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Mutations in XPA That Prevent Association with ERCC1 Are Defective in Nucleotide Excision Repair

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The human repair proteins XPA and ERCC1 have been shown to be absolutely required for the incision step of nucleotide excision repair, and recently we identified an interaction between these two proteins both in vivo and in vitro (L. Li, S. J. Elledge, C. A. Peterson, E. S. Bales, and R. J. Legerski, Proc. Natl. Acad. Sci. USA 91:5012–5016, 1994). In this report, we demonstrate the functional relevance of this interaction. The ERCC1-binding domain on XPA was previously mapped to a region containing two highly conserved XPA sequences, Gly-72 to Phe-75 and Glu-78 to Glu-84, which are termed the G and E motifs, respectively. Site-specific mutagenesis was used to independently delete these motifs and create two XPA mutants referred to as ΔG and ΔE. In vitro, the binding of ERCC1 to ΔE was reduced by approximately 70%, and binding to ΔG was undetectable; furthermore, both mutants failed to complement XPA cell extracts in an in vitro DNA repair synthesis assay. In vivo, the ΔE mutant exhibited an intermediate level of complementation of XPA cells and the ΔG mutant exhibited little or no complementation. In addition, the ΔG mutant inhibited repair synthesis in wild-type cell extracts, indicating that it is a dominant negative mutant. The ΔE and ΔG mutations, however, did not affect preferential binding of XPA to damaged DNA. These results suggest that the association between XPA and ERCC1 is a required step in the nucleotide excision repair pathway and that the probable role of the interaction is to recruit the ERCC1 incision complex to the damaged site. Finally, the affinity of the XPA-ERCC1 complex was found to increase as a function of salt concentration, indicating a hydrophobic interaction; the half-life of the complex was determined to be approximately 90 min.

Cells of living organisms, from *Escherichia coli* to mammals, are subjected to a wide variety of spontaneous and environmentally induced alterations in DNA; in response, they have developed highly efficient mechanisms to recognize and correct lesions in the genetic material. Nucleotide excision repair (NER) is one of the major DNA repair pathways and is responsible for the removal of a wide range of structurally unrelated lesions such as UV-induced photoproducts, chemical adducts, and protein-DNA cross-links (12, 15, 16). The NER mechanism appears to be highly conserved in eukaryotes and has been found to encompass five steps: damage recognition, incision on either side of the lesion, excision of the lesion-containing oligonucleotide, resynthesis of the gap, and ligation to extant DNA.

Defects in the NER pathway are known to give rise to a number of rare photosensitive human genetic diseases, including xeroderma pigmentosum, Cockayne's syndrome, and trichothiodystrophy (8, 9, 33, 34, 44). In addition to photosensitivity, patients with xeroderma pigmentosum exhibit a high predisposition to skin cancer and, in some cases, neurological symptoms. Patients with Cockayne's syndrome or trichothiodystrophy lack a high predisposition to cancer but exhibit unique spectra of developmental and growth defects, including mental retardation. Complementation analysis has revealed 10 distinct genetic groups in the human disorders that are involved in NER (16, 17, 34, 45), and a series of photosensitive rodent mutant cell lines has been found to contain 11 complementation groups (10, 28). A total of eight human repair genes that complement the mammalian NER mutants have now been cloned, and four human complementation groups have been found to overlap rodent groups (21, 31, 36, 37, 39, 40, 43, 44). Many of the expression products of these genes have been shown to be organized in two large multiprotein complexes, the basal transcription factor IIH (11, 30, 42) and the putative NER incision complex composed of ERCC1, ERCC4, ERCC11, and XPF (ERCC1 complex) (6, 27, 41), although it is not clear at present that XPF is distinct from either ERCC4 or ERCC11.

We have demonstrated both in vivo, using the yeast two-hybrid system, and in vitro, using recombinant proteins, that the human repair proteins XPA and ERCC1 specifically interact (22). Domains required for the interaction were mapped to Leu-92 to Val-119 on ERCC1 and to Gly-74 to Phe-114 on XPA. The functional relevance of the XPA-ERCC1 association, however, was not directly demonstrated. The XPA protein shows a greater binding affinity toward damaged double-stranded DNA than toward undamaged double-stranded DNA (18, 29), indicating its involvement in a damage recognition step. It is also plausible that XPA interacts with other repair factors to initiate the incision process. As indicated above, evidence based on in vitro complementation analysis that ERCC1 is a component of a putative multiprotein complex has been reported (6, 27, 41). Additional evidence for the existence of the complex is shown by the report that both ERCC1 and ERCC4 complementing activities can be purified by XPA affinity chromatography (25). The homologs of ERCC1 and ERCC4 in *Saccharomyces cerevisiae*, RAD10 and RAD1, respectively, are known to constitute a single-stranded DNA endonuclease (5, 35, 38), suggesting that the ERCC1 complex may be a mammalian exonuclease. Thus, the interaction between XPA and ERCC1 is likely to be a required step in NER in which the exonuclease is loaded onto the damaged site.

To evaluate this hypothesis, we identified highly conserved motifs in XPA in the region previously determined to contain

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the ERCC1-interactive domain. These motifs were deleted by site-directed mutagenesis, and the resulting mutants were examined both in vitro and in vivo for the ability to complement XPA-deficient cells. In addition, we studied the binding affinity of the XPA-ERCC1 complex as a function of salt concentration and determined the half-life of the complex.

MATERIALS AND METHODS

Materials. *E. coli* PR745, vector pMAL-c2, and amylose agarose beads were obtained from New England BioLabs. *E. coli* MutS was obtained from Pharmacia. Plasmids pMAL-XPA and pCDM8-ERCC1 were obtained as previously described (22). The pBSIIKS+ and pGEX-2T vectors were obtained from Stratagene and Pharmacia, respectively. XPA-deficient cell lines GM02345C (XP2OS lymphoblast) and GM04312A (XP2OS fibroblast) were purchased from the Human Genetic Mutant Cell Repository (Camden, N.J.). Lymphoid cell line WI-L2 was obtained from the American Type Culture Collection (Rockville, Md.). Prepacked heparin-agarose columns (HEP-II-5) and hygromycin B were obtained from Sigma Chemical Co. Isopropyl-β-D-thiogalactopyranoside (IPTG) was purchased from United States Biochemical.

Oligonucleotide-directed site-specific mutagenesis. A 893-bp *Sac*I-*Xba*I fragment containing the coding sequence of XPA was isolated from pMAL-XPA and cloned into pBSIIKS+. Single-stranded template DNA carrying the XPA nonsense strand was prepared as described previously (1). Oligonucleotides used for the mutagenesis of XPA had the following sequences: CCAAAGATAATTGA CACAATTTTAGAAGAGGAAGAA (ΔG) and GACACAGGAGGAGGCT TCATTTTACAGAAAATTGGAAAAGTTGTTCAT (ΔE). Mutagenesis reactions were performed by using the U.S.E. kit according to the manufacturer's protocol (Pharmacia). Mutations were confirmed by DNA sequencing, and the *Sac*I-*Xba*I fragment from each mutant was cloned into pMALc2 and the mammalian expression vector pEBS7 (26).

Preparation and purification of recombinant proteins. Maltose-binding protein (MBP) fusion proteins (from pMAL constructs) were expressed in *E. coli* PR745. Induction of the fusion proteins with IPTG, lysis of the cells by sonication, and subsequent purification by affinity chromatography on amylose resin were performed as recommended by the manufacturer (New England BioLabs). For additional purification, peak fractions containing MBP fusion proteins were pooled (3.5 ml), diluted 1:2 with buffer A (25 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid [HEPES]-KOH [pH 7.6], 1 mM EDTA, 10 mM β-mercaptoethanol, 10% [vol/vol] glycerol), and loaded onto a 2.5-ml heparin-agarose column equilibrated with buffer A containing 100 mM KCl at a flow rate of 10 ml/h. Sixteen milliliters of buffer A containing 100 mM KCl was used to wash the column. The bound protein was eluted with buffer A plus 1.0 M KCl. Fractions (0.3 ml) were collected and analyzed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE). Peak fractions were pooled (2.4 ml) and dialyzed against protein storage buffer (25 mM HEPES-KOH [pH 7.9], 100 mM KCl, 12 mM $MgCl_2$, 0.5 mM EDTA, 2 mM dithiothreitol, 16.5% [vol/vol] glycerol) overnight. Aliquots of 100 μl were frozen in dry ice-ethanol and stored at −80°C.

In vitro protein-protein interaction assay (pull-down assay). The interactions of proteins with MBP fusion proteins bound to amylose resin were examined as previously described (22). [^{35}S]methionine-labeled ERCC1 was prepared with 0.5 μg of supercoiled pCDM8-ERCC1 DNA by a coupled transcription-translation system in a total volume of 50 μl (TnT kit; Promega). Whole-cell extracts were also used as a source of ERCC1.

IDT assay. Immobilized DNA templates were prepared as described previously (2), with the following modifications. Two fragments of 622 and 485 bp were obtained from pBR322 by digestion with *Hin*dIII and *Hin*cII and labeled at the *Hin*dIII site with biotinylated dATP by incubation with Klenow fragment. After purification by centrifugation through Microcon-30 concentrators (Amicon), the fragments were irradiated with 254-nm UV light at a dose of 600 J/m^2. For the binding assay, 10 ng of either unirradiated or damaged DNA was incubated with streptavidin-magnetic beads (Dynal) for 30 min at room temperature in immobilized DNA template (IDT) buffer (45 mM HEPES [pH 7.8], 70 mM KCl, 7.4 mM MgCl, 0.4 mM EDTA, 1.0 mM dithiothreitol, 380 μg of bovine serum albumin per ml). The beads were washed three times, 100 ng of MBP-XPA protein in IDT buffer was added, and the mixture was incubated at 30°C for 30 min. The beads were washed four times with IDT buffer, and bound proteins were eluted by 1× SDS loading buffer and after separation by SDS-PAGE were analyzed by immunoblotting.

Whole-cell extract preparation. WI-L2 cells were cultured in suspension in RPMI medium supplemented with 10% fetal calf serum. Cells were harvested at late log phase. The cell extracts for use in the in vitro DNA repair synthesis assay were prepared as previously described (46).

Repair synthesis in cell extracts. The in vitro assay for DNA repair was performed essentially as previously described (46), with the following modifications. pGEX2T and pBSII supercoiled DNAs were used as the control and damaged substrates, respectively. *N*-(Guanin-8-yl)acetylaminofluorene (AAF) adducts were introduced into pBSII by treatment with *N*-acetoxy-*N*-2-acetylaminofluorene (AAAF) (kindly provided by Eric Tang, The University of Texas M. D. Anderson Cancer Center, Smithville) as previously described (20). Unincorporated AAAF was removed from the AAF-modified plasmids by repeated extractions with diethyl ether and subsequent ethanol precipitation. After DNA repair synthesis was performed as described previously (46), plasmids were linearized by *Eco*RI digestion and subjected to agarose gel electrophoresis. Dried gels were exposed to film to obtain autoradiograms.

Immunoblotting. Polyclonal anti-ERCC1 antiserum (41) was provided by Jan Hoeijmakers (Erasmus University, Rotterdam, The Netherlands). Immunoblotting was performed with an ECL kit (Amersham) according to the manufacturer's specifications.

DNA transfection and UV survival. Transfection of plasmid DNAs, selection of transformants with hygromycin B, and determination of UV survival were performed as previously described (21).

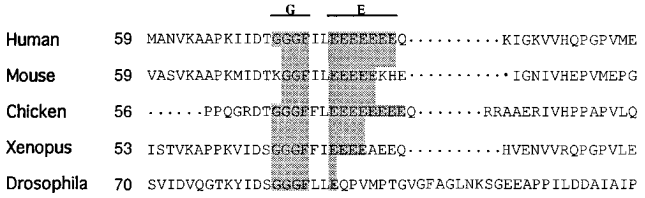

FIG. 1. Amino acid sequence alignment of the ERCC1-interactive domains of human, mouse, chicken, *Xenopus laevis*, and *Drosophila melanogaster* XPA homologs. As indicated, the G and E regions are conserved.

RESULTS

Identification of motifs in XPA that affect the interaction with ERCC1. We previously demonstrated a specific association between XPA and ERCC1 both in the yeast two-hybrid system and in vitro by a pull-down assay. In addition, a preliminary mapping of the ERCC1-interactive domain identified amino acid residues Gly-74 through Phe-114 as being necessary for the interaction of the XPA protein with ERCC1 (22). Tanaka and colleagues have cloned and sequenced a number of homologs of the *XPA* gene (32, 36), allowing examination of the ERCC1-interactive domain for highly conserved residues. Amino acid alignment of the Gly-72–Phe-114 region identified two highly conserved motifs designated the G and E motifs (Fig. 1). The G motif is highly conserved from mammals (human) through insects (*Drosophila* spp.); the E cluster is well conserved within vertebrate species but reduced to one glutamic acid residue in *Drosophila* species.

To determine whether these two highly conserved motifs are involved in mediating the XPA-ERCC1 interaction, site-specific mutagenesis was used to independently delete the two motifs. The resultant mutants, which lack either the G or the E motif, are referred to as ΔG or ΔE, respectively. The mutant proteins were purified as MBP fusion products (Fig. 2a) and analyzed by a pull-down assay to test their ability to interact with [^{35}S]methionine-labeled ERCC1. As shown in Fig. 2b, binding of ERCC1 to the ΔE mutant was reduced by approximately 70%, and binding of ERCC1 to the ΔG mutant was undetectable. These results suggest that one function of the highly conserved G and E motifs may be to mediate the XPA-ERCC1 association.

A number of investigators have reported indirect biochemical evidence that ERCC1 exists in a multiprotein complex involving ERCC4, ERCC11, and XPF (6, 27, 41), although it is not clear that XPF is distinct from either ERCC4 or ERCC11. To determine whether XPA interacts with another member of this complex in addition to ERCC1, the binding activity of ERCC1 in a whole-cell extract was evaluated in a pull-down assay with XPA and ΔG. As shown in Fig. 2c, XPA interacted with ERCC1 in the cell extract, but the ΔG mutant did not. This result suggests that of the four putative subunits of the ERCC1 complex, only ERCC1 directly interacts with XPA.

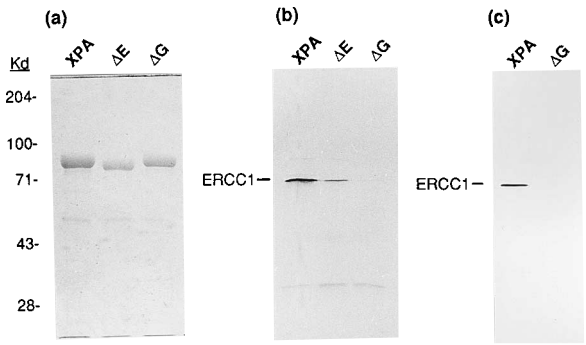

FIG. 2. In vitro binding of ERCC1 to the ΔE and ΔG mutants. (a) MBP fusion proteins were prepared as described previously (20), separated by SDS-PAGE, and stained with Coomassie blue. (b) Binding of in vitro-translated [^{35}S]methionine-labeled ERCC1 to MBP-XPA fusion proteins as determined in a pull-down assay. (c) Binding of ERCC1 from a WI-L2 cell extract to MBP-XPA fusion proteins as determined in a pull-down assay and analyzed by immunoblotting. Approximately 5 μg of either MBP-XPA or the ΔG proteins was bound to amylose beads and subsequently incubated with 100 μg of cell extract.

Evaluation of the ΔE and ΔG mutants in an in vitro DNA repair assay. To address the functional significance of the XPA-ERCC1 interaction, XPA mutant proteins were tested in an in vitro DNA repair assay (46) to determine whether reduction in ability to bind ERCC1 was accompanied by the loss of ability to complement XPA cell extracts. XPA, ΔE, and ΔG were purified as MBP fusion proteins and added to XPA-deficient cell extracts to measure the complementing activity (Fig. 3). XPA-deficient cell extracts complemented with wild-type XPA showed substantial increases in DNA repair activity, whereas those complemented with either the ΔE or the ΔG mutant protein did not, suggesting that the XPA-ERCC1 association is required for excision repair in vitro.

ΔG is a dominant negative mutant in vitro. XPA is a damage-specific DNA-binding protein and presumably plays a direct role in damage recognition as well as subsequent steps during excision repair. Therefore, the presence of an excess of ΔG protein in a normal cell extract may block NER at a preincision step because of its inability to recruit the ERCC1 incision complex to the site of damage. Presumably, an inability to complete the incision process would prevent turnover of the repair complex. To test this hypothesis, purified XPA and ΔG proteins were added to repair-proficient WI-L2 cell-free extracts and DNA repair synthesis was examined. As shown in Fig. 4, wild-type XPA slightly increased repair synthesis, whereas the ΔG mutant inhibited repair synthesis of the wild-type extracts in a dose-dependent manner. We estimate that approximately 10- to 20-fold excess of ΔG is required to effect a 50% reduction in repair synthesis. Thus, the deletion of the G motif in XPA has created a mutant that exhibits negative

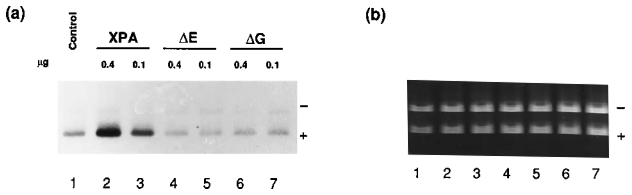

FIG. 3. In vitro complementation of XPA-deficient cell-free extracts by MBP-XPA fusion proteins as determined by the DNA repair synthesis assay (46). (a) Autoradiogram of the gel shown in panel b. +, pBSII DNA treated with AAAF; −, untreated pGEX-2T DNA. (b) Ethidium bromide-stained gel of plasmids subjected to the in vitro DNA repair synthesis assay.

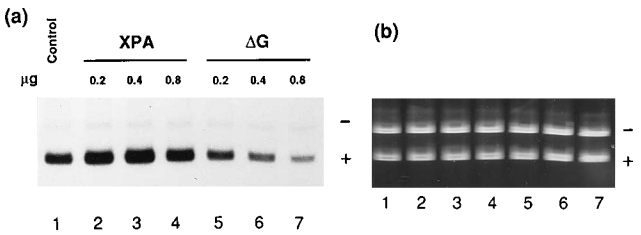

FIG. 4. The dominant negative effect of the ΔG mutant as analyzed by the in vitro DNA repair synthesis assay. (a) Autoradiogram of the gel shown in panel b. MBP-XPA and MBP-ΔG proteins were added to wild-type (WI-L2) cell extracts as indicated. + and − are as indicated in the legend to Fig. 3. (b) Ethidium bromide-stained gel of plasmids subjected to the in vitro DNA repair synthesis assay.

dominance in the in vitro repair assay, although high molar ratios of the mutant to wild-type protein are required. This result is consistent with the proposal that the XPA-ERCC1 association is required for the loading of the putative ERCC1 incision complex onto the damaged site.

Complementation activity of the ΔE and ΔG mutants in vivo. To examine the complementing abilities of the ΔE and ΔG mutants in vivo, XPA cDNAs bearing ΔE and ΔG mutations, as well as wild-type XPA, were subcloned into the mammalian expression vector pEBS7 (26) and introduced into the XPA-deficient cell line XP2OS by the calcium phosphate coprecipitation method (7). Transformants for each clone were selected by resistance to hygromycin B, and the level of complementation of UV resistance was determined for each construct (Fig. 5). The ΔG mutant exhibited little complementing activity in that the survival curve was similar to that of the parental cell line, whereas the ΔE mutant exhibited partial complementation in comparison with that of wild-type XPA. These results correlate strongly with the in vitro binding results discussed above in which the ΔE mutant exhibited an intermediate level of binding and the ΔG mutant exhibited no detectable binding. These results further support the in vitro findings that binding to ERCC1 is a required function of the XPA protein in NER.

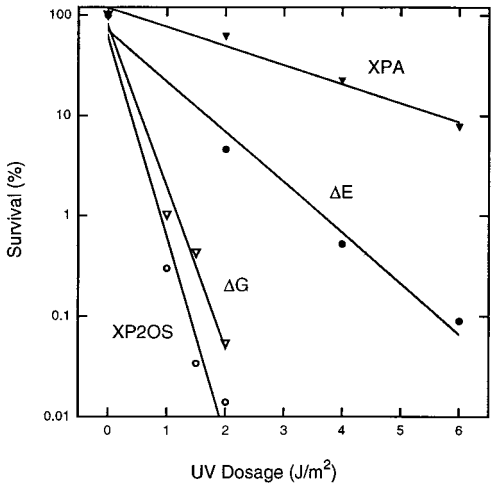

FIG. 5. Complementation of the XPA-deficient cell line XP2OS by the mammalian expression constructs pEBS7-XPA, pEBS7-ΔE, and pEBS7-ΔG. After establishment of cell lines by selection of transformants with hygromycin B, the survival of each line was determined as a function of UV dosage as described previously (21).

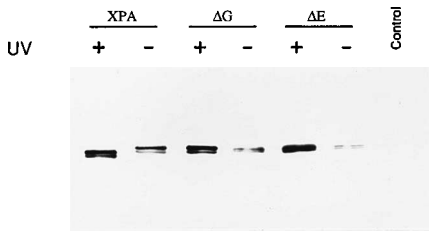

FIG. 6. Preferential binding of MBP-XPA and mutant proteins to damaged DNA as determined by the IDT assay followed by immunoblotting. The control lane indicates MBP-XPA protein added to streptavidin-magnetic beads in the absence of DNA.

The ΔG mutant was also examined for its ability to exhibit a dominant negative phenotype in vivo. However, this effect (results not shown) was quite marginal, presumably because, as shown above, high ratios of mutant to wild-type protein, which were not achieved in vivo, are required to detect the dominant negative phenotype.

The ΔE and ΔG mutants retain their damage-specific DNA binding activity. To determine if deletion of the E and G motifs affected other functions of the XPA protein, both mutants were assayed for the ability to preferentially bind to damaged double-stranded DNA. For this purpose, we used a modification of the IDT assay originally described by Arias and Dynan (2) for analysis of transcription complexes. As shown in Fig. 6, both mutants preferentially bound to UV-damaged DNA as well as the wild-type protein did, indicating that these deletions did not globally disrupt the secondary structure of the XPA protein.

Analysis of the stability of the XPA-ERCC1 complex in vitro. Purified MBP-XPA and [^{35}S]methionine-labeled ERCC1 were used to analyze the half-life of the XPA-ERCC1 complex in vitro. Five micrograms of MBP-XPA protein was bound to amylose beads and incubated with 20 ng of [^{35}S]methionine-labeled ERCC1 in protein binding buffer. After removal of unbound ERCC1 by repeated washing, the beads were diluted with 10 ml of the same buffer, and the amount of complex remaining over time was measured by eluting and examining the labeled ERCC1 on an SDS-polyacrylamide gel (Fig. 7). Dissociation of the preformed XPA-ERCC1 complex yielded a half-life of approximately 1.5 h, which reflects a stable interaction between these two proteins in vitro.

The effect of ionic strength on the XPA-ERCC1 association was examined in a pull-down assay by allowing the complex to form in the presence of increasing concentrations of KCl. The result (Fig. 8) suggests that the interaction is probably of a hydrophobic nature, since complex formation increased with increasing salt concentration.

DISCUSSION

In this study, we have identified two motifs (Fig. 1) in XPA, designated E and G, that may be involved in mediating its interaction with ERCC1. XPA mutants in which these motifs have been deleted showed reduced binding to ERCC1 in vitro, an inability to complement XPA-deficient cell extracts, and in the case of the ΔG mutant, a dominant negative inhibition of wild-type cell extracts. In vivo, the mutants exhibited a level of complementation of XPA cells that strongly correlated with their binding to ERCC1 in vitro. Taken together, these results provide strong evidence that the interaction between XPA and ERCC1 is a required step during damage processing by the NER system. These results are also consistent with the conclusion that the E and G motifs are involved in directly mediating the interaction with ERCC1. Alternatively, deletion of these motifs may affect the secondary structure of a domain elsewhere in the protein. In this regard, we note that a polypeptide containing residues 1 to 114 is able to bind ERCC1 (22) and that residues 1 to 58 have been shown previously to be dispensable for XPA function in vivo (23), suggesting that residues 59 to 114 contain the ERCC1-interactive domain.

The XPA protein has been shown to bind damaged double-stranded DNA in preference to undamaged double-stranded DNA (18, 29). This property suggests that XPA functions as an important element in the early damage recognition step of NER. In addition, XPA probably interacts with other DNA repair components to perform subsequent steps of damage processing. The *S. cerevisiae* homologs of ERCC1 and ERCC4, RAD10 and RAD1 (4, 40), tightly associate with each other and exhibit a single-stranded DNA endonuclease activity in vitro (3, 35, 38). In mammalian cells, ERCC1 is a component of a multifactorial complex involving ERCC4, ERCC11, and XPF (6, 27, 41) and thus is probably the mammalian counterpart of the RAD1-RAD10 complex. These findings and our results suggest that the interaction between XPA and ERCC1 is required to load the ERCC1 complex onto the site of damage for subsequent incision at one side of the lesion. Two

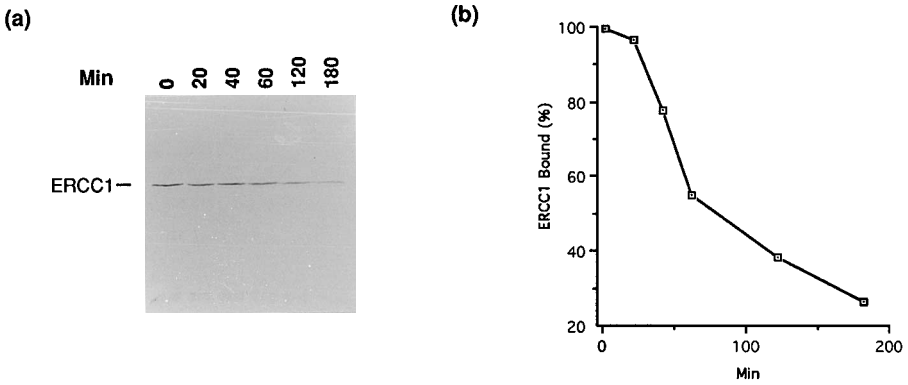

FIG. 7. Determination of the half-life of the XPA-ERCC1 complex. (a) The XPA-ERCC1 complex was formed in a pull-down assay. After removal of unbound ERCC1, the complex was diluted with protein binding buffer (40 mM HEPES [pH 7.9], 2% [vol/vol] glycerol, 50 mM KCl, 50 mM NaCl, 5 mM $MgCl_2$, 1 mM EDTA, 0.5 mM dithiothreitol, 0.2 mM phenylmethanesulfonyl fluoride, 0.5 μg each of leupeptin, pepstatin, and aprotinin per ml), and the samples were incubated for the indicated times and examined by SDS-PAGE. (b) Plot of data derived from densitometric analysis of film shown in panel a.

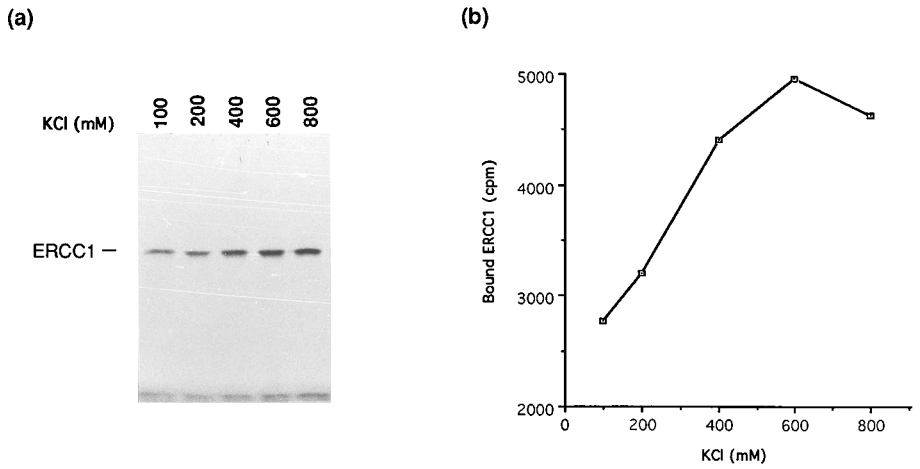

FIG. 8. Determination of the stability of the XPA-ERCC1 complex as a function of increasing salt concentration. (a) A pull-down assay was used to analyze the stability of the XPA-ERCC1 complex in the presence of increasing concentrations of KCl as indicated. After elution, samples were examined by SDS-PAGE. (b) Plot of data derived from densitometric analysis of film shown in panel a.

recent studies have shown that the human *XPG* (24) and the *S. cerevisiae RAD1* and *RAD10* (5) genes encode structure-specific DNA endonucleases. These enzymes specifically cleave at junctions between duplex and single-stranded DNA and implicate single-stranded bubble structures as intermediates in NER. In addition, XPG was shown to cleave only the 3′ side of a single-stranded bubble structure, and the RAD1-RAD10 complex was found to cleave only on the 5′ side. Presumably the latter finding also applies to the putative ERCC1 incision complex and suggests that the interaction with XPA orients this complex to the 5′ side of the damaged site.

Our results indicate that the deletion of the E and G motifs in XPA does not affect its damage-specific DNA-binding capability (Fig. 6). Thus, the mechanism of the dominant negative phenotype of the ΔG mutant can be explained by a model in which the ΔG protein maintains its damage-specific DNA-binding activity and possible interaction with other repair factors but fails to recruit the ERCC1 incision complex to the site of damage. The NER process is then stalled at a preincision stage, and turnover of the excision repair complex is prevented. In vivo, the dominant negative effect of the ΔG mutant is negligible, presumably because the phenomenon requires a high ratio of mutant to wild-type protein.

Mapping of the interaction domains on both XPA and ERCC1 proteins has revealed that the association is mediated by conserved regions in each protein. Additional mutational analysis has indicated that the G and E motifs in XPA may be directly involved in mediating the interaction with ERCC1. These two motifs, which are extensively conserved in higher eukaryotes, are absent from the *S. cerevisiae* homolog of XPA, RAD14. Furthermore, that RAD10 and RAD14 do not interact in a two-hybrid assay (13) as do ERCC1 and XPA may indicate that in this aspect of the NER process, there is a mechanistic difference between yeast and higher eukaryotic cells. However, Lambert et al. (19) have reported that RAD10 partially complements to a small degree both the UV and mitomycin sensitivity of ERCC1-deficient mammalian cells. This result suggests that RAD10 can interact, albeit imperfectly, with the mammalian NER apparatus, implying some conservation of RAD10-interactive motifs. Since RAD10 apparently does not interact with RAD14 and by implication not with XPA, then presumably the RAD1-RAD10 complex may interact with other NER components in order to be recruited to the site of damage. Alternatively, RAD10 may be able to interact with the mammalian homolog of RAD1, and this complex may in turn be able to recognize and cleave with low efficiency a single-stranded bubble structure without interacting with other components of the NER apparatus.

ACKNOWLEDGMENT

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