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ENHANCEMENT OF DAMAGE-SPECIFIC DNA BINDING OF XPA BY INTERACTION WITH THE ERCC1 DNA REPAIR PROTEIN

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The human XPA and ERCC1 proteins, which are involved in early steps of nucleotide excision repair of DNA, specifically interacted in an *in vitro* binding assay and a yeast two-hybrid assay. A stretch of consecutive glutamic acid residues in XPA was needed for binding to ERCC1. Binding of XPA to damaged DNA was markedly increased by the interaction of the XPA and ERCC1 proteins. ERCC1 did not enhance binding to DNA when a truncated XPA protein, MF122, was used in place of the XPA protein. MF122 retains damaged DNA binding activity but lacks the region for protein-protein interaction including the E-cluster region. These results suggest that the XPA/ERCC1 interaction may participate in damage-recognition as well as in incision at the 5' site of damage during nucleotide excision repair.

Nucleotide excision repair (NER) functions in many organisms to remove a great diversity of damage from DNA. The best known of the gene products that participate in NER are those that correspond to xeroderma pigmentosum (XP) complementation groups A through G, and a set of NER-defective rodent cell mutants which are corrected by human genes designated *ERCC* (excision repair cross complementing) genes. Most of the *XP* and many of the *ERCC* genes have been cloned. It has been shown that the *ERCC3, 2, 5* and *6* genes are equivalent to the *XPB* (XP group B), *XPD* (XP group D), *XPG* (XP group G) and Cockayne syndrome group

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Abbreviations: XP, xeroderma pigmentosum; NER, nucleotide excision repair; RPA, replicating protein A; PCNA, proliferating cell nuclear antigen.

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B (*CSB*) genes, respectively (1). The XPA protein consists of 273 amino acids, with a C_4-type zinc finger. It binds preferentially to UV-, cisplatin-, N-acetoxy-2-acetylaminofluorene- or osmium tetroxide-damaged DNA, suggesting that the XPA protein is involved in the damage recognition step of NER (2, 3, 4). The ERCC1 and ERCC4 (possibly equivalent to XPF) proteins make a tight complex (5, 6), which may serve to nick the DNA duplex on the 5' side of a damaged site. Here we found that the XPA protein directly bound with the ERCC1 protein through the E-cluster region of the XPA protein, and that the damaged DNA binding activity of XPA was increased by interaction with the ERCC1 protein. These results indicate that XPA/ERCC1/XPF complex formation may participate in the damage-recognition and incision steps of the NER process.

MATERIALS AND METHODS

Plasmids: pET11aGST-ERCC1 was provided by Akira Yasui (Tohoku University, Japan). The pGEX-truncated *XPA* cDNAs were prepared by ligating the *Eco* RI inserts of mutant *XPA* cDNAs described by Miyamoto *et al.* (7) to the pGEX vector. The pGEX-*XPA*4 - 137, *XPA*4 - 137(Δ61 - 92), and *XPA*4 - 137(Δ78 - 84) were prepared by inserting a stop codon linker (Nippon Gene) into the blunted *Hin* dIII site of the pGEX-*XPA*, Δ exon 2, and Δ E-cluster cDNAs (7), respectively.

Preparations of XPA and ERCC1 proteins: The XPA and GST-XPA proteins were expressed in *E. coli* and purified as described (3, 8). The GST-ERCC1 and GST-truncated XPA proteins were purified from soluble extracts of *E. coli*. For the preparation of the [^{35}S]methionine-labeled ERCC1 protein, *in vitro* transcription and translation of pSPERB was performed using the TNT coupled reticulocyte lysate system (Promega) in the presence of [^{35}S]methionine (Amersham; SJ1015) according to the manufacturer's instructions.

***In vitro* assay for the binding of the XPA and ERCC1 proteins:** *In vitro* binding assays using the GST-ERCC1, GST-XPA or GST-truncated XPA protein beads were performed as described (8).

Yeast two-hybrid assay: The yeast two-hybrid assay was carried out as described (8). pGBT9-XPA4-129 and PGAD424-XPA4-129 were generated by self-ligating the larger *Pst* I fragment of pGBT9-XPA and pGAD424-XPA, respectively.

DNA binding assay: To examine the DNA binding activity of the XPA and ERCC1 proteins, a nitrocellulose filter-binding assay was performed as described by Robins et al. (2) with some modifications (3).

RESULTS

Specific interaction of XPA and ERCC1 proteins: Many *XP*, *ERCC* and other genes are involved in the early steps of NER and so it is likely that protein interactions and/or protein complex formation are required for these steps. We therefore searched for direct interactions between XPA, XPB, XPC, XPD, XPG, ERCC1, the p34 subunit of RPA and PCNA using yeast two-hybrid assays. In most combinations, colonies were not detected. However, hybrid combinations between XPA and either ERCC1 or the p34 subunit of RPA yielded colonies that grew on plates lacking histidine. Transformants produced by the combination of XPA and the p34 subunit of RPA induced ß-galactosidase activity, while those produced by the combination of XPA and ERCC1 failed to induce as previously described (8). However, ß-galactosidase activity was induced in a transformant with pGAD424-ERCC1 and pGBT9-XPA4-129

Table 1
Protein-protein interactions determined by a liquid quantative β-galactosidase assay

DNA-binding domain	Activation domain	β-Galactosidase activity Miller units
pGBT9-XPA	pGAD424	>0.2
pGBT9-XPA	pGAD424-ERCC1	>0.1
pGBT9-ERCC1	pGAD424XPA	>0.1
pGBT9	pGAD424-XPA$_{4-129}$	>0.1
pGBT9-ERCC1	pGAD424-XPA$_{4-129}$	1.6 ± 0.5
pGBT9-XPA$_{4-129}$	pGAD424	2.3 ± 0.5
pGBT9-XPA$_{4-129}$	pGAD424-ERCC1	5.1 ± 0.6

Values reported are the average of 5-10 independent transformants.

containing amino acid residues 4 - 129 of XPA. ß-galactosidase activity was also induced in the transformant with pGBT9-ERCC1 and pGAD424-XPA4-129 (Table 1). These results suggest that the XPA protein specifically interacts with the ERCC1 protein as well as the p34 subunit of

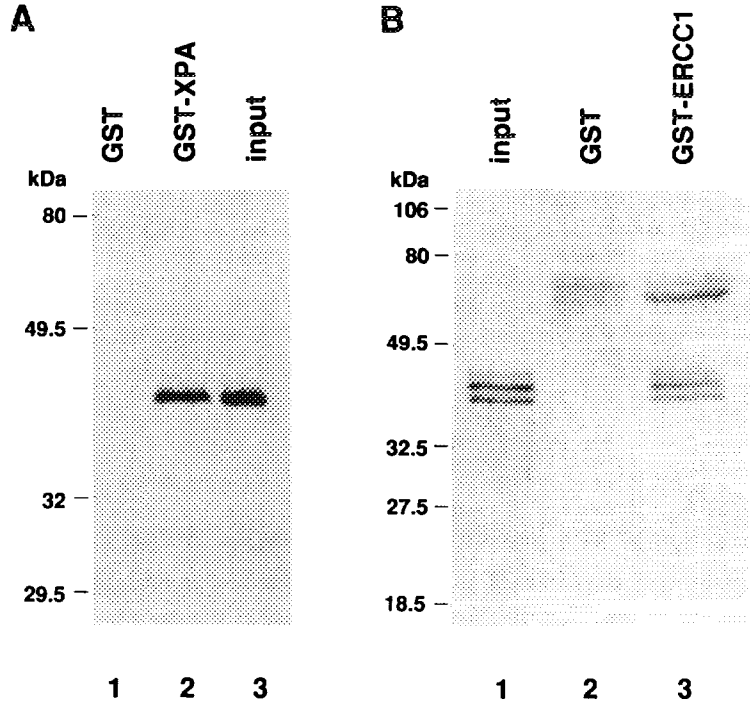

Fig. 1. *In vitro* assay for binding of the XPA and ERCC1 proteins. (A) HeLa whole cell extracts were incubated with GST (lane 1) or GST-XPA beads (lane 2). After washing the beads, the bound proteins were analysed by SDS-PAGE and immunoblotting with anti-ERCC1 antibody (ref. 5). As a control, HeLa whole cell extracts were loaded directly onto SDS-PAGE (lane 3). (B) Purified XPA protein was incubated with GST (lane 2) or GST-ERCC1 (lane 3) beads. After washing the beads, the bound proteins were extracted by boiling in SDS sample buffer, separated by SDS-PAGE and analyzed by immunoblotting with anti-XPA antibody . As a control, purified XPA protein was loaded directly onto SDS-PAGE (lane 1).

RPA, and that the ERCC1-binding domain of the XPA protein is located within an N-terminal region of 126 amino acids.

To confirm the interaction of the XPA and ERCC1 proteins, *in vitro* binding assays were performed. As shown in Figure 1, GST-XPA beads bound to the ERCC1 protein in whole cell extracts, and purified XPA protein directly bound to the GST-ERCC1 beads. The effect of ionic strength on the XPA-ERCC1 interaction was then examined. ERCC1 protein binding to GST-XPA beads decreased at higher concentrations of NaCl, but some ERCC1 protein was still bound to XPA beads at 1 M NaCl (Figure 2). These results suggest that some of the interaction between the ERCC1 and XPA proteins is brought by hydrophobic bonds rather than ionic bonds. With the constructs used here, we did not find the enhanced interaction between XPA and ERCC1 at higher salt concentrations that was recently reported by Li et al. (15).

ERCC1-binding region of the XPA protein: To identify a region of the XPA protein responsible for interaction with the ERCC1 protein, a series of GST-truncated XPA protein beads were mixed with *in vitro* translated and [^{35}S]methionine-labeled ERCC1 protein. The GST-XPA$_{91-273}$ mutant protein which contains amino acid residues 91 - 273 of the XPA protein was unable to bind with the ERCC1 protein, while the GST-XPA$_{4-137}$ mutant protein which contains amino acid residues 4 - 137 of the XPA protein was able to bind (Figure 3). These results further indicate that the N-terminal region of the XPA protein is involved in binding to ERCC1. We have found that the N-terminal region is not well-conserved among

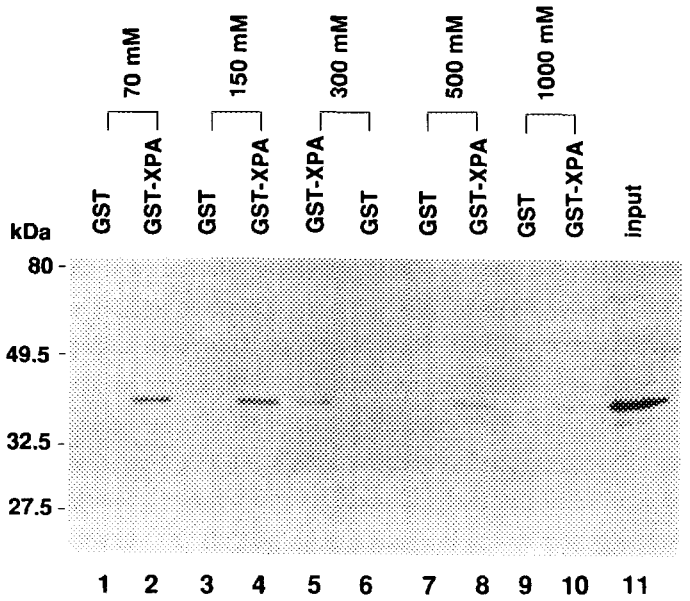

Fig. 2. Effect of salt concentration on the binding of ERCC1 proteins with the GST-XPA beads. HeLa whole cell extracts were incubated with GST (lanes 1, 3, 6, 7, 9) or GST-XPA beads (lanes 2, 4, 5, 8, 10) in the buffer containing 70 mM (lanes 1, 2), 150 mM (lanes 3, 4), 300 mM (lanes 5, 6), 500 mM (lanes 7,8) or 1000 mM (lanes 9, 10) NaCl. After washing the beads with each buffer, the bound proteins were separated by SDS-PAGE and analyzed by immunoblotting with anti-ERCC1 antibody. As a control, HeLa whole cell extracts were loaded directly onto SDS-PAGE (lane 11).

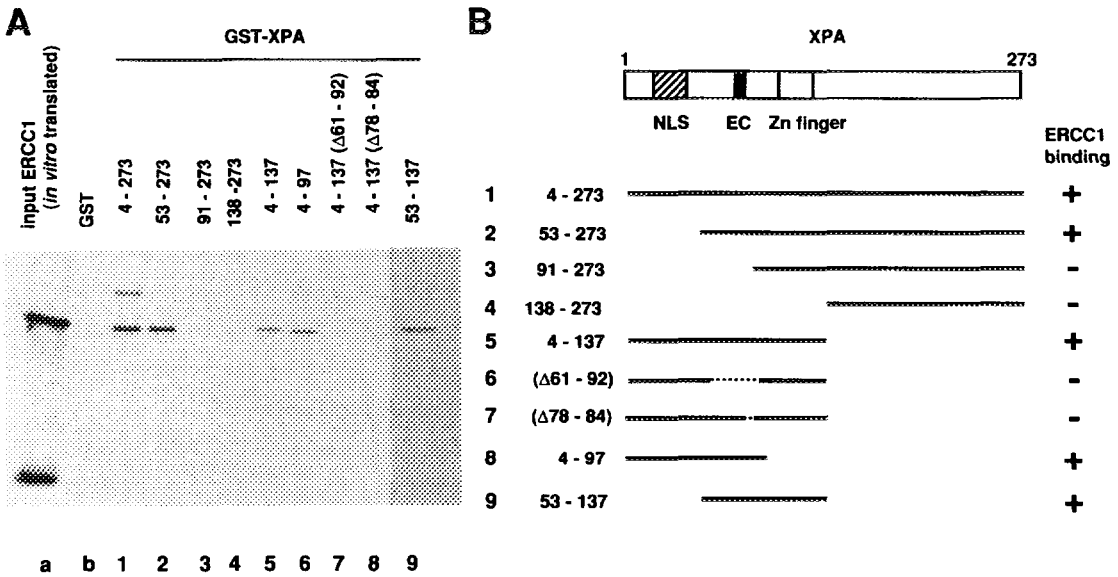

Fig. 3. Localization of the ERCC1-binding region of the XPA protein. (A) The *in vitro* translated and [^{35}S]methionine-labeled ERCC1 proteins were incubated with a set of GST-truncated XPA protein beads or GST beads. After washing with beads, bound ERCC1 proteins were analyzed by SDS-PAGE and image analyzer (FUJIX BAS2000). Lane a, ERCC1 protein directly loaded onto SDS-PAGE; lane b, GST beads; lane 1, GST-XPA$_{4-273}$ beads; lane 2, GST-XPA$_{53-273}$ beads; lane 3, GST-XPA$_{91-273}$ beads; lane 4, GST-XPA$_{138-273}$ beads; lane 5, GST-XPA$_{4-137}$ beads; lane 6, GST-XPA$_{4-97}$ beads; lane 7, GST-XPA$_{4-137(\Delta 61-92)}$ beads; lane 8, GST-XPA$_{4-137(\Delta 78-84)}$ beads; lane 9, GST-XPA$_{53-137}$ beads. (B) Schematic presentation of truncated XPA proteins and the results of their binding to the ERCC1 protein. Lane designations are the same as in (A).

different species except for the nuclear localization signal and the E-cluster region, in contrast to the well-conserved C-terminal region (9). These results prompted us to examine the possibility that the E-cluster region is involved in the interaction with ERCC1. The GST-XPA$_{4-137}$ mutant protein with a deletion of seven consecutive glutamic acids in the E-cluster region (residues 78-84) did not bind to ERCC1 (Figure 3). These results indicate that the E-cluster region of XPA is important for the interaction with ERCC1, although other regions of XPA may also be involved. This is consistent with the recent report that full-length XPA protein with a deletion of the E-cluster region reduced binding to ERCC1 by 70%, but did not abolish binding (15).

Effect of the interaction between XPA and ERCC1 proteins on damaged DNA binding activity: The interaction between the XPA and ERCC1 proteins might be expected to have biological significance in NER. We examined whether the binding activity of XPA to damaged DNA is affected by the interaction with ERCC1. As shown in Figure 4, the UV-damaged DNA binding activity of the XPA protein was markedly increased in the presence GST-ERCC1 protein. The ERCC1 protein alone bound neither UV-damaged nor undamaged double-stranded DNA (data not shown). When undamaged DNA was used as a probe, little or no enhancement of the DNA binding activity was observed in the concomitant presence of the XPA and ERCC1 proteins. The MF122 protein is a truncated XPA protein which lacks the protein-protein interaction regions (and the ability to correct the XPA repair defect) but retains

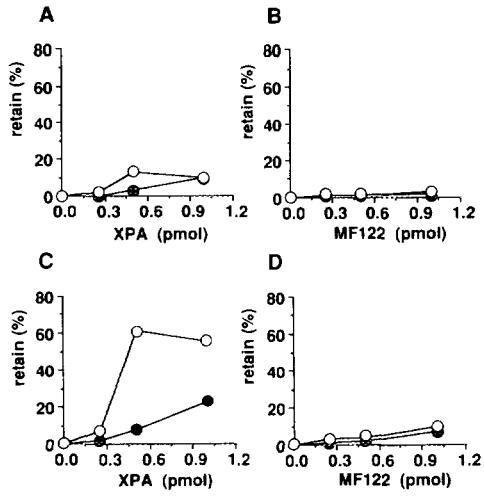

Fig. 4. Effect of the interaction of the XPA and ERCC1 proteins on DNA binding activity. DNA binding activities of the XPA protein (A, C) and the MF122 protein (B, D) were examined by a filter binding assay using unirradiated DNA (A, B) or DNA irradiated with 8 kJ/m^2 UV (C, D) or in the presence (○) or absence (●) of 1.5 pmol ERCC1 protein.

the damaged DNA binding activity (Kuraoka et al., unpublished results). When MF122 was used in place of the wild type XPA protein, ERCC1 did not enhance the UV-damaged DNA binding activity (Fig. 4D).

DISCUSSION

The ability of XPA protein to binds preferentially to damaged DNA suggests that it is involved in the damage-recognition step of NER. We recently found that the damaged DNA binding domain of the XPA protein is localized in a region from Met^{122} to Phe^{219} that includes the C$_4$ type zinc finger motif (Kuraoka et al., in preparation). The E-cluster and the C-terminal regions of the XPA protein, which are well conserved in different species, are not necessary for the damaged DNA binding, suggesting that these regions might have important NER function(s) other than DNA binding. It has been found that ERCC1, RPA, and TFIIH can each bind to the XPA protein. The ERCC1 protein and TFIIH bind to the E-cluster and C-terminal regions of the XPA protein, respectively (4, 8, 10, 11, 12). Thus the E-cluster and C-terminal regions may be domains for protein-protein interactions that help coordinate the NER process. Group A XP cells (XP12ROSV) expressing a mutant XPA protein with a deletion of seven consecutive glutamic acid residues of the E-cluster region or the C-terminal 48 amino acid residues showed only a marginal recovery of UV-sensitivity (7, 15), strongly suggesting that the interaction of the XPA protein with the ERCC1, TFIIH or RPA is indispensable for NER *in vivo*.

The budding yeast homologue of *ERCC1* is *Rad10*. The RAD10 protein forms a stable complex with the RAD1 protein and the RAD10/RAD1 complex is a duplex-3' single strand junction-specific endonuclease (13), indicating that it incises DNA 5' to damaged bases during NER. It has been shown that the ERCC1 protein forms a tight complex with ERCC4 and

ERCC11 activities (one of the latter probably being equivalent to XPF) (5, 6). The ERCC1/XPF/ERCC11 complex is likely to be responsible for an endonuclease activity in human cells similar to the RAD1/RAD10 complex in yeast. Thus the interaction of the XPA and ERCC1 protein may play a role in orienting the ERCC1/XPF/ERCC11 endonuclease in the repair complex. In addition, we showed that the ERCC1 and XPA interaction caused marked enhancement of the damaged DNA binding activity. Hence the interaction of XPA and ERCC1 may also participate in the damage-recognition step of NER.

It has been shown that RPA interacts with the XPG protein, indicating that RPA mediates the formation of an XPA/RPA/XPG ternary complex (4). The XPG protein is a duplex-5' single strand junction-specific endonuclease that incises 3' to DNA damage during NER (14). The XPA/RPA/XPG interaction may also play an important role in loading XPG endonuclease to the damaged sites. Damaged DNA binding activity has been found to be increased by the XPA/RPA interaction as well (4). All these results suggest that the XPA/ERCC1 and XPA/RPA interactions may play key roles in damage-recognition as well as in incisions at both sides of damage.

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