XRCC1 Protein Interacts with One of Two Distinct Forms of DNA Ligase III

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ABSTRACT: Human DNA ligase III (103 kDa) has been shown to interact directly with the 70 kDa DNA repair protein, XRCC1. Here, the binding sites have been defined. Subcloned fragments of XRCC1 have been expressed and assayed for their ability to associate with DNA ligase III by far Western and affinity precipitation analyses. The C-terminal 96 amino acids of XRCC1 are necessary and sufficient for the specific interaction with DNA ligase III. A similar approach with the 103 kDa DNA ligase III has identified the C-terminal 148 amino acids of this enzyme as containing the binding site for XRCC1. An alternative 96 kDa form of DNA ligase III, abundant in testes, has been described [Chen, J., *et al*. (1995) *Mol. Cell. Biol. 15*, 5412−5422]. These two forms of DNA ligase III have identical N-terminal regions but differ toward their C termini and may be alternatively spliced products of the same gene. Antipeptide antibodies directed against the different C termini of the two forms of the enzyme indicate that both of them occur *in vivo*. The C-terminal region of the 96 kDa derivative of DNA ligase III is not able to interact with XRCC1. These findings indicate that only the larger form of DNA ligase III acts together with XRCC1, suggesting a role for this isoform of the enzyme in base excision repair.

DNA ligases join DNA strand interruptions during DNA replication, repair, and recombination. In mammalian cells, unlike in prokaryotes, several DNA ligases are required for these diverse roles (Barnes et al., 1990; Chen et al., 1995; Wei et al., 1995). Cloned cDNAs have been identified for DNA ligases I, III, and IV and the genes assigned to chromosomes 19q13.2-13.3, 17q11.2-12 and 13q33-34, respectively; no cDNA is available for the 70 kDa DNA ligase II. The sequences of DNA ligases I, III, and IV show limited amino acid homology, which is confined to a core region. The enzymes share no apparent homology between terminal domains, which may be required for specific protein−protein interactions. DNA ligase I is localized in nuclear "replication factories" and is involved in the joining of Okazaki fragments during lagging-strand DNA replication (Lasko et al., 1990; Li et al., 1994; Prigent et al., 1994; Waga et al., 1994; Montecucco et al., 1995). The extended N terminus of DNA ligase I has been shown to mediate an essential role of the enzyme *in vivo* (Petrini et al., 1995), despite being dispensable for catalytic activity *in vitro*, suggesting its involvement in interactions with other replication proteins. DNA ligase III has been implicated in DNA repair and recombination processes (Jessberger et al., 1993; Caldecott et al., 1994; Kubota et al., 1996). DNA ligase IV is a 96 kDa DNA ligase of unknown function, but its unique extended C terminus apparently mediates interaction with another protein which may target it to a specific role in the cell (Robins & Lindahl, 1996).

The human XRCC1 protein fully corrects the mutant phenotype of the Chinese hamster ovary cell lines, EM9 and EM-C11 (Thompson et al., 1990; Zdzienicka et al., 1992). These cell lines are hypersensitive to simple alkylating agents and ionizing radiation, are inefficient at rejoining single-strand breaks resulting from treatment with such agents, and exhibit a 10-fold-elevated level of spontaneous sister chromatid exchanges (Thompson et al., 1990; Op het Veld et al., 1994). In addition, they show a reduction in the level of DNA ligase III; recombinant XRCC1 has been shown to interact directly with DNA ligase III *in vitro* to form an apparent 1:1 complex resistant to 2 M NaCl (Caldecott et al., 1994, 1995; Ljungquist et al., 1994). The phenotype of EM9 and EM-C11 cells suggests that XRCC1 functions in the repair of single-strand breaks in damaged DNA and, by association, a role for DNA ligase III in this process.

Recently, two DNA ligase III cDNA clones have been independently obtained. Surprisingly, they differ in the length and C-terminal sequence of the proteins they encode (Chen et al., 1995; Wei et al., 1995). One cDNA encodes a polypeptide of 922 amino acids (103 kDa), whereas the second cDNA encodes a polypeptide of 862 amino acids (96 kDa). Here, both isoforms are shown to occur *in vivo*. The regions of interaction between XRCC1 and DNA ligase III have been defined. The 103 kDa form of DNA ligase III, but not the 96 kDa form, is able to bind XRCC1.

EXPERIMENTAL PROCEDURES

XRCC1 Constructs. Bacterial expression construct pET16BXH or pET16BHX (Caldecott et al., 1995) was used to produce full length XRCC1 with a C- or N-terminal decahistidine tag, respectively. Constructs δ 56−374 and δ 286−536 were prepared from pET16BXH by cleavage at appropriate restriction enzyme sites, followed by rejoining with T4 DNA ligase; oligonucleotide linkers were used in some cases. Construct δ 527−633 was prepared from pET16BHX as a truncation produced by random transposon insertion of TnXR, a derivative of Tn1000 (Guyer, 1983; Sedgwick et al., 1991). *Escherichia coli* DH1 donors, carrying TnXR on F′ R388, were transfected with pET16BHX, resulting in insertion of the transposon into pET16BHX.

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Mating DH1638 with the recipient strain MH1578, a streptomycin-resistant derivative of DH1, stabilized the transposon integration. Clones with TnXR inserted into pET16BHX were detected by selection for streptomycin-resistant colonies, and those with TnXR in the XRCC1 gene were identified by restriction enzyme analysis. Transposon insertion in the open reading frame results in truncation due to stop codons at either end of the transposon. The strains used were kindly supplied by S. Sedgwick. Construct δ 1−537 was generated by PCR[1] using pET16BXH as a template but replacing the C-terminal His tag with a FLAG marker octapeptide, N-AspTyrLysAspAspAspAspLys-C (Eastman Kodak Co.). The site of transposon insertion and the authenticity of all PCR products were determined by DNA sequencing.

DNA Ligase III Constructs. The open reading frame encoded by the DNA ligase III cDNA clone HGS473238 (Wei et al., 1995) was placed in the bacterial expression vector pET16B with an N-terminal decahistidine tag, resulting in the construct pET16BHIII. Construct δ 1−773 of the 103 kDa form of DNA ligase III was derived from pET16BHIII by restriction enzyme cleavage and religation. The analogous C-terminal fragment of the 96 kDa form of DNA ligase III, δ 1−773, was generated by PCR using a λ library of human testes cDNA (Clontech) as a template. The sense primer contained a decahistidine tag; the antisense primer was specific to the unique C terminus of the 96 kDa form of DNA ligase III. The authenticity of PCR products was determined by DNA sequencing.

Purification of Recombinant Proteins. All constructs used were introduced into the *E. coli* strain BL21(DE3), and recombinant proteins were overproduced after IPTG induction. With the exception of XRCC1 δ 1−537-FLAG, all proteins were affinity purified using Ni−NTA−agarose (Qiagen, Chatsworth, CA) as described previously (Caldecott et al., 1995). Extracts of XRCC1 δ 1−537-FLAG were produced in the same way as the His-tagged proteins but were then affinity purified by binding to anti-FLAG M2 affinity gel followed by elution with FLAG peptide. Centricon microconcentrators (Amicon Inc.), preblocked in 1% dried milk, were used to concentrate and buffer exchange proteins produced. In the case of XRCC1 δ 1−537-FLAG, the FLAG peptide, used for the elution of the fusion protein, was also removed at this stage.

Affinity Precipitation Assay. This was performed as described (Caldecott et al., 1995). In brief, XRCC1-His (0−1 μg) was incubated with partially purified DNA ligase III (10 μg of total protein) or crude HeLa nuclear extract (90 μg of total protein), after which Ni−NTA−agarose beads (25 μL bed volume) were added to affinity bind XRCC1-His. The agarose beads were washed to remove nonspecifically associated proteins prior to elution of XRCC1-His and bound proteins with 250 mM imidazole. Fractions were analyzed by assays for DNA ligase−adenylate formation and SDS−PAGE, silver staining of proteins, or Western blotting. A slightly modified form of the assay was used for measuring binding of DNA ligase III polypeptides by XRCC1 δ 1−537-FLAG. Recombinant His-tagged DNA ligase III polypeptides (2 μg) were mixed with recombinant δ 1−537-FLAG (2 μg) and BSA (1 μg) in a 10 μL reaction volume. Following 20 min of incubation at 20 °C, the proteins were added to a 25 μL bed volume of Ni−NTA−agarose beads in 1 mM imidazole at pH 8 and incubated for a further 20 min with mixing. One hundred five microliters of 25 mM imidazole wash buffer was added to the beads and the supernatant removed. After six washes in 25 mM imidazole, 35 μL of 250 mM imidazole elution buffer was added twice. In all cases, 10% glycerol was included in the buffers. Thirty microliters of each of the six washes and the two elutions were examined by SDS−PAGE, silver staining of proteins, or Western blotting.

Formation of DNA Ligase−Adenylate Reaction Intermediates. Protein samples were incubated at 20 °C for 10 min in the presence of [α-^{32}P]ATP to allow formation of a covalent DNA ligase−AMP complex (Tomkinson et al., 1991); the reaction was stopped by addition of SDS−PAGE loading buffer and heating at 90 °C for 10 min. After gel electrophoresis, protein−adenylate complexes were visualized by autoradiography or analyzed on a phosphorimager (Molecular Dynamics).

Western Blotting Analysis and Antipeptide Antibodies. Polypeptides separated by SDS−PAGE were electroblotted onto nitrocellulose and detected by immunostaining with appropriate antibodies. δ 1−537-FLAG was detected using anti-FLAG M2 monoclonal antibody as a primary antibody. DNA ligase III polypeptides were detected by antipeptide antibodies. DNA ligase III antibodies were produced by injecting synthetic peptides coupled to keyhole limpet hemocyanin with Freund's adjuvant into rabbits by standard procedures (Harlow & Lane, 1988). The peptide used to raise antibodies specific for the 103 kDa form was (C)-EFDMTSATHVLGSRDK, corresponding to amino acids 882−897 of the 103 kDa form of DNA ligase III; these antibodies were used at a dilution of 1:100. The peptide used to raise antibodies specific to the 96 kDa form of DNA ligase III was (C)RRPASEQRGRTVPAGRR, corresponding to amino acids 846−862 of this polypeptide; these antibodies were used at 1:100 dilution. Alkaline phosphatase-conjugated secondary antibodies (BioRad) and BCIP/NBT substrate (Sigma) were employed for detection in all cases.

Far Western Blotting. Nitrocellulose blots were processed as described previously (Caldecott et al., 1995; Wei et al., 1995) using either fragments of XRCC1 or the full length protein as probes. Briefly, proteins were fractionated by SDS−PAGE, electroblotted onto nitrocellulose, denatured (using guanidine hydrochloride), renatured, and incubated with XRCC1 probes that had been phosphorylated by casein kinase II (Boehringer Mannheim) in the presence of [γ-^{32}P]-ATP. After removal of excess probe, blots were dried and subjected to autoradiography.

RESULTS

The C-Terminal 97 Amino Acids of XRCC1 Contain the Binding Site for DNA Ligase III. His-tagged XRCC1 protein with a large N-terminal deletion, δ 56−374 (Figure 1A), was produced so the region of interaction between DNA ligase III and XRCC1 could be located. Full length XRCC1, and fragments of the protein, were tested for their ability to bind to DNA ligase III in an affinity precipitation assay (Caldecott

[1] Abbreviations: BSA, bovine serum albumin; BCIP, 5-bromo-4-chloro-3-indolyl phosphate; IPTG, isopropyl β-D-thiogalactopyranoside; NBT, nitro blue tetrazolium; NTA, nitrilotriacetic acid; PCR, polymerase chain reaction; RT, reverse transcriptase; SDS−PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis.

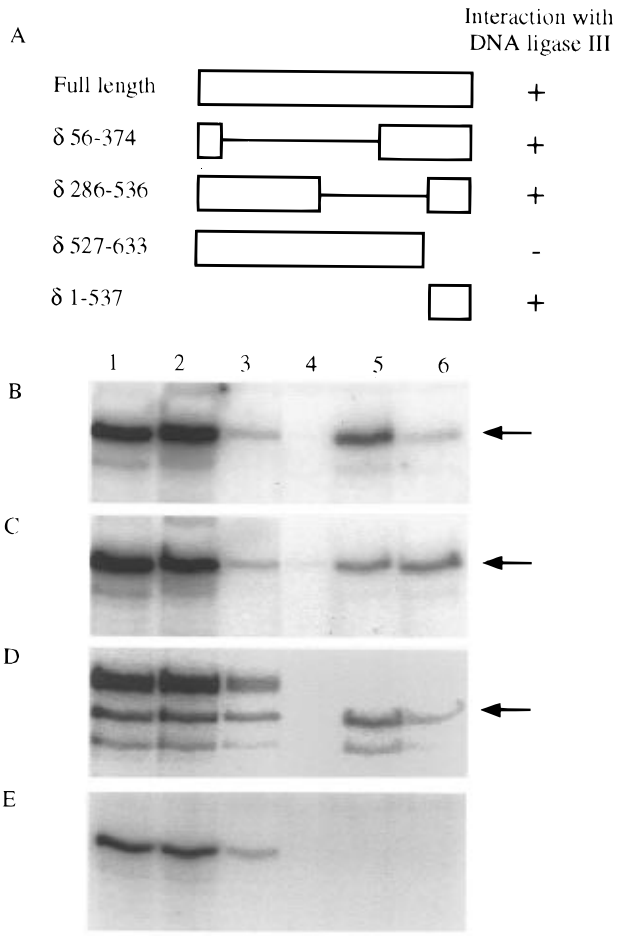

FIGURE 1: Affinity precipitation of DNA ligase III by XRCC1 proteins. (A) Schematic representation of full length XRCC1, 633 amino acids, and its derivatives, where δ 56−374 indicates that this protein lacks amino acids 56−374. The ability of each protein to interact with full length DNA ligase III is summarized (for δ 1−537, see Figure 3). Open boxes depict portions of XRCC1 which are expressed; single lines indicate internal deletions. Each protein contains a histidine tag at the N or C terminus, except δ 1−537 which contains a FLAG epitope at its C terminus. (B−E) Affinity precipitation analyses. Full length XRCC1 protein (B) and fragments δ 56−374 (C), δ 286−536 (D), and δ 527−633 (E) were incubated with either HeLa nuclear extract (D) or partially purified DNA ligase III (B, C, and E). After further incubation with Ni−NTA−agarose beads, an aliquot (load) of this suspension was removed (lane 1), and the remaining beads were pelleted. The supernatant was removed (nonadsorbed material) (lane 2), and the beads were washed with 25 mM imidazole (lanes 3 and 4). XRCC1-His proteins and any associated proteins were eluted with two 250 mM imidazole washes (lanes 5 and 6). Fractions were tested for the presence of DNA ligase III by enzyme−adenylate formation in the presence of [α-^{32}P]ATP and then subjected to SDS−PAGE. Gels were either silver stained, blotted for Western analysis (data not shown), or analyzed on a phosphorimager. The DNA ligase III−adenylate complex visible in the eluate is indicated by an arrow. DNA ligase III was not present in the eluate in the absence of XRCC1-His (data not shown), in agreement with previous experiments (Wei et al., 1995). DNA ligase I is present in crude HeLa nuclear extract and appears as a large adenylated band above the DNA ligase III band (D, lanes 1−3). The 87 kDa active fragment of DNA ligase III is also present in nuclear extract (D, lanes 1−3). Full length DNA ligase III protein in the eluate is indicated by an arrow (lanes 5 and 6). Full length XRCC1 was able to bind DNA ligase III and its 87 kDa fragment in crude HeLa nuclear extract (data not shown).

et al., 1995). XRCC1 proteins were incubated with partially purified DNA ligase III, after which XRCC1-His and associated proteins were selectively precipitated using nickel−agarose beads. Eluted fractions were incubated with [α-^{32}P]-ATP to generate enzyme−AMP intermediates of DNA ligase molecules that cofractionated with XRCC1-His. Recovery of XRCC1 in each of the experiments was detected by silver staining and Western blotting with an XRCC1-specific antibody (data not shown). Full length XRCC1 bound DNA ligase III, as indicated by the recovery of an ~100 kDa DNA ligase III−adenylate complex in the imidazole eluate (Figure 1B, lanes 5 and 6). Similarly, XRCC1 δ 56−374 (Figure 1C, lanes 5 and 6) and another XRCC1 fragment lacking the N-terminal 401 amino acids (data not shown) were able to interact with DNA ligase III, despite lacking large N-terminal regions of XRCC1. These data suggest that the binding site for DNA ligase III is located in the C-terminal 232 amino acids of XRCC1. Further XRCC1 fragments were analyzed to confirm and extend this observation.

His-tagged XRCC1 proteins with an internal deletion, δ 286−536, or a C-terminal truncation, δ 527−633 (Figure 1A), were analyzed for their ability to coprecipitate DNA ligase III in the affinity precipitation assay. XRCC1 fragment δ 286−536 retained the capacity to associate with DNA ligase III (Figure 1D, lanes 5 and 6). A truncation which removed the last 107 amino acids, δ 527−633, prevented interaction with DNA ligase III (Figure 1E). These data suggest that the binding site for DNA ligase III is within the C-terminal 97 amino acids of XRCC1 (see below). Attempts to assign the binding site to a smaller region using three other truncations of XRCC1 were unsuccessful.

The C-Terminal 148 Amino Acids of DNA Ligase III (103 kDa Form) Contain the Binding Site for XRCC1. The region of DNA ligase III that binds XRCC1 was also defined. The human DNA ligase III cDNA, isolated from a HeLa cell library that encodes the 103 kDa form of the protein, was inserted into a bacterial expression construct and used to produce soluble full length recombinant DNA ligase III. The C-terminal region of XRCC1 shows weak homology with the C-terminal 144 amino acids of DNA ligase III (Wei et al., 1995). A DNA ligase III fragment lacking the C-terminal 148 amino acids was made and was shown by far Western analysis to differ from the full length protein in being unable to bind XRCC1 (data not shown). DNA ligase I, DNA ligase IV, and XRCC1 did not interact with XRCC1 (Wei et al., 1995). The results suggest that the site which binds XRCC1 is in the C-terminal region of the 103 kDa form of DNA ligase III, and this was supported by data on further constructs (see below).

Differential XRCC1 Binding by Two Forms of DNA Ligase III. Two cDNAs encoding human DNA ligase III which differ at their C termini have been obtained (Figure 2A). This suggests the existence of two alternatively spliced forms of DNA ligase III, since a gene encoding either form has been mapped to chromosome 17q (Chen et al., 1995; Wei et al., 1995). Intriguingly, the divergence between the two forms of DNA ligase III occurs within the region which apparently interacts with XRCC1 (Wei et al., 1995). The alternative sequence in the shorter form shows no apparent homology to XRCC1, while the corresponding region in the 103 kDa form shows 27% identity to XRCC1 (Figure 2A). To confirm that the C-terminal 148 amino acids of DNA ligase III (103 kDa form) are sufficient for interaction with the XRCC1 protein, the DNA ligase III truncation δ 1−773 was generated (Figure 2A). In order to determine whether the 96 kDa form of DNA ligase III was able to associate with XRCC1, the C-terminal sequence encoding the 96 kDa

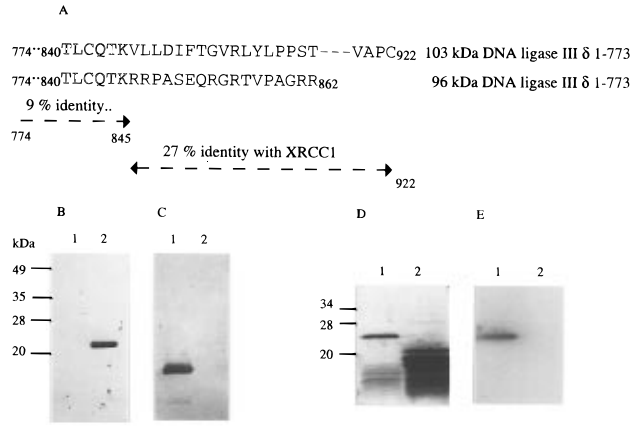

FIGURE 2: Two forms of DNA ligase III; detection of the proteins by specific antipeptide antibodies and their analysis by far Western blotting with a full length XRCC1 probe. (A) Alignment of the C-terminal amino acids of the two forms of DNA ligase III. Amino acid residues are shown in the single-letter code; identity between the sequences of the two forms of DNA ligase III is shown in outline type (the two forms are identical from amino acid 1 to 845). Only part of the sequence is shown for the 103 kDa form of DNA ligase III. Amino acids 774−845 show 9% identity to XRCC1; amino acids 845−922 of the 103 kDa form of DNA ligase III show 27% identity to XRCC1. (B and C) Detection of the two C-terminal fragments by Western analysis. δ 1−773, 96 kDa form (lane 1), and δ 1−773, 103 kDa form (lane 2), were probed with antipeptide antibodies against amino acids 882−897 of the 103 kDa form (B) or against amino acids 846−862 of the 96 kDa form (C). (D) Coomassie-stained gel of the proteins used for the far Western blot. (E) Far Western blot with full length [^{32}P]XRCC1 probe. (D and E) δ 1−773, 103 kDa derivative (lane 1), and δ 1−773, 96 kDa derivative (lane 2).

DNA ligase III was amplified by PCR from a human testes cDNA library. PCR amplification and DNA sequencing confirmed the presence of an mRNA for the shorter 96 kDa form of DNA ligase III *in vivo*. The protein fragment corresponding to the region of amino acids 774−862 was subsequently expressed (denoted δ 1−773, 96 kDa derivative, Figure 2A). Antibodies raised against a synthetic peptide corresponding to amino acids 882−897 of the 103 kDa form specifically recognized these C-terminal amino acids by Western blotting (Figure 2B). Similarly, antibodies raised against a synthetic peptide corresponding to amino acids 846−862 of the 96 kDa form specifically recognized δ 1−773, the 96 kDa derivative (Figure 2C). These data show that there are two distinct forms of DNA ligase III protein. Fragment δ 1−773 (103 kDa derivative) was able to bind XRCC1 as detected by far Western blotting using full length [^{32}P]XRCC1 as a probe (Figure 2E, lane 1). In contrast, δ 1−773 (96 kDa derivative) was unable to bind XRCC1 (Figure 2E, lane 2). Attempts were made to more closely define the binding site required for association with XRCC1; two more DNA ligase III fragments representing amino acids 774−845 and 839−922 were expressed, but neither protein was capable of associating with XRCC1 (data not shown).

To confirm that the C-terminal region of XRCC1 is sufficient for binding to DNA ligase III (103 kDa form) and to further compare the two isoforms of DNA ligase III in their ability to bind XRCC1, the protein fragment corresponding to the C-terminal 96 amino acids of XRCC1, XRCC1 δ 1−537, was produced (Figure 1A), with a FLAG epitope at its C terminus. His-tagged DNA ligase III proteins δ 1−773 (103 kDa derivative) and δ 1−773 (96 kDa derivative) were tested for their ability to bind to XRCC1 δ 1−537 in a modified form of the affinity precipitation assay. DNA ligase III δ 1−773 (103 kDa derivative) and DNA ligase III δ 1−773 (96 kDa derivative) were detected by silver staining (Figure 3A, lanes 7 and 8; Figure 3B, lanes 7 and 8, respectively) and also by Western analysis with the antipeptide antibodies specific for each form (data not shown). XRCC1 δ 1−537 was visualized by immunodetection using the FLAG M2 monoclonal antibody. The C-terminal 148 amino acids of DNA ligase III (103 kDa form) were able to associate with the 96-amino acid C-terminal region of XRCC1, as shown by coelution of XRCC1 δ 1−537 (Figure 3C, lanes 7 and 8). However, in agreement with the far Western data, the C-terminal region of the 96 kDa form of DNA ligase III did not interact with the C-terminal region of XRCC1 (Figure 3D, lanes 7 and 8). This demonstrates that the different C termini of the two forms of DNA ligase III result in proteins which differ in their ability to bind XRCC1 and that the C-terminal 96 amino acids of XRCC1 are sufficient for binding to the 103 kDa form of DNA ligase III.

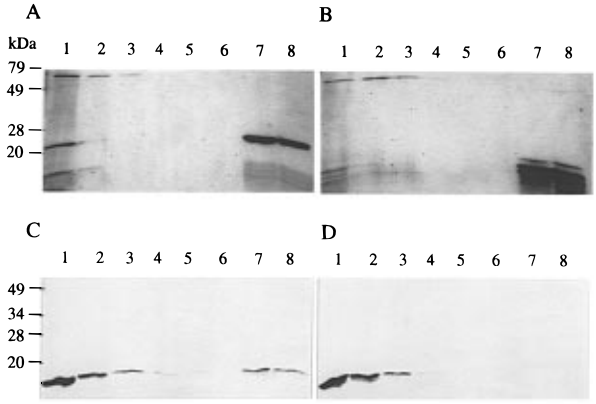

FIGURE 3: Affinity precipitation of minimal fragments of XRCC1 and the two forms of DNA ligase III. (A and B) Silver-stained gels of affinity precipitation assay. (C and D) Western blots of affinity precipitation assay using anti-FLAG M2 monoclonal antibody. XRCC1 δ 1−537-FLAG was incubated with DNA ligase III δ 1−773, 103 kDa derivative (A and C), or DNA ligase III δ 1−773, 96 kDa derivative (B and D), in the presence of BSA. After further incubation with Ni−NTA−agarose beads, the beads were washed six times with 25 mM imidazole (lanes 1−6). DNA ligase III-His proteins were eluted by two 250 mM imidazole washes (A and B, lanes 7 and 8). XRCC1 δ 1−537-FLAG was detected in the washes (C and D, lanes 1−3) and also in the eluate (C, lanes 7 and 8) by the anti-FLAG M2 antibody. BSA (69 kDa) was removed from the beads in the washes (A and B, lanes 1−3).

DISCUSSION

Two independently derived human cDNAs encoding DNA ligase III have been obtained from a HeLa cell library *vs* a testes library. The enzymes have identical catalytic properties on standard DNA ligase substrates but differ at their C termini (Chen et al., 1995; Wei et al., 1995). DNA ligase III protein fragments corresponding to the 89 C-terminal amino acids of the 96 kDa form (Chen et al., 1995), or the 148 C-terminal amino acids of the 103 kDa form (Wei et al., 1995), have been generated here from appropriate expression constructs. Antipeptide antibodies corresponding to unique sequences in each protein were shown to be able to specifically detect the alternative C termini of the 96 or 103 kDa forms of DNA ligase III. These reagents were used to show the presence *in vivo* of both forms of DNA ligase

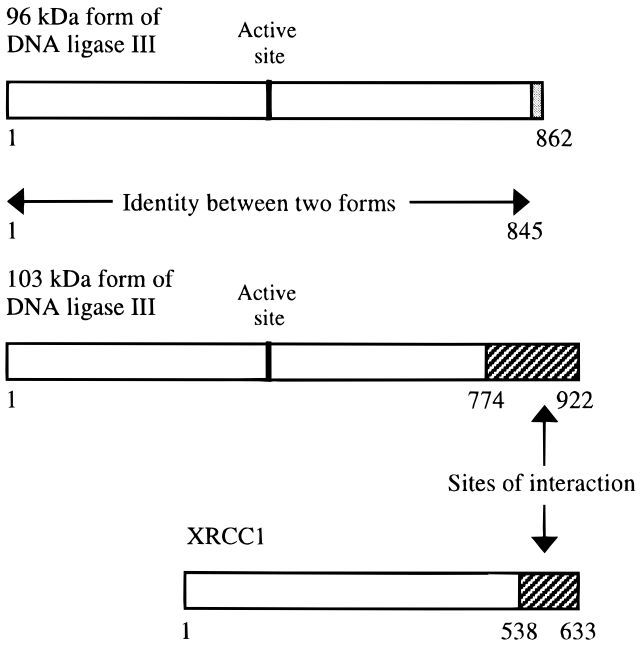

FIGURE 4: Schematic representation of the 96 and 103 kDa forms of DNA ligase III and the interaction of the latter with XRCC1. The active site is indicated by a solid bar. The regions of identity between the two forms of DNA ligase III are delineated by arrows, and the unique sequences in the 96 kDa form are represented by a shaded box. The regions required for interaction between the 103 kDa form of DNA ligase III and XRCC1 are indicated by slashed boxes and are marked with arrows; amino acid residues indicate the exact positions of these regions within the proteins.

III. The two forms appear to be transcribed from a common gene on chromosome 17 (Chen et al., 1995; Wei et al., 1995), suggesting that they correspond to alternatively spliced forms of DNA ligase III. Recently, the 96 kDa form was shown to occur only in testes during latter stages of meiotic prophase, whereas the 103 kDa form is present at similar levels in several tissues, including testes (Mackey et al., 1997). In agreement with this, DNA ligase III in HeLa cell nuclear extracts eluted together with XRCC1, in the expected position of a heterodimer, on gel filtration in the presence of 1 M NaCl, and no monomeric DNA ligase III was detected (our unpublished data).

The C-terminal 96 amino acids of XRCC1 are both necessary and sufficient for interaction with DNA ligase III. Similarly, the C-terminal 148 amino acids of the 103 kDa form of DNA ligase III are necessary and sufficient for interaction with XRCC1 (Figure 4). In contrast, the corresponding C terminus from the 96 kDa form of DNA ligase III was unable to interact with XRCC1. These data indicate that a functional difference exists between the two isoforms. One role of XRCC1 appears to be to serve as a scaffold protein, recruiting DNA polymerase β and DNA ligase III to DNA reaction intermediates during base excision repair (Kubota et al., 1996; Caldecott et al., 1996). DNA polymerase β binds to an N-terminal region in XRCC1, different from the C-terminal region that binds DNA ligase III (Kubota et al., 1996).

No counterparts to DNA ligase III or XRCC1 have been detected in either budding yeast or fission yeast. In mammalian cells, four distinct DNA ligases have now been identified, one of which is shown here to have two functionally different forms. A larger number of DNA ligases may have evolved in higher eukaryotes to allow more specialized roles through specific interactions with other nuclear proteins.

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