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An Interaction between the DNA Repair Factor XPA and Replication Protein A Appears Essential for Nucleotide Excision Repair

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Replication protein A (RPA) is required for simian virus 40-directed DNA replication in vitro and for nucleotide excision repair (NER). Here we report that RPA and the human repair protein XPA specifically interact both in vitro and in vivo. Mapping of the RPA-interactive domains in XPA revealed that both of the largest subunits of RPA, RPA-70 and RPA-34, interact with XPA at distinct sites. A domain involved in mediating the interaction with RPA-70 was located between XPA residues 153 and 176. Deletion of highly conserved motifs within this region identified two mutants that were deficient in binding RPA in vitro and highly defective in NER both in vitro and in vivo. A second domain mediating the interaction with RPA-34 was identified within the first 58 residues in XPA. Deletion of this region, however, only moderately affects the complementing activity of XPA in vivo. Finally, the XPA-RPA complex is shown to have a greater affinity for damaged DNA than XPA alone. Taken together, these results indicate that the interaction between XPA and RPA is required for NER but that only the interaction with RPA-70 is essential.

Nucleotide excision repair (NER) is a ubiquitously expressed DNA repair pathway that removes a wide range of structurally unrelated DNA damage from the genome. Three human genetic diseases, xeroderma pigmentosum (XP), Cockayne's syndrome, and trichothiodystrophy, have been identified as being caused by defects in this pathway, and much progress has been made recently in identifying and cloning the genes involved in this pathway by using cell lines from these patients or mutant rodent cell lines (for recent reviews, see references 19 and 40). The gene that complements XP group A (XPA) cells encodes a zinc finger protein (45) that preferentially binds to DNA damaged by irradiation with UV light or by treatment with chemical agents such as *cis*-diamminedichloroplatinum(II) (21, 39). The damage recognition activity of XPA is an essential function in NER, since XPA cells in which the protein has been inactivated by mutation are completely deficient in the incision step of NER. Furthermore, XP group A patients are afflicted by the most severe forms of the disease, which include highly elevated levels of skin cancer and progressive mental retardation. In addition to its function in the early damage recognition step of NER, XPA may also play a further role in subsequent steps of damage processing. In this regard, we and others have reported that XPA interacts with the repair protein ERCC1 (26, 29, 36). ERCC1 forms a complex with ERCC4 and possibly other factors (2, 38, 46) and contains a putative excision nuclease (excinuclease) activity that makes an incision 5′ to the site of damage (1). Thus, an additional function of XPA is to load and orient the ERCC1-ERCC4 excinuclease complex at the site of damage.

Replication protein A (RPA; also called human single-stranded DNA-binding protein [HSSB]) is a three-subunit complex consisting of polypeptides p70 (RPA-70), p34 (RPA-34), and p14 (RPA-14) (12, 49, 50) and is required for the initiation and elongation steps of simian virus 40 (SV40)-directed DNA replication in vitro. The RPA-70 subunit contains the single-stranded DNA-binding activity (11, 22), while the function of the other two subunits is unknown, although the RPA-34 subunit is phosphorylated at the G_1/S phase transition and dephosphorylated during mitosis (7, 10, 13, 15). In addition to RPA, two other factors, SV40 large T antigen and polymerase alpha-primase, are essential for initiation of replication at the SV40 origin (20, 44, 48). In the first step of initiation, T antigen binds to the origin as a double hexameric complex in an ATP-dependent process and induces unwinding of the DNA by a small number of turns (29). In the presence of RPA and ATP, T antigen extensively unwinds the helix bidirectionally from the origin, and subsequently polymerase alpha-primase is recruited to form the initiation complex (4, 16). This complex then synthesizes an RNA-DNA primer at the SV40 origin. Direct interactions have been demonstrated between RPA and T antigen, between RPA and polymerase alpha-primase, and between T antigen and polymerase alpha-primase (8, 31, 43). In addition to its role in DNA replication, RPA has also been shown to be required for the incision step of NER in vitro (5, 6, 42). This latter result was somewhat unexpected, since the most plausible role for RPA was in the later repair synthesis stage of NER, as was found for proliferating cell nuclear antigen (PCNA) (34, 42), rather than the earlier incision steps. The function of RPA in the incision process has not been elucidated and, as suggested previously (5), may only be required for turnover of the incision complex and not for the actual incision reaction. In vivo evidence of a function for RPA in NER is shown by the recent report that temperature-sensitive mutations in *Saccharomyces cerevisiae* RPA-70 result in cellular UV sensitivity at the restrictive temperature (28).

The yeast two-hybrid system (3, 14) has been highly useful for investigating interactions among proteins involved in complex biochemical pathways. We have used the two-hybrid system to search for interactions among DNA repair factors involved in NER. Using XPA as the bait in a two-hybrid library

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screen, we have identified a number of proteins that interact with XPA, including, as previously reported, ERCC1 (26, 27). Here, we report that a two-hybrid screen revealed that XPA also interacts with RPA-34 and RPA-70, the two largest subunits of RPA. Furthermore, XPA interacts with RPA in vitro, as determined in a pull-down assay. When individual subunits of RPA were examined in vitro, both RPA-34 and RPA-70, but not RPA-14, were found to interact with XPA. Mutational and functional analysis of the RPA-interactive regions in XPA indicate that XPA-RPA interaction is likely to be required for damage processing by the NER system. The interaction between XPA and RPA also suggests that RPA is involved in the early steps of processing a damaged site for subsequent incision in addition to its likely involvement in the later repair synthesis steps. It also indicates a model by which the incision stage of the repair reaction might be coupled to the subsequent gap-filling step.

MATERIALS AND METHODS

Plasmids and strains. Plasmid pMAL-XPA and a pMAL construct expressing XPA (59-273) were kindly provided by Aziz Sancar (University of North Carolina at Chapel Hill). *Escherichia coli* PR745 and the vector pMAL-c2 were obtained from New England Biolabs (Beverly, Mass.). The vectors pBSIIKS$^+$ and pGEX-2T were obtained from Stratagene (La Jolla, Calif.) and Pharmacia (Piscataway, N.J.), respectively. *E. coli* MutS was obtained from Pharmacia.

Two-hybrid assay. The preparation of the pAS1-XPA construct has been previously described (26). Two-hybrid screenings of a human lymphocyte cDNA library in the pACT vector, the β-galactosidase assay, and rescue of plasmids from yeast clones into bacterial hosts were performed as described previously (9). Manual sequencing of rescued plasmids was done by the dideoxy method with Sequenase 2.0 according to the manufacturer's (United States Biochemical, Cleveland, Ohio) specifications. Homology searches were performed with FASTA (Genetics Computer Group, Madison, Wis.).

Deletion mutants. Deletion mutants expressing truncated forms of maltose-binding protein (MBP)-XPA were prepared by the exonuclease III method (18) as previously described (27). MBP-XPA mutants containing small internal deletions were prepared by oligonucleotide-directed site-specific mutagenesis as described previously (27). The oligonucleotides used for the mutagenesis of XPA had the following sequences: ΔEYLL, 5′ CCAAAACAGAGGCAAAACAAT TAGAAAAAAGAGAGCCACC 3′; ΔKREP, 5′ GAAAGACTGTGATTTAG AACCTCTTAAATTTATTGTGA 3′; ΔLKFI, 5′ TAGAAAAAAGAGAGCCA CCTGTGAAGAAGAATCCACATC 3′; and ΔKNPH, 5′ CTCTTAAATTTAT TGTGAAGCATTCACAATGGGGTGAT 3′.

Preparation and purification of recombinant proteins. MBP-XPA fusion proteins were expressed in *E. coli* PR745. Induction of the expression of the fusion proteins with IPTG (isopropylthiogalactopyranoside), lysis of the cells by sonication, and subsequent purification by affinity chromatography on amylose resin were performed according to the manufacturer's recommendations (New England Biolabs). Additional purification of the fusion proteins was obtained by chromatography on heparin-agarose as previously described (27).

In vitro protein-protein interaction assay (pull-down assay). The interaction of proteins with MBP-XPA fusion proteins bound to amylose resin was examined as previously described (26, 27). Sources for RPA were either whole-cell extracts, purified RPA from mammalian cultured cells (George Brush, Johns Hopkins University; Zhengqiang Pan, Memorial Sloan-Kettering Cancer Center), or purified recombinant RPA (Zhengqiang Pan) or subunits (Emma Gibbs, Memorial Sloan-Kettering Cancer Center; Friedemann Muller, Memorial Sloan-Kettering Cancer Center) from *E. coli.*

IDT assay. To determine preferential binding of DNA repair proteins to UV-damaged DNA, an immobilized DNA template (IDT) assay was performed as described previously (27). Briefly, two fragments obtained by digestion of pBR322 were labeled at one end by the addition of biotinylated dATP with Klenow fragment. After purification, a portion of the fragments was irradiated with 600 J/m^2, and another portion was left undamaged. The fragments were bound to streptavidin-magnetic beads, and after a wash, the beads were incubated with various amounts of repair proteins. After another wash, the bead-bound proteins were eluted with sodium dodecyl sulfate (SDS) and examined by SDS-polyacrylamide gel electrophoresis (PAGE) followed by immunoblotting.

Immunoblotting. Antisera to RPA-70, RPA-34, and RPA-14 were kindly provided by Bruce Stillman (Cold Spring Harbor Laboratory) and George Brush and Christopher Umbrischt (Johns Hopkins University), respectively. Immunoblotting was performed with an enhanced chemiluminescence kit (Amersham, Arlington Heights, Ill.) according to the manufacturer's specifications.

Repair synthesis in cell extracts. Whole-cell extracts were prepared from WI-L2 lymphoblastoid cells as previously described (52). The in vitro assay for DNA repair was performed essentially as described previously (52) with the following modifications. pGEX2T and pBSII supercoiled DNAs were used as the control and damaged substrates, respectively. *N*-(Guanin-8-yl)acetylaminofluorene adducts were introduced into pBSII by treatment with *N*-acetoxy-*N*-2-acetylaminofluorene (AAAF) (kindly provided by Eric Tang, University of Texas M. D. Anderson Cancer Center, Smithville, Tex.) as previously described (24). Unincorporated AAAF was removed from the *N*-(guanin-8-yl)acetylaminofluorene-modified plasmids by repeated extractions with diethyl ether and subsequent ethanol precipitation. DNA repair synthesis was performed in a volume of 50 μl with 200 μg of whole-cell extract for 3 h at 30°C. Subsequently, plasmids were linearized by *Eco*RI digestion and subjected to agarose gel electrophoresis. Dried gels were exposed to film to obtain autoradiograms.

DNA transfection and UV survival. Transfection of plasmid DNAs into SV40-immortalized XP2OS-SV cells, selection of transformants with hygromycin B, and determination of UV survival were performed as previously described (25).

TABLE 1. Two-hybrid interactions between XPA and RPA[a]

pAS1	pACT	
	RPA-34	RPA-70
XPA	+	+
SNF1	−	−
tat	−	−
p53 gene	−	−
Lamin gene	−	−

[a] The pAS1 and pACT plasmids have been described previously (9). A plus sign indicates activation of the *HIS3* and *lacZ* reporter genes in strain Y190.

RESULTS

Association between RPA subunits and XPA in vivo. To identify proteins that interact with XPA, we screened a human peripheral lymphocyte cDNA library by a modified two-hybrid assay (9). Partial sequencing of one positive clone indicated that it contained the cDNA that encoded RPA-34. As shown in Table 1, this interaction was specific to XPA, as other tested proteins failed to give a positive result. We next performed a two-hybrid assay to determine if the large subunit of RPA, RPA-70, directly cloned into the pACT vector interacted with XPA as well. This experiment also yielded a positive result, indicating that both of the two largest subunits of RPA interact with XPA in vivo.

Association between XPA and RPA in vitro. To determine if the interaction observed in vivo between XPA and subunits of RPA also occurred in vitro, we used a pull-down assay, in which an MBP-XPA fusion protein bound to amylose beads was used as an affinity reagent. We first used the pull-down assay to determine if XPA interacted with RPA. As shown (Fig. 1A), MBP-XPA but not MBP alone interacted with RPA contained in whole-cell extracts. Typically, approximately 20% of the RPA contained in the whole-cell extract bound to the MBP-XPA affinity column. Furthermore, MBP-XPA also interacted individually with purified preparations of recombinant RPA-34 and glutathione *S*-transferase (GST)–RPA-70, while MBP did not (Fig. 1B). Control experiments (not shown) indicated that GST itself did not bind to MBP-XPA. The third subunit, RPA-14, did not interact with MBP-XPA. Since both XPA and RPA are DNA-binding proteins, it was possible that rather than a direct interaction between these two proteins, the association was mediated by contaminating DNA in the protein preparations. To ensure that this was not the case, a pull-down assay was performed in the presence of concentrations of either DNases or RNases sufficient to completely digest endogenous nucleic acids present in the extracts. As shown (Fig. 1C), the presence of nucleases did not affect the binding of RPA to MBP-XPA. Together, these results confirm the in vivo findings obtained with the two-hybrid assay and

FIG. 1. In vitro binding of RPA and its subunits to XPA as determined by a pull-down assay and subsequent immunoblotting. (A) Binding of RPA from HeLa cell extract to MBP-XPA and MBP. (B) Binding of the individual recombinant subunits of RPA to MBP-XPA and MBP. Note that the recombinant RPA-70 subunit has been tagged with GST. (C) Binding of RPA to MBP-XPA is not affected by treatment with either DNase I (80 U), micrococcal nuclease (2 U), or an RNase cocktail (2 μg). For all panels, the heading HeLa extracts indicates direct loading of extract for use as a marker without prior incubation with amylose beads.

suggest that the interaction between XPA and RPA may be involved in damage processing by the NER pathway.

Identification of an RPA-interactive domain in XPA. To make a preliminary determination of the RPA-interactive domain in XPA, we prepared a series of deletion mutants of MBP-XPA that were truncated at the carboxy terminus of XPA. These mutants were then analyzed by a pull-down assay for interaction with RPA. As shown in Fig. 2a, an initial assay indicated that XPA polypeptides containing residues 1 to 219 or longer bound to RPA but that polypeptides containing residues 1 to 153 or shorter were deficient in binding. To further refine the region involved in binding RPA, three additional mutants that were truncated between residues 153 and 219 were prepared. As shown (Fig. 2b), all three of these deletion mutants bound to RPA, which indicated that the amino-terminal 64% of XPA contains a region sufficient for binding to RPA and suggests that a region between residues 153 and 176 contains a domain that is required for the interaction (Fig. 2c). Also note that the XPA deletion mutants that were defective in interacting with RPA nevertheless bound a residual amount of RPA that was not detected with the MBP control. This latter finding suggests that there may be a second, weaker RPA-interactive domain located upstream of the one identified between residues 153 and 176.

Identification of conserved motifs in XPA that affect the interaction with RPA. To further define the RPA-interactive domain between XPA residues 153 and 176 identified above, we examined this region for conserved motifs in a number of XPA homologs (Fig. 3A) (41). Each of the four conserved motifs shown in Fig. 3A was independently deleted by site-specific mutagenesis and subsequently examined for interaction with RPA. For clarity, each deletion mutant will be referred to by the first four amino acids of the deletion. All of the mutants were examined in a pull-down assay for interaction

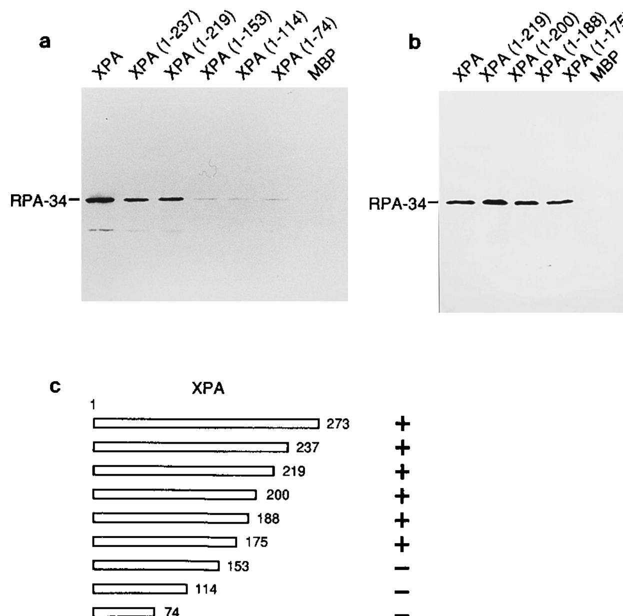

FIG. 2. Mapping of the RPA-interactive domain in XPA. (a and b) Binding of RPA from HeLa cell extracts to carboxy-truncated MBP-XPA polypeptides, as determined by a pull-down assay and subsequent immunoblotting. (c) Schematic illustration of XPA deletion mutants and the results of their interaction with RPA. Plus and minus signs indicate interaction and no interaction, respectively, with MBP-XPA polypeptides.

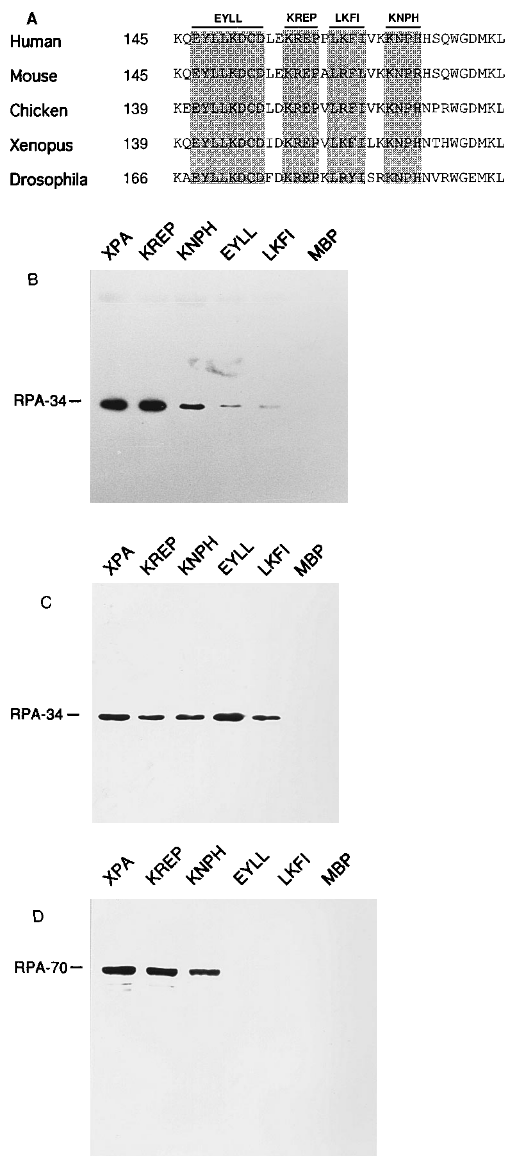

FIG. 3. Conserved motifs in XPA that affect binding with RPA. (A) Amino acid sequence alignment of the RPA-interactive domain for various XPA homologs. Highlighted regions indicate conserved motifs. (B) In vitro binding of RPA from HeLa cell extracts to small deletion mutants of XPA as determined in a pull-down assay. (C) In vitro binding of recombinant RPA-34 to small deletion mutants of XPA as determined in a pull-down assay. (D) In vitro binding of recombinant RPA-70 to small deletion mutants of XPA as determined in a pull-down assay. All bound proteins were subsequently assayed by immunoblotting.

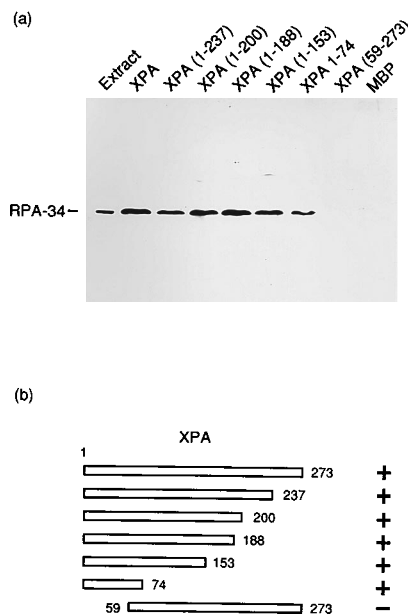

FIG. 4. Mapping of the RPA-34-interactive domain in XPA. (a) In vitro binding of recombinant RPA-34 to various deletion mutants of MBP-XPA as determined in a pull-down assay and subsequent immunoblotting. (b) Schematic illustration of XPA deletion mutants and the results of their interaction with RPA-34. Plus and minus signs indicate interaction and no interaction, respectively, with MBP-XPA polypeptides.

with RPA. Of the four mutants tested, one, ΔKNPH, showed moderately reduced binding and two, ΔEYLL and ΔLKFI, showed highly reduced binding; the fourth, ΔKREP, showed no change in binding to RPA (Fig. 3B). These results are in agreement with the domain mapping studies described above and indicate either that the deleted motifs are directly involved in mediating the interaction with RPA or that these deletions prevent binding to RPA by altering the secondary structure of XPA.

To determine if these mutant proteins affected binding to either or both of the RPA-34 and RPA-70 subunits, a pull-down assay was performed with purified recombinant forms of these subunits. As shown in Fig. 3C, binding of the RPA-34 subunit was not affected by these deletions; however, binding of RPA-70 (Fig. 3D) was decreased to an even greater extent than what was found above for RPA. Thus, the region identified above between residues 153 and 176 is involved in mediating the interaction with the large but not the middle subunit of RPA.

Since an interaction between XPA and RPA-34 was found in the two-hybrid system as described above, we used deletion analysis of XPA to identify a domain involved in binding the middle subunit of RPA. As shown (Fig. 4a), XPA-(1–74) bound RPA-34, but an amino-terminally truncated polypeptide lacking the first 58 amino acid residues of XPA failed to bind to RPA-34, indicating that this region contains an RPA-34-interactive domain (Fig. 4b). Interestingly, this region of XPA contains a putative nuclear location signal and has previously been shown to be dispensable for the complementing activity of XPA in vivo (32).

XPA mutants that exhibit reduced binding to RPA-70 are defective in NER in vitro. To determine the ability of the XPA mutants identified above as being defective in interacting with RPA-70 to complement XPA whole-cell extracts, an in vitro repair synthesis assay was performed (52). Wild-type XPA and the mutant proteins ΔLKFI and ΔEYLL were purified as MBP fusion proteins through two chromatographic steps. Each protein was then assayed for its ability to complement whole-cell extracts prepared from XPA cells. Wild-type XPA showed a high level of complementation of these extracts in contrast to that of the mutant proteins, which exhibited no detectable complementation (Fig. 5). These results suggest that the interaction between XPA and RPA is required for NER in vitro.

XPA mutants that exhibit reduced binding to RPA-70 are defective in NER in vivo. Since the ΔLKFI and ΔEYLL mutants exhibited a reduced level of activity in vitro, we examined the ability of these mutants to complement XPA cells in vivo.

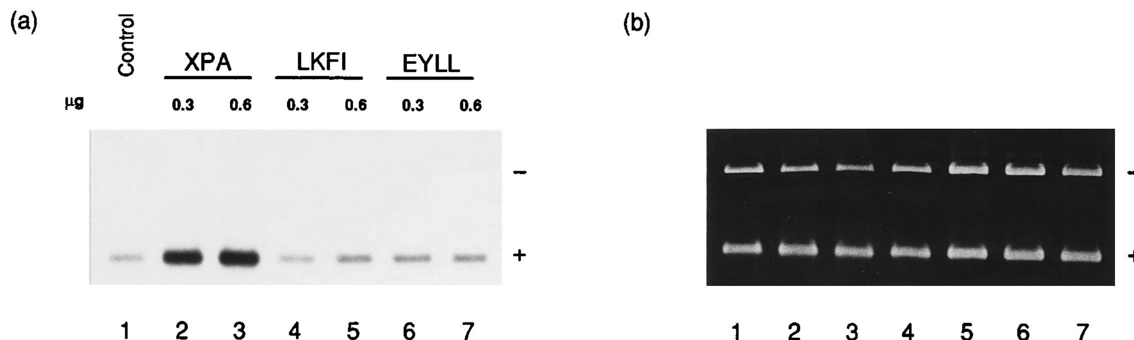

FIG. 5. In vitro complementation of XPA-deficient cell extracts by MBP-XPA fusion proteins as determined by the DNA repair synthesis assay (49). (a) Autoradiogram of gel shown in panel b. +, pBSII DNA treated with AAAF; −, untreated pGEX-2T DNA. (b) Ethidium bromide-stained gel of plasmids subjected to the in vitro DNA repair synthesis assay.

Each mutant gene was subcloned into the mammalian expression vector pEBS7 (37) and transfected into SV40-immortalized XP2OS cells. After selection for transformants, the cultures were examined for sensitivity to UV irradiation by a colony survival assay. Consistent with the in vitro results, both the ΔLKFI and the ΔEYLL mutants complemented XPA cells very poorly in vivo (Fig. 6). Immunoblotting of extracts from cells transfected with either XPA or the mutant clones indicated that the inability of the mutants to complement was not due to lack of expression of the mutant proteins (results not shown).

As described above, in addition to the interactive region identified for the RPA-70 subunit, the RPA-34 subunit also interacts with XPA at another site near the amino terminus. Deletion of the first 58 amino acid residues of XPA prevents interaction with RPA-34; however, previous studies have shown that this deletion has no effect on the complementation of XPA cells in vivo (32). However, in our experiments, deletion of the first 58 residues does partially reduce the ability of XPA to complement XPA cells (Fig. 6). These discrepancies in results may be due to the use of different expression vectors or different recipient XPA cell lines. A double mutant that has both the first 58 residues and the LKFI motif deleted exhibits the same low level of complementation as the ΔLKFI mutant (Fig. 6).

RPA stimulates binding of XPA to DNA. XPA exhibits a preference for binding to damaged double-stranded DNA over undamaged DNA (21, 39). To determine if the XPA-RPA complex has a greater preference for damaged DNA than XPA alone, we performed an IDT assay (Materials and Methods) in the presence of UV-irradiated or untreated DNA. As shown (Fig. 7), the presence of RPA stimulated the binding of MBP-XPA to damaged DNA more than fivefold. However, RPA also stimulated, although not to the same extent, the binding of MBP-XPA to undamaged DNA. Binding of MBP alone to either damaged or undamaged DNA was not detected.

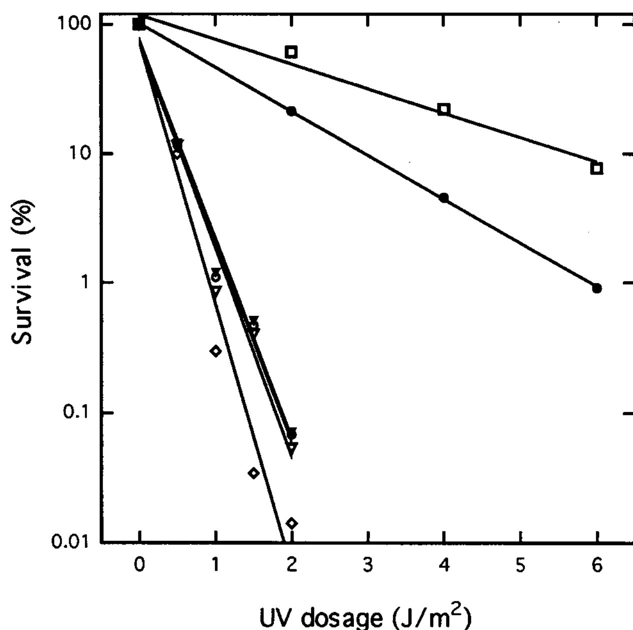

FIG. 6. Complementation of the immortalized XPA-deficient cell line XP2OS by various XPA constructs in the mammalian expression vector pEBS7. After establishment of cell lines by selection with hygromycin B, the survival of each line was determined as a function of UV dosage by a colony survival assay. □, XPA; ●, Δ58; ○, Δ58-LKFI; ▽, ΔEYLL; ▼, ΔLKFI; ◇, XP2OS cells.

DISCUSSION

RPA has been shown to function in vitro in the elongation and initiation stages of DNA replication and in the incision step of NER. In this report, we show that both of the two largest subunits of RPA, RPA-34 and RPA-70, interact with XPA. These interactions were detected both in vivo, by use of the two-hybrid system, and by direct binding in vitro. Mapping studies to identify the domains in XPA that mediate the interaction with the RPA subunits revealed that RPA-70 interacts within a highly conserved region in the carboxy-terminal half of

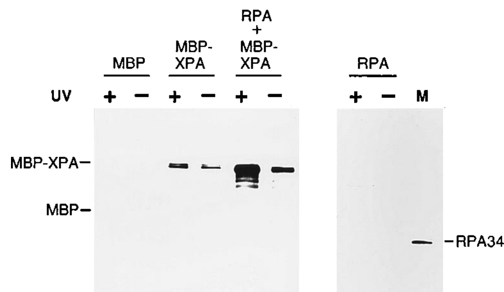

FIG. 7. IDT assay demonstrating enhanced binding of XPA to DNA in the presence of RPA. (Left) Immunoblot in which XPA and MBP were blotted simultaneously. (Right) Immunoblot for RPA-34. Lane M, RPA for use as a marker. The concentrations of reagents used were 10 ng of biotinylated DNA template, 25 ng of XPA proteins, and 100 ng of RPA.

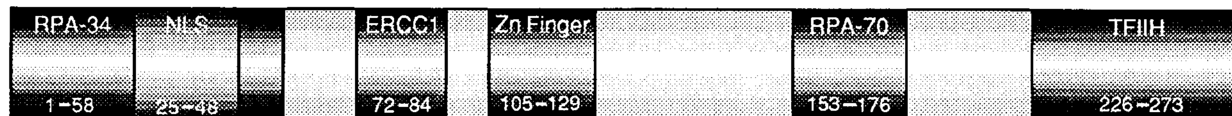

FIG. 8. Schematic of identified motifs in XPA from results reported in this paper and in reports by others (17, 26, 45). NLS, nuclear location signal; Zn finger, zinc finger.

XPA and that RPA-34 interacts within a region of XPA at the amino terminus. Deletion mutants of XPA that fail to interact with RPA-70 are highly defective in NER both in vitro and in vivo; however, mutants that fail to bind to RPA-34 are only partially defective in vivo. This latter finding may not be due to the inability of this mutant to bind RPA-34, but rather to the fact that it also lacks a nuclear location signal. Taken together, these results strongly suggest that the interaction between XPA and RPA is required for damage processing by the NER pathway, but that only the interaction with RPA-70 is essential. In a recent report, Matsuda et al. (30) showed that XPA interacts in vivo with RPA-34 and in vitro with RPA.

As shown in Fig. 3A, we have identified three motifs in XPA just downstream of the zinc finger motif that, when deleted, affect the interaction with RPA and RPA-70. The ΔEYLL and the ΔLKFI deletions affect the interaction with RPA the most severely, while the ΔKNPH mutation, which lies downstream, only moderately affects the interaction with RPA. The ΔKREP deletion, which lies between the EYLL and LKFI motifs, had no effect on binding to RPA. Our data do not discriminate between whether these motifs are directly involved in mediating the interaction with RPA or whether these deletions alter the secondary structure of XPA so as to affect its interaction with RPA. However, we can rule out the possibility that these deletions have altered the global structure of XPA in the sense that other interaction sites have been affected. The binding of XPA both to damaged DNA and to ERCC1, like the interaction with the RPA-34 subunit, is unaffected by these deletions (24) (results not shown). A summary of identified interaction domains in XPA is shown in Fig. 8.

XPA exhibits a preference for damaged double-stranded DNA over undamaged double-stranded DNA (21, 39). However, this preferential binding is not sufficient to account for the ability of the NER apparatus to recognize and remove DNA lesions in the mammalian genome. RPA binds strongly to single-stranded DNA but weakly to double-stranded DNA (11, 22). Our results indicate that the XPA-RPA complex has a much greater affinity for double-stranded DNA than XPA alone. Thus, RPA stimulates the affinity of XPA for damaged double-stranded DNA. Similar results have also recently been reported by He et al. (17), who showed that both RPA-70 and RPA-34 interact with XPA in vitro and that RPA stimulates the binding of the XPA-RPA complex to damaged DNA.

Previous results have shown that extracts depleted of RPA are deficient in making incisions at sites of damage (42) and that extracts supplemented with *E. coli* UvrABC do not require RPA for repair synthesis (5). These results suggest that RPA is required in steps preceding the incision reaction of NER. In addition, a recent report has shown that in a reconstituted repair assay, RPA is required for the dual endonucleolytic incisions that occur on either side of a lesion (33). Our finding, as well as that of others (18, 31), that RPA interacts with XPA provides a mechanistic basis for the role of RPA in the early steps of NER.

Current models of SV40-directed DNA replication indicate that large T antigen first binds to the origin of replication and unwinds the duplex by several turns. RPA is then recruited to this site by an interaction with both T antigen and the single-stranded region created by the action of T antigen. If an analogous scheme is followed in NER, then RPA would be recruited to a damaged site previously unwound, presumably by one of the helicases present in TFIIH and by interaction with XPA. Alternatively, RPA may interact with XPA at the beginning of NER and function to assist XPA and possibly TFIIH to recognize the lesion and unwind the helix at the site of damage. This latter model is supported by our finding that RPA greatly stimulates the binding of XPA to damaged DNA. In both models, one function of RPA would be to stabilize the open complex until the incision and repair synthesis steps can occur. Both models are supported by a recent report in which it was shown that TFIIH interacts with the XPA (17). Another plausible function of RPA is to link the early damage recognition and incision stages of NER to the later repair synthesis stage. RPA has been shown (23) to form a nucleoprotein complex with primed DNA and polymerase epsilon, which has been implicated as a repair polymerase (49). RFC and PCNA were also shown to interact with this complex, although they are not required for the binding of polymerase epsilon to the complex as they are for the binding of polymerase delta (23). Thus, RPA, which is initially recruited to the damaged site by interaction with XPA, may in a subsequent step recruit factors required for repair synthesis.

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