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DNA Repair Protein XPA Binds Replication Protein A (RPA)*

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XPA is a zinc finger DNA-binding protein, which is missing or altered in group A xeroderma pigmentosum cells and known to be involved in the damage-recognition step of the nucleotide excision repair (NER) processes. Using the yeast two-hybrid system to search for proteins that interact with XPA, we obtained the 34-kDa subunit of replication protein A (RPA, also known as HSSB and RFA). RPA is a stable complex of three polypeptides of 70, 34, 11 kDa and has been shown to be essential in the early steps of NER as well as in replication and recombination. We also demonstrate here that the RPA complex associates with XPA. These results suggest that RPA may cooperate with XPA in the early steps of the NER processes.

Xeroderma pigmentosum (XP)[1] is an autosomal recessive human disease characterized by hypersensitivity to sunlight and a high incidence of skin cancer in sun-exposed areas (1). Cells from XP patients are hypersensitive to UV irradiation and have a defect in nucleotide excision repair (NER). Complementation analysis by cell fusion has identified seven different complementation groups (A–G) and a variant form in XP (2, 3). In addition, there are 11 different complementation groups (groups 1–11) in UV-sensitive rodent mutant cell lines. The *XPA* (XP group A) and *XPC* (XP group C) genes have been directly cloned by transfections of XP cells with mouse genomic DNA and a human cDNA library, respectively (4, 5). Additionally, human genes that can correct the repair deficiency of the UV-sensitive rodent mutant cell lines have been cloned by the same transfection cloning strategy and are denoted *ERCC* (excision repair cross-complementing rodent repair deficiency) genes. The *ERCC3*, *ERCC2*, *ERCC5*, and *ERCC6* genes have shown to be equivalent to *XPB* (XP group B), *XPD* (XP group D), *XPG* (XP group G), and Cockayne syndrome group B (*CSB*) genes, respectively (6–11). These genes are involved in the early steps of NER, suggesting that protein interactions and/or protein complex formations are required for the early steps of NER processes, including the recognition of DNA damage, generation of dual incisions on either side of the damage, and excision of the oligonucleotides containing the damaged sites (12). It has been shown that ERCC1 forms a complex with ERCC4 (XPF) and ERCC11 (15, 16), and that XPC associates tightly with a human homologue of yeast RAD23 (17), although the biological meaning of these interactions are not clear yet. Moreover, it is known that XPB is a component of TFIIH (13), a part of the RNA polymerase II transcription initiation complex, and that XPD associates with TFIIH (14), indicating a tight connection between NER and the transcription processes.

We have previously cloned the *XPA* gene and shown that it encodes a protein of 273 amino acids containing a C_4 zinc finger motif (4). The XPA protein (XPA) binds preferentially to UV- or chemical carcinogen-damaged DNA (18–20), suggesting that XPA is involved in the recognition of several types of DNA damage in the NER processes. In light of the observation that the defect in NER in group A XP cells is particularly severe, we hypothesized that XPA might have an important DNA repair function other than damage recognition. We expected that XPA might have domains that interact with other proteins to coordinate the NER processes. Recently, it has been reported that XPA and ERCC1 specifically interact (21, 22). Here, we have used yeast two-hybrid system to identify other XPA-associated proteins by screening a HeLa cDNA library and found that the 34-kDa subunit of replication protein A (RPA, also known as HSSB and RPF) binds to XPA. RPA is composed of three tightly associated subunits of 70, 34, and 11 kDa (p70, p34, and p11) (23–25). We also demonstrate on interaction between the RPA complex and XPA. It has been shown that RPA is involved in NER (26, 27) as well as in replication and recombination (28). These results strongly suggest that a specific interaction between XPA and RPA is required for the early steps of the NER process.

MATERIALS AND METHODS

Yeast Strains—The yeast strain used for the library screening was HF7c (*MATa, ura3–52, his3–200, ade2–101, lys2–801, trp1–901, leu2–3, 112, gal4–542, gal80–538, LYS2::GAL1-HIS3, URA3::(GAL4 17-mers)$_3$-CYC1-lacZ*) (29) and that used for β-galactosidase assays was SFY526 (*MATa, ura3–52, his3–200, ade2–101, lys2–801, trp1–901, leu2–3, 112, can^r, gal4–542, gal80–538, URA3::GAL1-lacZ*) (30). Both strains were obtained from Clontech Laboratories, Inc. (Palo Alto, CA).

Two-hybrid Plasmid Constructions—Plasmid vectors for the two-hybrid system (MATCHMAKER Two-Hybrid System) were purchased from Clontech Laboratories, Inc. The GAL4 DNA-binding domain vector was pGBT9 and the GAL4 activation domain was pGAD424 (31). pGBT9 encodes GAL4 residues 1 to 147 and contains the yeast *TRP1* gene as selectable marker. pGAD424 encodes GAL4 residues 768–881 and contains the yeast *LEU2* gene as a selectable marker. The XPA hybrids with either the GAL4 DNA-binding domain, pGBT9-XPA, or the GAL4-activation domain, pGAD424-XPA, were constructed by ligating the *Bam*HI fragment from pET3c-XPA (20) into *Bam*HI-digested pGBT9 or pGAD424. The entire XPB cDNA was isolated from a λgt10 HeLa cDNA library (Clontech Laboratories, Inc.) by plaque hybridiza-

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[1] The abbreviations used are: XP, xeroderma pigmentosum; RPA, replication protein A; NER, nucleotide excision repair; ERCC, excision repair cross-complementing rodent repair deficiency; PCR, polymerase chain reaction; PMSF, phenylmethylsulfonyl fluoride; PAGE, polyacrylamide gel electrophoresis; GST, glutathione *S*-transferase.

tion using oligonucleotides probes 5'-AACGAATCTTGCTGTACAC-CATGAACCCCA-3'. The entire XPC cDNA was isolated from a λZapII neonatal embryonic carcinoma cDNA library kindly provided by Dr. Hiroshi Nojima (Osaka University) by plaque hybridization using the oligonucleotides probes 5'-TCCTCCAGCGAGGAAGATGAGGGC-CCAGGA-3' and 5'-ATACCCAAGGATGGCAGCTGTCTCGGGCTC-3'. The entire XPD cDNA was isolated from a pcD2 human fibroblast cDNA library kindly provided by Dr. Hiroshi Nojima (Osaka University) by plaque hybridization using a PCR fragment probe (PCR primers: 5'-CGCGAATTCCGCCATGAAGCTCAACGTGGA-3', 5'-GGGCCACATT-GGCATGCAGGATTGA-3'). The hybrid plasmids, pGBT9-ERCC1, pGAD424-ERCC1, pGBT9-XPB, pGAD424-XPB, pGBT9-XPC, pGAD-424-XPC, pGBT9-XPD, and pGAD424-XPD were generated in-frame.

Two-hybrid Screening—Yeast strain HF7c was sequentially transformed with pGBT9-XPA and the GAL4 activation domain pGAD GH HeLa cDNA library (Clontech Laboratories, Inc.) (33). Transformants were plated on yeast drop-out media lacking leucine, tryptophan and histidine. A small fraction of the transformation mixture was spread on yeast drop-out media lacking leucine and tryptophan as a control plate. The transformation frequency was obtained by scoring the colony number on the control plate. After 5 days of growth on yeast drop-out media lacking leucine, tryptophan and histidine, the filter assay was performed to measure β-galactosidase activity and positive colonies were picked up.

β-Galactosidase Assays—β-Galactosidase activity was measured in a filter assay and in a liquid assay using *o*-nitrophenylgalactopyranoside according to the protocol provided by Clontech Laboratories, Inc.

Purification of the GST-XPA—The XPA cDNA encoding amino acid 4–273 was isolated as a 854-base pair *Xma*III-*Aha*III fragment from pcD2h19 (4), blunted with Klenow fragment and ligated into the *Bam*HI site of the expression vector pGEX-2T (Pharmacia Biotech Inc.) using a *Bam*HI linker. The resulting plasmid was designated pGEX-XPA. *Escherichia coli* (JM109 strain) cells transformed with pGEX-XPA were grown to an A_{600} of 0.6, and isopropyl-1-thio-β-D-galactopyranoside was added to a final concentration of 0.1 mM. After 3 h of additional growth, *E. coli* cells were pelleted, lysated, and treated as described by Miura *et al.* (32).

After cells were lysed by sonication, the lysates was centrifuged at 15,000 rpm for 20 min (Sorvall type SS-34 rotor) at 4 °C. The pellet was saved and resuspended in buffer A (50 mM Tris-HCl, pH 7.5, 6 M guanidine HCl, 0.5 mM PMSF and 1 mM dithiothreitol) in order to denature the inclusion bodies. The suspension was stirred at 4 °C for 1 h and then centrifuged at 50,000 rpm for 1 h (Hitachi RP 65T rotor) at 4 °C. The supernatant was loaded onto a Superose CL-6B (Pharmacia) gel filtration column equilibrated with buffer A. The protein was eluted with buffer A (flow rate, 0.5 ml/h) and then the GST-XPA peak fraction was dialyzed against buffer B (20 mM Tris-HCl, pH 7.5, 0.5 M NaCl, 1 mM $MgCl_2$, 20 mM zinc acetate, 0.5 mM PMSF, and 1 mM dithiothreitol) at 4 °C. The purity was checked by SDS-PAGE (11%) and Coomassie staining. The DNA repair activity of the GST-XPA protein was checked by the restoration of UV-induced unscheduled DNA synthesis in group A XP cells (GM5509 cells obtained from NIGMS Human Genetic Mutant Cell Repository, Camden, NJ) after microinjection of the protein (20).

In Vitro Transcription and Translation of p34—The p34 cDNA fragment which we cloned lacked the N-terminal 4 amino acids. To obtain the cDNA with the entire p34 coding region, poly(A)$^+$ RNA from WI 38 VA 13 cells was reverse transcribed and amplified using 5'-ACCT-TCTCGGCCTCTTTGC-3' and 5'-GCCCATCTCCCTCTGAGC-3' as primers. The PCR product was ligated to the *Sma*I site of pBluescript II KS(+) (Stratagene, California). *In vitro* transcription and translation was performed using the TNT coupled reticulocyte lysate system (Promega) according to the manufacturer's instructions.

Purification of RPA from HeLa Cells—RPA was purified to homogeneity from HeLa cells by sequential column chromatography (phosphocellulose, hydroxyapatite, single-stranded DNA-cellulose, Mono Q), with the active fractions identified by their ability to stimulate DNA polymerase α activity and SV40 synthesis in crude extracts as described previously (23–25). The purified fraction prepared from 2×10^{10} HeLa cells contained 1.4 mg of the protein.

Preparations of Nuclear Extracts and Whole Cell Extracts from HeLa Cells—HeLa cells were washed once with solution A (20 mM Tris-HCl, pH 7.5, 3 mM $MgCl_2$, 1 mM EDTA, and 0.5 mM PMSF), suspended in solution A containing 0.05% Triton X-100 at a concentration of 1×10^7 cells/ml, and stood at 0 °C for 20 min to be lysed. Nuclei were separated by centrifugation for 5 min at 1,000 × *g* and washed once with solution A. Nuclear extracts were prepared by adding NETN (50 mM Tris-HCl, pH 7.8, 150 mM NaCl, 1 mM EDTA, 1% Nonidet P-40, 0.5 mM PMSF, 0.5 μg/ml leupeptin, and 0.5 μg/ml pepstatin) at a concentration of 2×10^7 nuclei/ml and incubated at 0 °C for 20 min. The extracts were centrifuged for 10 min at 13,000 × *g*, and the supernatant was recovered. For radiolabeling, HeLa cells were incubated with methionine-free medium for 1 h and then metabolically radiolabeled by the addition of [^{35}S]methionine (3.7 MBq/ml; Tran^{35}S-label, DuPont NEN) for 3 h. Whole cell extracts were prepared by adding NETN at a concentration of 2×10^7 cells/ml and incubated at 0 °C for 20 min. The extracts were centrifuged for 10 min at 13,000 × *g*, and the supernatant was recovered.

In Vitro Assay for Binding of the XPA Protein to p34 or RPA—The GST-XPA (500 ng) adsorbed to glutathione-Sepharose (10 μl) was incubated with *in vitro* translation products, 300 ng of purified RPA in 1 ml of NETN containing 50 mg/ml skim milk, or HeLa nuclear extract prepared from 2×10^7 cells at 4 °C for 2 h and then washed five times with NETN. The bound proteins were extracted by boiling in SDS sample buffer and separated by SDS-PAGE and analyzed by fluorography or immnoblotting with anti-p70 or anti-p34 monoclonal antibodies.

Immunoprecipitation—The supernatant media of hybridoma (1 ml) or anti-XPA polyclonal antibody (2 μg) in 1 ml of NETN were mixed with 10 μl of protein G plus/protein A-agarose (Oncogene Science) at 4 °C for 1 h. After being washed three times with NETN, the agarose beads were incubated with whole cell extract prepared from 2×10^7 cells at 4 °C for 2 h and washed five times with NETN.

RESULTS

Two-hybrid Screening—The yeast two-hybrid protein interaction screening procedure was carried out to isolate cDNAs encoding proteins that could associate with XPA. Yeast strain HF7c was sequentially transformed with pGBT9-XPA and pGAD GH HeLa cDNA library (33). Transformants (1.2×10^6) were subjected to under dual selection (HIS$^+$, LacZ$^+$), and 39 independent clones were isolated. We failed to rescue plasmid DNA from seven clones, but classified the remaining 32 clones into three groups by dot blot hybridizations of each plasmid using the cDNA insert of each isolated plasmid as a probe. To confirm the above protein-protein interactions, representative candidate plasmids from each of the three groups were retransfomed into HF7c in the following combinations: candidate plasmid alone, pGBT9 plus the candidate, pGBT9-XPA plus the candidate, or pVA3(murine $p53_{[72-390]}$ in pGBT9, Clontech Laboratories, Inc., California) plus the candidate. These transformants were tested for β-galactosidase activity in the filter assay. Only colonies containing the combination of pGBT9-XPA and each candidate turned blue, suggesting that all of the candidates encode proteins that specifically interact with XPA (data not shown). To further characterize these cDNAs, we determined their partial DNA sequences and carried out homology searches using GenBank and EMBL data bases. Two of the three groups were found to be unknown. However, the open reading frame that was in-frame to GAL4 activation domain in clone 16 (third group by a single isolate) perfectly matched to a portion of the cDNA sequence of the 34-kDa subunit (p34) of replication protein A (RPA, also known as HSSB and RFA). These results suggest that XPA could interact with p34.

Specific Interaction of XPA and p34 in Yeast Cells—Since RPA was shown to be involved in an early step of NER processes (27), it is possible that p34 might interact with NER proteins other than XPA. We tested interactions among p34, ERCC1, XPA, XPB, XPC, and XPD using the two-hybrid assay (Fig. 1 and Table I). When cells were transformed with a combination of pGBT9-XPA and pGAD424-ERCC1, colonies grew on plates lacking histidine, indicating an interaction between XPA and ERCC1 as reported (Fig. 1) (21, 22). However, these colonies failed to induce β-galactosidase activity in our filter and liquid quantitative assays (Table I). The same results were obtained using the transformants with a combination of pGBT9-ERCC1 and pGAD424-XPA (data not shown). On the other hand, as stated above, β-galactosidase activity was observed in the transformants with either combination of pGBT9-XPA and pGAD424-p34 or pGBT9-p34 and pGAD424-XPA

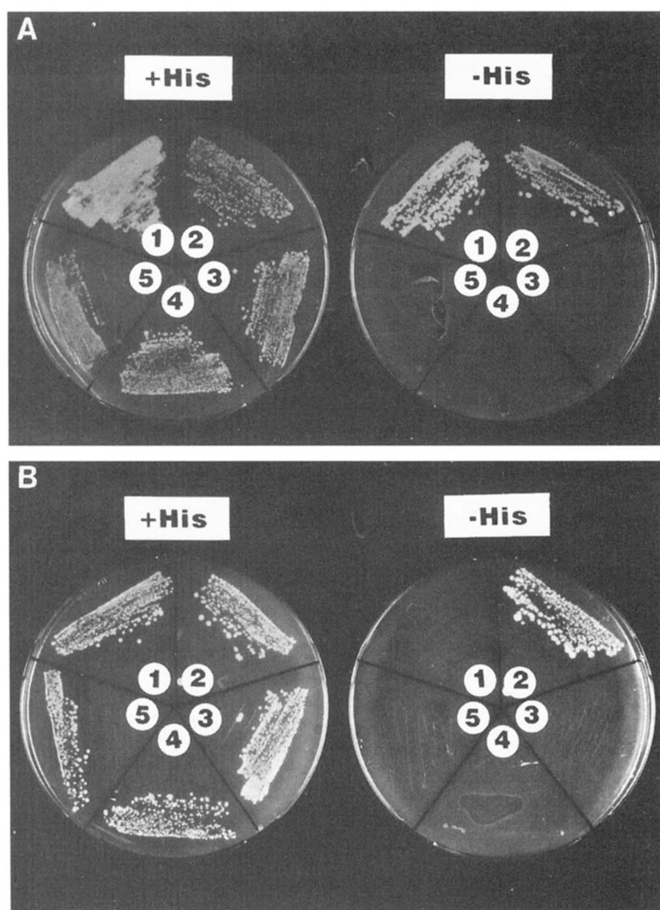

FIG. 1. *Panel A*, interaction of XPA protein. Yeast cells (HF7c) were simultaneously transformed with pGBT9-XPA and plasmids expressing the pGAD424 fused to the NER genes *ERCC1* (*section 1*), *XPB* (*section 3*), *XPC* (*section 4*), *XPD* (*section 5*), or to pGAD424 fused to p34 (*section 2*). *Panel B*, interaction of the 34-kDa subunit of RPA. Yeast cells (HF7c) were simultaneously transformed with pGAD424-p34 and plasmids expressing the pGBT9 fused to the NER genes *ERCC1* (*section 1*), *XPA* (*section 2*), *XPB* (*section 3*), *XPC* (*section 4*), and *XPD* (*section 5*). Cells containing both plasmids were streaked on plates with or without histidine. The ability to grow in the absence of histidine depends on the expression of the HIS3 gene under the control of a Gal1-responsive promoter.

TABLE I
Protein-protein interactions determined by a liquid quantitative β-galactosidase assay

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

DNA-binding domain	Activation domain	β-Galactosidase activity
		Miller units
pGBT9-XPA	pGAD424	0.2
pGBT9	pGAD GH-p34	0.1
pGBT9-XPA	pGAD GH-p34	4.6
pGBT9-XPA	pGAD424-ERCC1	0.1
pGBT9-p34	pGAD424	0.7
pGBT9-p34	pGAD424-XPA	3.0

(Table I). With the exception of pGBT9-p34, which induced a small amount of β-galactosidase activity, these plasmids alone induced essentially no β-galactosidase activity. Moreover, no protein-protein interactions were detected in the yeast two-hybrid system other than the combination of XPA and p34 or ERCC1 (Fig. 1).

Interaction of XPA with p34 in Vitro—To further confirm the association of XPA and p34, *in vitro* binding assays were performed. We constructed a glutathione *S*-transferase (GST) and GST-XPA fusion protein. The GST or GST-XPA was immobilized onto glutathione-Sepharose beads. The *in vitro* translated and [^{35}S]methionine-labeled p34 was incubated with either GST or GST-XPA beads. After washing the beads, the GST or GST-XPA bound proteins were extracted and analyzed by SDS-PAGE and fluorography. As shown in Fig. 2, the p34 was bound to GST-XPA but not to GST.

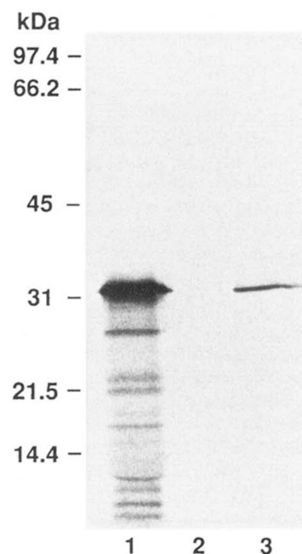

FIG. 2. **Interaction of XPA with p34 *in vitro*.** *In vitro* translated [^{35}S]methionine-labeled p34 (*lane 1*) was incubated with GST beads (*lane 2*) or GST-XPA beads (*lane 3*). After washing the beads, the bound proteins were separated by SDS-PAGE and analyzed by fluorography.

Interaction of XPA with RPA Complex—RPA is known to be a stable complex of three polypeptides of 70, 34, and 11 kDa. To examine whether XPA could associate with the RPA complex, nuclear extracts from HeLa cells were mixed with GST or GST-XPA beads. The proteins bound to the GST or GST-XPA beads were analyzed by SDS-PAGE and subsequent immunoblotting with anti-p34 and anti-p70 antibodies. As shown in Fig. 3*a*, both p34 and p70 were detected in the fraction bound to GST-XPA but not in that bound to GST. To further confirm the association of XPA with the RPA complex, the nuclear extracts from HeLa cells which have been metabolically labeled with [^{35}S]methionine were incubated with GST or GST-XPA beads, and the bound proteins were eluted with the buffer containing 1 M NaCl. The eluate was immunoprecipitated either with anti-p34 or anti-p70 antibody. All three subunits (70, 34, and 11 kDa) of RPA complex were detected in both immunoprecipitants (Fig. 3*b*). These results confirmed that RPA complex binds to XPA.

To examine whether the interaction between RPA and XPA is direct or is mediated through other factors, purified RPA complex was incubated with GST or GST-XPA beads. The GST or GST-XPA bound fractions were analyzed by SDS-PAGE and immunoblotting with anti-p70 or anti-p34 monoclonal antibody. As shown in Fig. 3*c*, both p34 and p70 bound directly to GST-XPA but not to GST.

Co-immunoprecipitation of RPA with XPA—We further examined the specific interaction between XPA and RPA by co-immunoprecipitation analysis using anti-XPA antiserum. HeLa whole cell extracts were immunoprecipitated with anti-XPA polyclonal antibody. The immunoprecipitants were analyzed by SDS-PAGE and subsequent immunoblotting with anti-p34 and anti-p70 antibodies. As shown in Fig. 4, both p70 and p34 were co-immunoprecipitated with XPA by anti-XPA antiserum. These results suggest that XPA may interact with RPA *in vivo*.

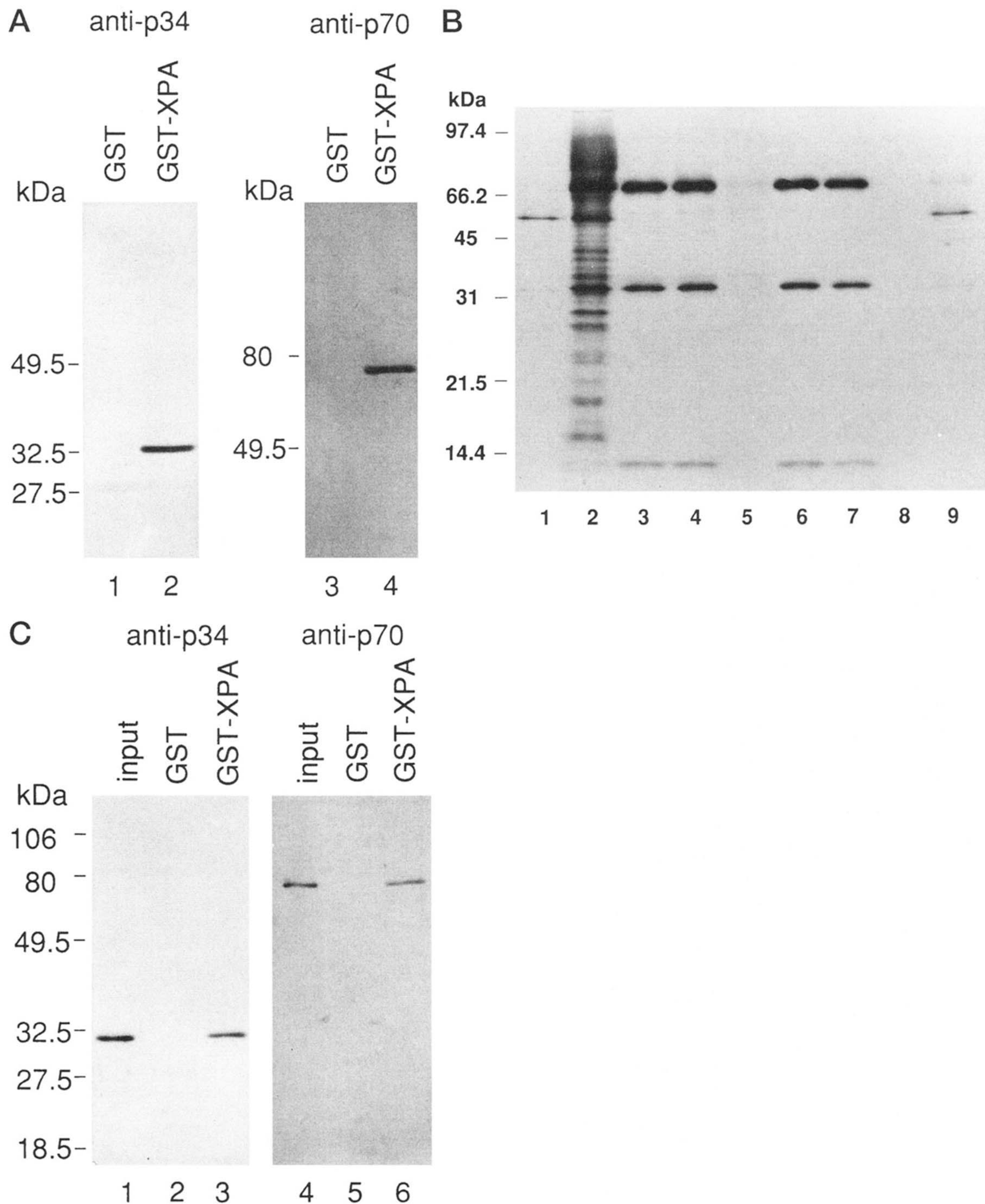

FIG. 3. **Interaction of XPA with the RPA complex *in vitro*.** *Panel A*, HeLa nuclear extract was incubated with GST beads (*lanes 1* and *3*) or GST-XPA beads (*lanes 2* and *4*). After washing the beads, the bound proteins were analyzed by SDS-PAGE and immunoblotting with either anti-p70 or anti-p34 antibody. *Lanes 1* and *2*, immunoblotting with anti-p34 antibody; *lanes 3* and *4*, immunoblotting with anti-p70 antibody. *Panel B*, [^{35}S]methionine-labeled HeLa nuclear extracts were incubated with GST (*lane 1*) or GST-XPA (*lane 2*) beads, and the bound proteins were analyzed by SDS-PAGE and fluorography. The HeLa nuclear extracts were immunoprecipitated with the corresponding antibodies, directly loaded onto SDS-PAGE, and analyzed by fluorography (*lane 3*, immunoprecipitation with anti-p70 antibody; *lane 4*, immunoprecipitation with anti-p34 antibody; *lane 5*, immunoprecipitation with control supernatant media). On the one hand, the proteins bound to GST-XPA beads were eluted with NETN containing 1 M NaCl, immunoprecipitated with the protein G plus/protein A-agarose beads, which have been bound with the corresponding monoclonal antibodies (*lane 6*, anti-p70 antibody; *lane 7*, anti-p34 antibody; *lane 8*, control supernatant media). *Lane 9*, residual GST-XPA beads after the elution. The bound proteins were separated by SDS-PAGE and analyzed by fluorography. *Panel C*, purified RPA was incubated with GST beads (*lanes 2* and *5*) or GST-XPA beads (*lanes 3* and *6*). After washing the beads, the bound proteins were extracted by boiling in SDS sample buffer and separated by SDS-PAGE. As a control, purified RPA was loaded directly onto an SDS-polyacrylamide gel (*lanes 1* and *4*). The 70- and 34-kDa subunits of RPA were detected by immunoblotting with the corresponding monoclonal antibodies. *Lanes 1–3*, immunoblotting with anti-p34 antibody; *lanes 4–6*, immunoblotting with anti-p70 antibody.

DISCUSSION

XPA is a zinc metalloprotein and binds preferentially to UV-, cisplatin-, or osmium tetroxide-damaged DNA (18–20), indicating that it is involved in the damage-recognition step of the NER processes. We recently identified the DNA-binding domain of XPA. The region of XPA containing a C_4 type zinc

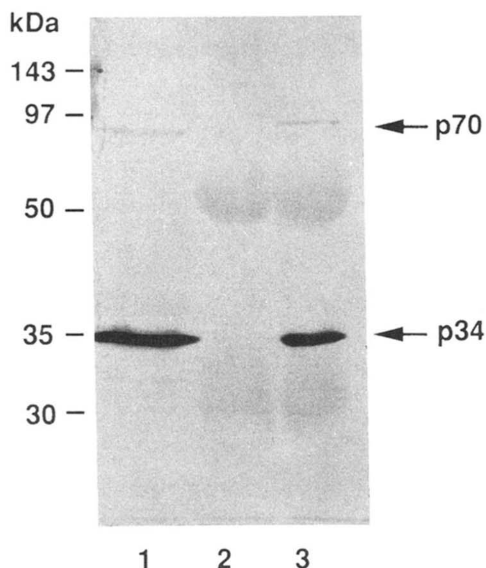

FIG. 4. **Co-immunoprecipitation of RPA with XPA.** HeLa whole cell extracts were immunoprecipitated with control (*lane 2*) or anti-XPA (*lane 3*) antibody. The precipitates were immunoblotted with anti-p70 and anti-p34 antibodies. *Lane 1*, whole cell extract from 2.5 × 10^6 cells.

finger domain is sufficient for its preferential binding to damaged DNA. The E-cluster and the C-terminal regions are not necessary for the DNA binding activity of XPA.[2] In addition, we have cloned homologues of the human *XPA* cDNA from mouse, chicken, *Xenopus laevis*, and *Drosophila melanogaster* (34). A comparison of the amino acid sequences of these homologues revealed that the N-terminal region is not well conserved except in its nuclear localization signal and E-cluster region, whereas the C-terminal region is highly conserved. These results suggest that the E-cluster and the C-terminal regions may have an important DNA repair function separate from DNA binding. We speculated that they might be domains for protein-protein interactions that coordinate the NER processes.

We therefore searched for proteins that bind to XPA using the yeast two-hybrid system, and found that the p34 subunit of RPA bound to XPA. The direct association between XPA and the RPA complex was confirmed by *in vitro* experiments. Furthermore, the RPA complex was co-immunoprecipitated from HeLa whole cell extracts with XPA by anti-XPA antiserum, suggesting the association of XPA and the RPA complex *in vivo*.

RPA was originally purified as a factor essential for the *in vitro* replication of SV40 (23–25). It is a three-subunit protein complex and binds strongly to single-stranded DNA and weakly to RNA and double-stranded DNA (35, 36). It is requisite for the helicase action of the virus-encoded T antigen to open the SV40 origin, and it stimulates *in vitro* DNA synthesis by DNA polymerases on artificial templates (37–40). It has been demonstrated that the p70 subunit of RPA, which retains single-stranded DNA binding activity (35, 36, 39), can interact with the tumor suppressor p53 and the transcription activators GAL4, VP16, and E2, functioning synergistically to enhance replication (41–43). SV40 large T antigen also can bind to intact RPA but not to any of the separated subunits of RPA (44). Less is known about the functions of the p34 and p11 subunits of RPA. It has been speculated that the two acidic regions of p34 are involved in protein-protein interactions with other proteins involved in replication or other functions (45). Our results indicate that XPA is one of these proteins that bind to the p34 subunit of RPA.

It has been found that RPA is involved in NER (26, 27) as well as in DNA replication and recombination (28). Neutralizing monoclonal antibodies which recognize the 70- and 34-kDa subunits inhibited DNA repair synthesis *in vitro* and the addition of an excess amount of RPA into an *in vitro* NER assay system caused a 2–3-fold stimulation of DNA repair synthesis. Interestingly, when damaged DNA is incised by Uvr ABC excinuclease, human cell extracts can carry out DNA repair synthesis even when RPA has been neutralized with antibodies (27), suggesting that RPA might be involved in the early step of the NER process. All these results taken together suggest that XPA might cooperate with RPA for the recognition of DNA damages.

It has been shown that XPA forms a complex with the ERCC1/ERCC4 (XPF) heterodimer (22). The ERCC1/ERCC4 (XPF) heterodimer might be a major component of the incision complex (15, 16). XPA might play a role in loading the incision complex onto a damaged DNA site. Thus, XPA is a multifunctional protein that coordinates the NER processes. Similarly, RPA also plays multiple roles during NER. RPA might also bind to the single-stranded gap created by the excision of damaged nucleotides. The binding of RPA to the gap protects the single-stranded region and DNA termini from degradation by other nucleases and could facilitate the recycling of the incision protein complex by means of protein-protein interactions (27). Therefore, the interaction between XPA and RPA is likely to have plural biological significance in the early steps of the NER processes.

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