeLa CFE, respectively, as positive controls.

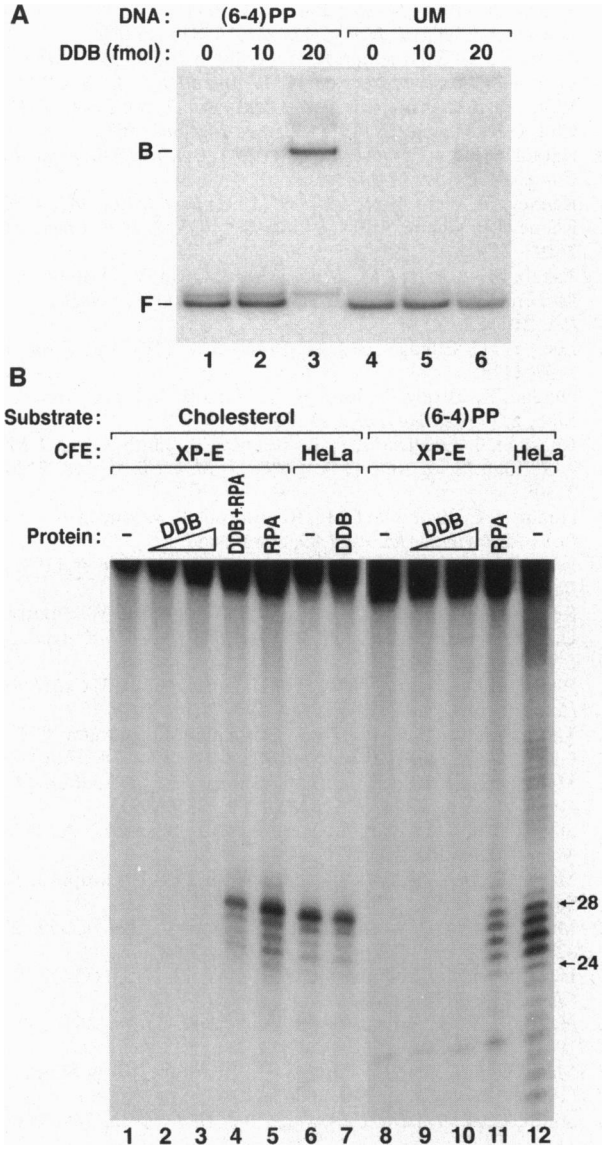

FIG. 5. Effect of DDB and RPA on excision activity of a (DDB⁻) XP-E CFE. *(A)* Gel retardation experiment with DDB and substrate (25 fmol) containing a (6-4) photoproduct or unmodified DNA (UM). *B.* Effects of DDB and RPA on excision. Using substrates containing cholesterol (lanes 1–7) or (6-4) photoproduct (lanes 8–12), excision assays were conducted with 10 or 20 fmol of DDB or 125 ng of RPA added to the reaction mixtures as indicated.

activity to CFE from DDB⁻ cell lines on substrates containing either cholesterol (lanes 2 and 3) or (6-4) photoproduct (lanes 9 and 10), whereas RPA restored the activity with both substrates (lanes 6 and 11). In fact, the addition of DDB to RPA-supplemented XP-E extract (lane 4) or to HeLa CFE (lane 7) had a moderate inhibitory effect. When the order of addition was changed by mixing DDB with CFE for 30 min before addition of damaged DNA substrate, the results were the same (data not shown). Thus, we conclude that RPA but not DDB complements XP-E (DDB⁻) CFE for excision activity on a substrate that is recognized by DDB with high affinity.

To further investigate the role of DDB in nucleotide excision repair, we supplemented the excision nuclease system reconstituted from highly purified proteins with DDB. Because biochemical properties of DDB suggest that it might participate in damage recognition, we carried out the excision reaction with the reconstituted system under limiting amount of XPA or RPA that are known to be involved in damage

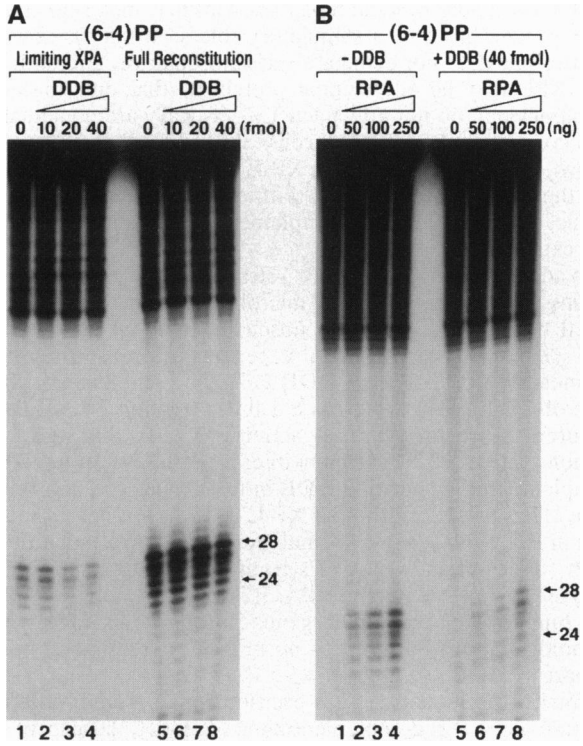

FIG. 6. Effect of DDB on excision of (6-4) photoproduct by the reconstituted system. *(A)* Reactions with limiting XPA. Lanes 1–4 contained about 1 ng and lanes 5–8 contained 25 ng of XPA. The percent of (6-4) photoproduct excised was determined using PhosphorImager (Molecular Dynamics) and was as follows: lane 1, 0.45; lane 2, 0.42; lane 3, 0.16; lane 4, 0.10; lanes 5–7, 6.0; and lane 8, 5.0. *(B)* Reactions with limiting RPA. Excision reaction with reconstituted system was carried out in the absence (lanes 1–4) or presence (lanes 5–8) of 40 ng of DDB and the indicated amounts of RPA. The percent of (6-4) photoproduct excision were as follows: lane 1, 0.0; lane 2, 1.0; lane 3, 1.5; lane 4, 3.0; lane 5, 0.0; lane 6, 0.4; lane 7, 0.6; and lane 8, 1.2.

recognition (2–4). As shown in Fig. 6*A*, lanes 1–4, under XPA limiting conditions inhibition rather than stimulation of excision was observed with increasing amount of DDB. Under optimal conditions for excision with the reconstituted system, the inhibitory effect of DDB was less pronounced (Fig. 6, lanes 5–8). Similarly, when we attempted to substitute DDB for RPA in the defined system, it failed to reconstitute the excision nuclease activity, and as in the case of XPA-limiting conditions DDB inhibited the excision activity under RPA-limiting conditions (Fig. 6*B*). These results fail to implicate DDB in excision nuclease activity, consistent with the data obtained using CFE.

RPA Is not Mutated in XP-E. The data presented so far is consistent with a mutation in one of the RPA subunits as the cause of the XP-E phenotype. Because RPA is an essential protein for replication, a missense mutation was a likely possibility. Therefore, we sequenced the cDNAs of all three subunits from XP-E 2RO multiple times (see *Materials and Methods*) and found that the sequences were identical to published sequences of RPA cDNAs. The codon for Ser535 of the p70 subunit was TCT in 5 and TCC in 4 out of 9 clones sequenced suggesting silent sequence polymorphism at this position. This also provides direct evidence that, at least for this subunit, the cDNAs from both parents were analyzed.

DISCUSSION

The genetics and biochemistry of XP-E pose a unique problem. The pertinent facts are briefly discussed below.

First, patients in the XP-E complementation group, as a rule, have a mild clinical form of XP and all of the XP-E patients

exhibit about 50% residual repair (5). This may mean either that XPE protein has only a stimulatory role such that its absence reduces but does not eliminate excision repair or, alternatively, that XPE may be an essential protein so that only missense mutations that do not affect activity drastically are nonlethal.

Second, even though XP-E cells exhibit 50% residual activity *in vivo*, the excision assay with XP-E CFEs reveals at most only 5% the level of excision seen with either a normal fibroblast extract or an XP-E extract complemented with another mutant cell extract (see Fig. 2).

In addition, the XP-E(DDB$^-$) strains but not XP-E(DDB$^+$) strains are complemented by microinjection of DDB as measured by ultraviolet-induced unscheduled DNA synthesis *in vivo* (14). In contrast, with a repair synthesis assay and permeabilized cell system DDB failed to complement either type of XP-E strains whereas a cellular fraction free of DDB restored the repair synthesis activity (35). In this study, we demonstrate that CFEs from neither type of XP-E mutants are complemented by purified DDB in the *in vitro* excision repair assay. However, even though XP-E cells do not have a mutation in RPA and express normal levels of all three subunits of RPA (data not shown), CFEs from both DDB$^-$ and DDB$^+$ cells are complemented by RPA. It is also pertinent that all of the human excision repair genes and proteins have yeast homologs (1, 36) but there is no known budding yeast counterpart to DDB.

Finally, the human *in vitro* excision repair system does not contain any XP-E complementing activity (21, 22) except the activity intrinsic to RPA. Similarly, the yeast *in vitro* excision repair system (37) is analogous to the human system in that the proteins required for reconstitution are those defined by the XPA, XPB, XPC, XPD, XPF, XPG, and ERCC1 homologs, the other subunits of TFIIH (in addition to XPB and XPD), and RPA.

It is not easy at present to propose a model for the function of XPE gene and protein consistent with all of these findings. However, the fact that XP-E CFE are defective in excision repair *in vitro* suggests that XPE is not a regulatory gene exerting its effect on cell survival by affecting cell cycle checkpoints. Similarly, we have found (data not shown) by Western blot analysis that XP-E cell strains contain normal levels of the following excision repair proteins: XPA, XPB, XPC, XPD, ERCC1, XPG, and the p62, cyclin H, and CDK7 subunits of TFIIH. Therefore, the reduced excision in XP-E cell lines is not caused by an overall decrease in excision nuclease level as a result of a mutation in a global regulator. The fact that the defect can be complemented by RPA, which is involved in damage recognition, is consistent with the notion that the XPE gene product acts as a molecular chaperone or a molecular matchmaker to aid in the assembly of RPA with the other subunits of the excision nuclease at the damage site (38–40). In the presence of high concentration of RPA, the repair complex may form without the aid of the matchmaker. Naturally, this model does not address the unusual relation between the XP-E phenotype and the DDB protein. Further work is needed on DDB gene and protein to understand whether or not it plays a direct role in repair.

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1. Friedberg, E. C., Walker, G. C. & Siede, W. (1995) *DNA Repair and Mutagenesis* (Am. Soc. Microbiol., Washington, DC), pp. 191–365.
2. Tanaka, K. & Wood, R. D. (1994) *Trends Biochem. Sci.* **19,** 83–86.
3. Sancar, A. (1994) *Science* **266,** 1954–1956.
4. Sancar, A. (1995) *J. Biol. Chem.* **270,** 15915–15918.
5. Cleaver, J. E. & Kraemer, K. H. (1989) in *The Metabolic Basis of Inherited Disease*, eds. Scriver, C. R., Beaudet, A. L., Sly, W. S. & Valle, D. (McGraw-Hill, New York), Vol. 2, pp. 2949–2971.
6. Chu, G. & Chang, E. (1988) *Science* **242,** 564–567.
7. Kataoka, H. & Fujiwara, Y. (1991) *Biochem. Biophys. Res. Commun.* **175,** 1139–1143.
8. Keeney, S., Wein, H. & Linn, S. (1992) *Mutat. Res.* **273,** 49–56.
9. Keeney, S., Chang, G. J. & Linn, S. (1993) *J. Biol. Chem.* **268,** 21293–21300.
10. Takao, M., Abramic, M., Moos, M., Jr., Otrin, V., Wooton, J. C., McLenigan, M., Levine, A. S. & Protic, M. (1993) *Nucleic Acids Res.* **21,** 4111–4118.
11. Lee, T. H., Elledge, S. J. & Butel, J. S. (1995) *J. Virol.* **69,** 1107–1114.
12. Dualan, R., Brody, T., Keeney, S., Nichols, A. F., Admon, A. & Linn, S. (1995) *Genomics* **29,** 62–69.
13. Reardon, J. T., Nichols, A. F., Keeney, S., Smith, C. A., Taylor, J.-S., Linn, S. & Sancar, A. (1993) *J. Biol. Chem.* **268,** 21201–21308.
14. Huang, J. C., Svoboda, D. L., Reardon, J. T. & Sancar, A. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 3664–3668.
15. Svoboda, D. L., Taylor, J. S., Hearst, J. E. & Sancar, A. (1993) *J. Biol. Chem.* **268,** 1931–1936.
16. Keeney, S., Eker, A. P. M., Brody, T., Vermeulen, W., Bootsma, D., Hoeijmakers, J. H. J. & Linn, S. (1994) *Proc. Natl. Acad. Sci. USA* **81,** 4053–4056.
17. Reardon, J. T., Thompson, L. H. & Sancar, A. (1993) *Cold Spring Harbor Symp. Quant. Biol.* **58,** 605–617.
18. Thompson, L. H., Busch, D. B., Brookman, K., Mooney, C. L. & Glaser, D. A. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 3736–3737.
19. Manley, J.l., Fire, A., Cano, A., Sharp, P. A. & Gefter, M. L. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 3855–3859.
20. Sibghat-Ullah, Husain, I., Carlton, W. & Sancar, A. (1989) *Nucleic Acids Res.* **17,** 4471–4484.
21. Mu, D., Park, C.-H., Matsunaga, T., Hsu, D. S., Reardon, J. T. & Sancar, A. (1995) *J. Biol. Chem.* **270,** 2415–2418.
22. Mu, D., Hsu, D. S. & Sancar, A. (1995) *J. Biol. Chem.* **271,** 8285–8294.
23. Henricksen, L. A., Umbricht, C. B. & Wold, N. S. (1994) *J. Biol. Chem.* **269,** 11121–11132.
24. Huang, J. C. & Sancar, A. (1994) *J. Biol. Chem.* **269,** 19034–19040.
25. Matsunaga, T., Mu, D., Park, C.-H., Reardon, J. T. & Sancar, A. (1995) *J. Biol. Chem.* **270,** 20862–20869.
26. Smith, C. A. & Taylor, J.-S. (1993) *J. Biol. Chem.* **268,** 11143–11151.
27. Erdile, L. F., Heyer, W.-D., Kolodner, R. & Kelly, T. J. (1991) *J. Biol. Chem.* **266,** 12090–12098.
28. Erdile, L. F., Wold, M. S. & Kelly, T. J. (1990) *J. Biol. Chem.* **265,** 3177–3182.
29. Umbricht, C. B., Erdile, L. F., Jabs, E. W. & Kelly, T. J. (1993) *J. Biol. Chem.* **268,** 6131–6138.
30. Wold, M. S. & Kelly, T. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 2523–2527.
31. Kenny, M. K., Lee, S.-H. & Hurwitz, J. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 9757–9761.
32. Coverley, D., Kenny, M. K., Lane, D. P. & Wood, R. D. (1992) *Nucleic Acids Res.* **20,** 3873–3880.
33. Coverley, D., Kenny, M. K., Munn, M., Rupp, W. D., Lane, D. P. & Wood, R. D. (1991) *Nature (London)* **349,** 538–541.
34. Fairman, M. P. & Stillman, B. (1988) *EMBO J.* **7,** 1211–1218.
35. Keeney, S. N. (1993) PhD thesis (Univ. California, Berkeley).
36. Prakash, S., Sung, P. & Prakash, L. (1993) *Annu. Rev. Genet.* **27,** 33–70.
37. Guzder, S. N., Habraken, Y., Sung, P., Prakash, L. & Prakash, S. (1995) *J. Biol. Chem.* **270,** 12973–12976.
38. He, Z., Henricksen, L. A., Wold, M. S. & Ingles, C. J. (1995) *Nature (London)* **374,** 566–569.
39. Matsuda, T., Saijo, M., Kuraoka, I., Kobayashi, T., Nakatsu, Y., Nagai, A., Enjoji, T., Masutani, C., Sunagawa, K., Hanaoka, F., Yasui, A. & Tanaka, K. (1995) *J. Biol. Chem.* **270,** 4152–4157.
40. Li, L., Lu, X., Peterson, C. A. & Legerski, R. J. (1995) *Mol. Cell. Biol.* **15,** 5396–5402.