Specific Interactions between the Human RAD51 and RAD52 Proteins*

Zhiyuan Shen‡, Kieran G. Cloud, David J. Chen, and Min S. Park

From the Life Sciences Division, Los Alamos National Laboratory, Los Alamos, New Mexico 87545

Processing of DNA damage by the DNA double-strand break repair pathway in mammalian cells is accomplished by multiprotein complexes. However, the nature of these complexes and details of the molecular interactions are not fully understood. Interaction of the yeast RAD51 and RAD52 proteins plays a crucial role in yeast DNA homologous recombination and DNA double-strand break repair. Here, specific interactions between human RAD51 and RAD52 proteins are demonstrated both *in vivo*, using the yeast two-hybrid system and immunoprecipitation of insect cells co-infected with RAD51 and RAD52 recombinant viruses, and *in vitro*, using affinity chromatography with purified recombinant proteins. These results suggest that RAD52 may modulate the catalytic activities of RAD51 protein such as homologous pairing and strand exchange through a direct physical interaction. In addition, the domain in RAD52 that mediates this interaction was determined *in vitro* and *in vivo*. The RAD51-interacting region (amino acids 291–330) of the human RAD52 protein shows no homology with the yeast RAD52 protein, indicating that the interaction between RAD51 and RAD52 is species-specific.

The genome of living organisms is constantly damaged by exposure to exogenous and endogenous agents. The inherent stability of the DNA molecule provides the first level of protection from such damage. Moreover, organisms have developed intricate machineries of DNA repair to fix various types of damage. During the last 2 decades, investigators have identified and characterized different DNA repair pathways using various approaches such as yeast genetic studies. Three epistasis groups have been identified in *Saccharomyces cerevisiae* that control nucleotide excision repair (RAD3 epistasis group), error prone/post-replication repair (RAD6 epistasis group), and recombinational repair (RAD52 epistasis group). Both genetic and molecular studies on the yeast RAD52 epistasis group have clearly indicated close links between the mechanism of homologous recombination and DNA double-strand break (DSB)[1]

repair. Mutants belonging to this group exhibit elevated sensitivity to ionizing radiation and methyl methanesulfonate. In addition, this group of mutants is defective in both meiotic and mitotic recombination processes (1–5).

Most biochemical processes in various mammalian DNA repair require multiprotein complexes. Efficient removal of photodamages or bulky adducts requires more than a dozen proteins in the nucleotide excision repair (6, 7). This is also the case for mismatch repair machineries (8). Recently, a mammalian protein complex that repairs double-strand breaks by recombination has been identified (9). Genetic and physical evidence for the interaction of yeast RAD52 and RAD51 suggests that at least a subset of the RAD52 epistasis group proteins interact with each other (10–12). Additional evidence for a multiprotein repair complex comes from the dominant negative phenotypes exhibited by mutant alleles of RAD52. Some *rad52* dominant-negative alleles act via nonproductive interaction with RAD51, whereas others can act independently of RAD51, perhaps associating with other repair proteins. Therefore, RAD52 epistasis group-mediated DSB repair appears to require multiprotein complexes for damage processing.

The null mutant of RAD51 is partially defective in the formation of physical recombinants and accumulates double-strand breaks at a meiotic recombination hot spot (10, 13). RAD51 from yeast and other eukaryotes has considerable homology to bacterial RecA (10, 14–21), which has been shown to play a major role in recombination-mediated DNA repair in bacteria. RecA promotes homologous pairing and DNA strand exchange by forming nucleofilaments in the presence of ATP (22–24). Recently, yeast RAD51 has been shown to form nucleofilaments (25) and possess biochemical characteristics similar to RecA (26). RAD51 can catalyze homologous pairing and strand exchange activities *in vitro* (26). The human RAD51 protein has also been shown to form nucleofilaments with DNA (27), and forms nuclear foci after ionizing radiation (28).

Most of the information regarding the functions of the RAD52 protein has come from studies in yeast. Some studies have suggested that the RAD52 protein is not required for the initiation of recombination, but is essential for the intermediate stage following the formation of DSBs but before the appearance of stable recombinants (10). Most recently, human RAD52 protein has been shown to confer resistance to ionizing radiation and induce homologous recombination in monkey cells (29).

Although interaction between yeast RAD52 and RAD51 proteins has been observed both *in vitro* (10) and *in vivo* (11, 12), two observations prompted us to study the interaction between human RAD51 and RAD52 proteins. First, while the human RAD51 protein is highly homologous to the yeast RAD51 protein (83%) throughout the whole polypeptide, the human and yeast RAD52 proteins show significant homology only in the amino-terminal one-third. The carboxyl-terminal two-thirds of human RAD52 has little or no homology with the yeast RAD52

‡ Supported by the Director's postdoctoral fellowship of Los Alamos National Laboratory. To whom correspondence should be addressed: LS-6, MS M888, Life Sciences Division, Los Alamos National Laboratory, Los Alamos, NM 87545. Tel.: 505-665-6874; Fax: 505-665-3024; E-mail: zshen@telomere.lanl.gov.

[1] The abbreviations used are: DSB, DNA double strand break; Gal4-DA, Gal4 DNA activation domain (amino acids 768–881 of Gal4 protein); Gal4-DB, Gal4 DNA binding domain (amino acids 1–147 of Gal4 protein); *lacZ*, β-galactosidase gene; BSA, bovine serum albumin; PCR, polymerase chain reaction.

protein (20, 30, 31). However, it is the carboxyl terminus of yeast RAD52 that is involved in the interaction with RAD51 (11, 12). This raises the question of whether human RAD52 and RAD51 interact at all. Second, neither human RAD51 cDNA (20) nor yeast *Kluyveromyces lactis* RAD51 complements the *Rad51* defect of yeast *S. cerevisiae* (12). This suggests that the interaction between RAD51 and RAD52 via the carboxyl terminus of RAD52 may be species-specific.

In this study, we show that human RAD52 physically associates with human RAD51 *in vitro* and *in vivo*. Additionally, the region of the RAD52 protein that provides this interaction with RAD51 has been determined. This RAD52 region shows no homology with yeast *S. cerevisiae* RAD52. These results suggest that a species-specific interaction between human RAD51 and RAD52 may be important for homologous recombination and/or DSB repair in mammalian cells.

MATERIALS AND METHODS

Yeast Two-hybrid Strain and Plasmid Vectors—The yeast MATCH-MAKER two-hybrid system (Clontech Laboratories, Palo Alto, CA) was used as one of the systems to test protein-protein interaction *in vivo*. Yeast strain, SFY526 (MATa; ura3-52; his3-200; ade 2-101; lys2-801; trp1-901; leu2-3, 112; canr; gal4-542; gal80-538; URA3::GAL1-lacZ) (32) was used as the host for the *in vivo* interaction studies. Plasmid pGBT9 (TRP1⁺) was used to fuse the Gal4 DNA binding domain (Gal4-DB, residues 1–147 of Gal4) to the amino terminus of either RAD52 or RAD51 protein and pGAD424 (LEU2⁺) to fuse the Gal4 activation domain (Gal4-DA, residues 768–881 of Gal-4) (33).

Two-hybrid Plasmid Constructs—To clone the RAD51 or RAD52 cDNA into the two-hybrid vector, PCR was used to generate the inserts. These inserts contained a *Bam*HI site at the 5′ ends and a stop codon and a *Sal*I site at the 3′ ends. The PCR products digested with *Bam*HI and *Sal*I were ligated into the two-hybrid vectors that had been pre-digested with the same enzymes. Final constructs were sequenced by automated sequencing (Applied Biosystems Inc.). The full-size RAD51 cDNA insert was PCR-amplified from a cDNA library, and its sequence was compared to published cDNA sequences (19, 20). The full-length or truncated human RAD52 cDNAs that were fused with either the Gal4-DB (pGBT-52) or the Gal4-DA (pGAD-52) were constructed by ligating the RAD52 cDNA synthesized by PCR from a RAD52 cDNA clone (30).

Two-hybrid Protein-Protein Interaction Assay—To determine the potential interactions between RAD51 and RAD52 proteins, plasmids for the two fusion constructs (one fused with the Gal4-DB, the other fused with Gal4-DA) were co-transfected into the SFY526 using the polyethylene glycol/lithium acetate method (34). Transformed yeast cells were selected on Trp⁻/Leu⁻ synthetic agar plates for 3 days at 30 °C. Three independent transformants were then transferred to gridded filter paper and grown overnight on Trp⁻/Leu⁻ plates. These clones were then frozen in liquid nitrogen for 5–10 s, placed on top of another filter which was presoaked in Z-buffer (60 mM $Na_2HPO_4 \cdot 7H_20$, 40 mM $NaH_2PO_4 \cdot H_2O$, 100 mM KCl, 1 mM $MgSO_4 \cdot 7H_2O$, pH 7.0) containing 0.27% (v/v) 2-mecapthoethanol and 0.334% (w/v) of 5-bromo-4-chloro-3-indolyl-β-D-galactoside, and incubated at 30 °C. Either 5 ml or 1.5 ml of Z-buffer was used for a 150-mm or a 100-mm filter, respectively. Interaction between these two fusion protein was monitored by color development at 1 h, 2 h, 4 h, 8 h, and 24 h postincubation. Clones that developed visible blue color by 1 h were registered as +++, 2–4 h as ++, and 8–16 h as +−. If no color developed after 16 h, clones were recorded as −.

Purification of RAD51 and RAD52 Proteins Overexpressed in Bacteria—Coding regions of both proteins were cloned into a T7 promoter-based bacterial expression vector (35), pET28, purchased from Novagene, Inc. (Madison, WI). The resulting plasmids were designated pET-hRAD51 and pET-hRAD52. BL21(DE3) strain of *E. coli* was transformed with either pET-hRAD51 or pET-hRAD52, induced with isopropyl-1-thio-β-D-galactoside and harvested for protein purification. Proteins were purified by a combination of metal-chelate affinity and Mono Q ion exchange column chromatography. The biochemical activities of RAD51 including ATP hydrolysis and DNA binding were demonstrated.[2]

Co-expression of RAD51 and RAD52 in Insect Cells—*Spodoptera*

frugiperda cells (Sf9) were kindly provided by Glenn Godwin at Life Technologies, Inc. and grown in Sf-900 II SFM medium (Life Technologies, Inc.). Cells were maintained and infected either as monolayer or in suspension on a rotary shaker in a low temperature incubator set at 27 °C. Cells were routinely infected with 10 plaque-forming units of recombinant virus per ml of culture at a density of 1×10^6 cells (>95% viability) per ml. Linearized wild type baculovirus (BaculoGold™) and transfer vector pVL1393 were purchased from Pharmingen (San Diego, CA) and Invitrogen (San Diego, CA), respectively. The recombinant transfer vectors pVL1393-hRAD51 and pVL1393-hRAD52 were constructed from the transfer vector pVL1393 and the plasmid pET-hRAD51 or pET-hRAD52. The resulting constructs were then co-transfected with the linearized wild type baculovirus DNA (BaculoGold™) into Sf9 cells by the cationic liposome method, as described by the manufacturer's instruction (Invitrogen). Recombinant BaculoGold-hRAD51 or BaculoGold-hRAD52 baculoviruses were detected by visual inspection and were further screened by PCR. For the co-expression of RAD51 and RAD52 in single Sf9 cells, an equal number of each recombinant virus was used (multiplicity of infection = 10). All methods pertaining to the growth, transfection, screening, and manipulation of the Sf9 cells and baculovirus have been described elsewhere (36, 37) unless otherwise indicated.

Antibody Production—Recombinant RAD52 proteins purified from *Escherichia coli* were used as antigens to immunize rabbits. Antibody was affinity-purified using protein A membranes (Amicon). Anti-yeast RAD51 was kindly provided by Dr. Patrick Sung (26) (University of Texas, Galveston).

SDS-Polyacrylamide Gel Electrophoresis and Immunoblotting—After electrophoresis in SDS (10–20%) polyacrylamide gels, proteins were transferred onto nitrocellulose filters using a semi-dry electroblot apparatus. Nitrocellulose sheets were probed for 1 h with a 1:3000 dilution of affinity-purified anti-RAD51 or anti-RAD52 antibodies (stock of $A_{280} = 3$) and developed using the indirect alkaline phosphatase procedure (38).

Immunoprecipitation of RAD51 and RAD52 from Insect Cells Co-infected with RAD51 and RAD52 Recombinant Virus—For the immunoprecipitation experiments, insect cells (2×10^6) overexpressing both proteins were harvested after a 48-h postinfection with BaculoGold-hRAD51 and BaculoGold-hRAD52 recombinant viruses. Cells were lysed in buffer A (50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.02% sodium azide, 1% Nonidet P-40) and protease inhibitors (aprotinin, chymostatin, pepstatin, and leupeptin, 1 μg/ml). Cells were homogenized and clarified by centrifugation ($15,000 \times g$, 30 min) and incubated with either anti-RAD51 or anti-RAD52 antibodies. The supernatant was mixed with 50 μl of protein G-agarose beads at 4 °C for 1 h and washed twice with buffer B (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.1% Nonidet P-40, 1 mM EDTA, 0.25% gelatin, 0.02% sodium azide) and once with buffer C (10 mM Tris-HCl, pH 7.5, 0.1% Nonidet P-40). Proteins were eluted from the immunoprecipitate using 100 μl of 2% SDS for 5 min at 37 °C, and 5 μl of the eluates was analyzed by immunoblotting using the aforementioned antibodies. Immunoprecipitation of RAD51 and RAD52 from HeLa cell extracts was performed in a similar manner, except the HeLa cells were metabolically labeled with [³⁵S]methionine.

RAD52 Affinity Column—RAD52 protein and BSA were coupled to AminoLink-Plus gel (Pierce) following the manufacturer's instructions. RAD52 protein (4 mg) was dialyzed against buffer D (0.1 M sodium phosphate, 0.15 M NaCl, pH 7.2) and mixed with 0.5 ml of AminoLink slurry equilibrated with buffer D. After incubation overnight at 4 °C by end-over-end rocking, the slurry was washed with buffer D, and the buffer was replaced by buffer D with 0.1 M cyanoborohydride. The slurry was further incubated overnight at 4 °C, quenched with 1 M Tris-HCl, pH 7.4, and washed with buffer E (25 mM Tris-HCl, pH 7.6, 1 mM EDTA, 5 mM $MgCl_2$, 0.05% sodium azide). BSA (5 mg) was coupled to Amino-Link gel following the same procedure.

RAD51 protein was incubated in batch with the AminoLink resin coupled with either RAD52 or BSA. RAD51 was dialyzed against buffer E and concentrated by centrifugation through an Amicon Centricon 30 filter. The concentrated RAD51 (250 μg in 250 μl) was mixed with 0.25 ml (drained volume) of the AminoLink resin covalently linked to either RAD52 or BSA. After incubating the mixture for 2 h at 4 °C with gentle mixing, the slurry was applied to a polyethylene column. The column was washed with buffer E. The RAD51 were eluted from the column with a step gradient of increasing NaCl concentration; each step (1 ml) contained buffer E plus either 50 mM, 0.15 M, 0.25 M, or 0.5 M NaCl. All steps are carried out at 4 °C and a flow rate of 0.2 ml/min. Fractions of 200 μl were collected, the absorbance at 280 nm was measured, and aliquots were used for immunoblot analysis using anti-RAD51 or anti-BSA antibodies to confirm the presence of relevant proteins in each

---

[2] M. S. Park, manuscript in preparation.

TABLE I
Yeast two-hybrid analysis of RAD51 and RAD52 interaction

DNA binding domain	Activation domain	β-Galactosidase (*lacZ*) activity[a]
pGBT9	pGAD424	−
pGBT9	pGAD51[b]	−
pGBT9	pGAD52[d]	−
pGBT51[c]	pGAD424	−
pGBT52[e]	pGAD424	−
pGBT51	pGAD52	+ −
pGBT52	pGAD51	+ +
pGBT51	pGAD51	+ + +
pGBT52	pGAD52	+ + +
p53 (pVA3)	RAD51	−
p53 (pVA3)	RAD52	−
pGBT51	T-antigen (pTD1)	−
pGBT52	T-antigen (pTD1)	−
p53 (pVA3)	T-antigen (pTD1)	+ +

[a] See text for the symbols of *lacZ* activity.
[b] Full size RAD51 protein fused to Gal4-DA (pGAD51).
[c] Full size RAD51 protein fused to Gal4-DB (pGBT51).
[d] Full size RAD52 protein fused to Gal4-DA (pGAD52).
[e] Full size RAD52 protein fused to Gal4-DB (pGBT52).

fraction.

Generation of Deletion Mutants for in Vitro Assay—Deletion mutants expressing the truncated form of RAD52 were made by generating a series of nested deletions of pET-hRAD52 from the amino terminus of RAD52 protein using the ExoIII method (39). To use the ExoIII approach to generate deletions in RAD52 cDNA, the original pET-hRAD52 construct was modified by inserting a linker (sense strand: 5′-CATATGGGTACCACTAGTCGGGATCC-3′; antisense strand: 5′-GGATCCCGACTAGTGGTACCCATATG-3′) into the pET-hRAD52 that was restriction-digested with *Nde*I and *Bam*HI. The modified pET-hRAD52 was restriction-digested with *Kpn*I and *Bam*HI and subjected to ExoIII/mung bean nuclease treatment and subsequent ligation. To further map the interacting domain, two mutants containing a part of the RAD52 protein (amino acids 260–340 and 270–330) were made by ligating the PCR products to pET28b that was restriction-digested with *Nco*I and *Xho*I. Mutant constructs were first transformed into an *E. coli* strain, XL2-Blue (Stratagene), and subsequently screened for clones with a proper RAD52 reading frame. Then, BL21(DE3) was transformed with deletion construct plasmids isolated from XL2-Blue, induced, harvested, and subjected to protein purification. These mutant constructs produced truncated RAD52 proteins with 6 histidine residues at the amino terminus of the protein. Mutant proteins were purified by metal-chelate affinity columns.

RAD51 Affinity Chromatography—Purified RAD51 protein was covalently linked to AminoLink resins as described for RAD52 protein. Two hundred μg of mutant RAD52 were mixed with 50 μl of AminoLink slurry coupled with RAD51, incubated for 1 h at 4 °C with gentle end-over-end shaking, washed 3 times with buffer F (25 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.1% Nonidet P-40), and RAD52 mutant proteins were eluted with buffer G (25 mM Tris-HCl, pH 7.5, 500 mM NaCl, 0.1% Nonidet P-40). The eluted proteins were analyzed by SDS-polyacrylamide gel electrophoresis.

RESULTS

Association of RAD51 and RAD52 in Vivo—To determine whether the human RAD52 protein interacts with human RAD51 protein, the following four constructs that contained the full-length coding regions of either human RAD52 or RAD51 cDNA were made: pGBT52, pGAD52, pGBT51, and pGAD51. Plasmids pGAD424 and pGBT9 were used as negative control. Two plasmids, one derived from pGBT9 (first column in Table I) and the other from pGAD424 (second column in Table I) were co-transfected into SFY526 yeast cells by different combinations of these constructs. The enzymatic activity of β-galactosidase (*lacZ*) resulting from the association of these fusion proteins is shown (Table I). Table I demonstrates that 1) human RAD51 and RAD52 protein can each self-associate, 2) human RAD52 protein associates with the human RAD51 protein.

The self-association of yeast RAD51 protein and the region responsible for this activity have been reported (12). However,
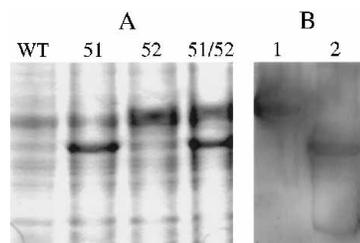

Fig. 1. **Co-expression and co-immunoprecipitation of human RAD51 and RAD52 protein in Sf9 cells.** *A*, a Coomassie Blue-stained gel. *lane WT*, Sf9 cells infected with wild type virus; *lane 51*, Sf9 cells infected with recombinant RAD51 virus; *lane 52*, Sf9 cells infected with recombinant RAD52 virus; *lane 51/52*, Sf9 cells coinfected with RAD51 and RAD52 recombinant viruses. *B*, immunoprecipitation of the human RAD51 and RAD52 complex in Sf9 cells co-transfected with RAD51 and RAD52 recombinant baculoviruses. *Lane 1*, immunoprecipitated with anti-RAD51 and immunoblotted with anti-RAD52 antibody; *lane 2*, immunoprecipitated with anti-RAD52 antibody and immunoblotted with anti-RAD51 antibody.

self-association of RAD52 protein has not been reported. A comprehensive analysis of the human RAD52 self-association will be reported elsewhere.[3] Interestingly, we consistently observed weaker interactions between pGAD52 and pGBT51 as compared to interaction between pGBT52 and pGAD51. Plasmid pVA3 that contains mouse p53 protein (amino acids 72–390)/Gal4-DB fusion, and pTD1 that contains SV40 large T-antigen (amino acids 64–708)/Gal4-DA fusion were used as experiment controls. Although p53 interacts with large T-antigen, p53 (amino acids 72–390) and T-antigen (amino acids 64–708) did not interact with human RAD51 or RAD52 in this yeast two-hybrid assay (Table I).

Although the two hybrid data indicated *in vivo* interaction of human RAD52 and RAD51 proteins in yeast cells, this interaction was relatively weak as compared to the self-association of either protein (Table I). Therefore, another *in vivo* experiment was conducted to confirm the association of RAD51 and RAD52. RAD51 and RAD52 were expressed independently or simultaneously in insect cells (Fig. 1*A*) using recombinant baculoviruses. The RAD51 and RAD52 complex was immunoprecipitated with antibodies specific to either RAD51 or RAD52 (Fig. 1*B*). The immunoprecipitates prepared by anti-RAD51 antibody contained a polypeptide that cross-reacted with the anti-RAD52 antibody. The immunoprecipitates prepared with anti-RAD52 contained a polypeptide that cross-reacted with the anti-RAD51 antibody. Furthermore, immunoprecipitation experiments with both antibodies on ^{35}S-labeled HeLa cell extracts also indicated physical association of RAD51 and RAD52 (data not shown). These results show that RAD51 and RAD52 interact with each other *in vivo*.

Association of RAD51 and RAD52 in Vitro—Although the physical association between RAD52 and RAD51 was demonstrated *in vivo*, it was not clear whether this interaction was a direct one or was mediated by another protein(s) *in vivo*. In order to distinguish between these possibilities, *in vitro* interaction studies using purified RAD51 and RAD52 proteins were conducted. For this purpose, RAD51 and RAD52 proteins were expressed in *E. coli* (Fig. 2*A*) and purified to near-homogeneity (Fig. 2*B*). The corresponding antibodies reacted with the purified RAD51 and RAD52 proteins (Fig. 2*C*). A RAD52 affinity column was constructed by coupling purified RAD52 protein to AminoLink resin at a concentration of 4 mg of RAD52 protein per ml of resin. The resin (0.25 ml) was mixed with 0.25 mg of RAD51 and, after incubating for 2 h at 4 °C, washed with increasing concentrations of NaCl. In the absence of NaCl,

---

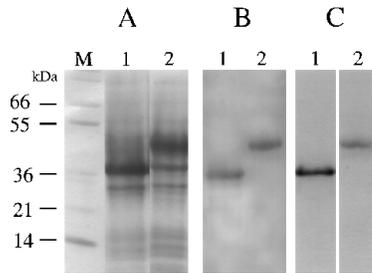[3] Z. Shen, submitted for publication.

FIG. 2. **Overexpression and purification of RAD51 and RAD52.** *A*, Coomassie Blue-stained total cell extracts of *E. coli* overexpressing RAD51 (*lane 1*) and RAD52 (*lane 2*). *B*, Coomassie Blue-stained purified RAD51 (2 μg, *lane 1*) and RAD52 (2.5 μg, *lane 2*) proteins. *C*, Western analysis of purified RAD51 (500 ng, *lane 1*) and RAD52 (500 ng, *lane 2*).

greater than 90% of RAD51 bound to the resin (Fig. 3). Less than 10% of RAD51 was eluted with NaCl concentrations of 50 and 150 mM. However, upon addition of >250 mM NaCl, ~60% of the bound RAD51 eluted from the column (Fig. 3). The corresponding fractions contained RAD51 as determined by immunoblot (data not shown).

As a control, an identical experiment was carried out with RAD51 protein using AminoLink gel, that had been coupled with BSA instead of RAD52 protein (Fig. 3). In this case, most of the RAD51 protein eluted in the absence of NaCl, with only a low level (less than 5%) bound to the column. Thus, the affinity of RAD51 for the resin coupled to RAD52 protein is due to an interaction of RAD51 with RAD52 protein and not to any nonspecific interactions between the RAD51 and the resin. Therefore, this experiment shows that the interaction between human RAD52 and RAD51 is direct.

Identification of RAD52 Domain That Interacts with RAD51—To identify the region(s) in the RAD52 protein that mediates the demonstrated interaction, *in vitro* and *in vivo* experiments were performed. For the *in vitro* experiment, a series of deletion mutants of RAD52 was generated and purified to apparent homogeneity (Fig. 4*A*). Mutant RAD52 proteins were tested by affinity chromatography with the RAD51 column. But this time the concentration of NaCl was fixed to 0.5 M to elute RAD52 proteins that were bound to the RAD51 column. As shown (Fig. 4*B*), a small region in RAD52 (amino acids 291–330) was accountable for the interaction of RAD52 with the RAD51 column.

For the *in vivo* experiment, a series of truncated RAD52 cDNAs were fused to the Gal4-DB in vector pGBT9. These constructs were co-transfected with pGAD51 into SFY526 cells. *lacZ* activity resulting from the interaction of Gal4-DB/RAD52 fusion constructs with full size RAD51/Gal4-DA fusion protein was assayed. Table II indicates that the amino acids 287–333 are responsible for the interaction in yeast cells. Based on the *in vivo* and *in vitro* results taken together, we conclude that the RAD51 interaction domain of human RAD52 protein resides in the region of 291–330.

DISCUSSION

The data presented in this study clearly indicate that human RAD51 and RAD52 proteins physically associate both *in vivo* and *in vitro*. Similar results have been described for yeast RAD51 and RAD52 proteins (10–12). The present study further mapped the human RAD52 region that interacts with the human RAD51 protein. A region of approximately 40 amino acids near the carboxyl terminus of RAD52 is required for interaction with RAD51 *in vitro*. Previous reports have shown that the human RAD51 protein cannot complement the *Rad51* defect in yeast *S. cerevisiae* (10), and yeast *K. lactis* RAD51 has little ability to restore the *Rad51* defect in yeast *S. cerevisiae* (12). Since the RAD51 interaction region (291–330) of human
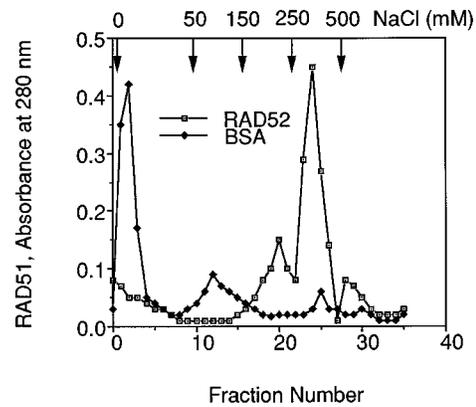

FIG. 3. **Human RAD52 protein affinity chromatography of human RAD51 protein.** RAD51 protein was applied to AminoLink resin that had been coupled with either RAD52 or BSA as described under "Materials and Methods." RAD51 protein was eluted from the column using 1-ml step gradients containing 0 mM, 50 mM, 150 mM, 250 mM, and 500 mM NaCl (*arrows*). Two hundred-μl fractions were collected, and the absorbances at 280 nm were determined.
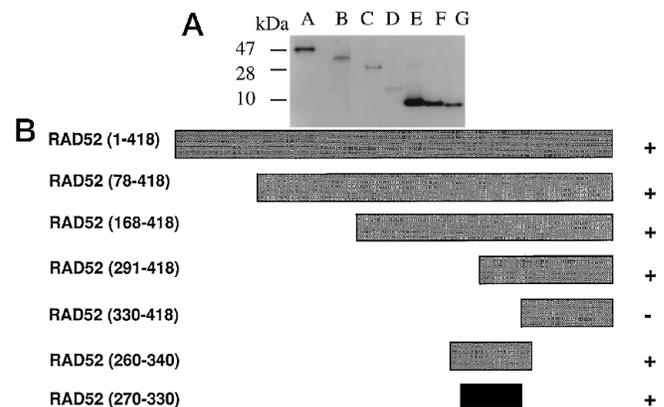

FIG. 4. **Determination of human RAD52 protein region interacting with RAD51.** *A*, SDS-polyacrylamide gel electrophoresis analysis of purified RAD52 deletion mutant proteins. *Lane A*, RAD52 (1–418); *B*, RAD52 (78–418); *C*, RAD52 (168–418); *D*, RAD52 (291–418); *E*, RAD52 (330–418); *F*, RAD52 (260–340); *G*, RAD52 (270–330). *B*, schematic illustration of RAD52 deletion mutants and results of their interaction with AminoLink-RAD51. RAD52 deletion mutants were tested for interaction with RAD51 coupled to AminoLink resin as described under "Materials and Methods." *Plus* or *minus sign* indicates interaction or no interaction with AminoLink-RAD51, respectively.

TABLE II
Yeast two-hybrid analysis of RAD52 regions that interact with RAD51

	pGAD424	pGAD51
pGBT9	−	−
pGBT52D(1–418)[a]	−	++
pGBT52A(1–124)	−	−
pGBT52B(1–165)	−	−
pGBT52C(1–221)	−	−
pGBT52G(1–301)	−	−
pGBT52K(124–222)	−	−
pGBT52Q(124–301)	−	−
pGBT52S(166–333)	−	++
pGBT52H(166–418)	−	++
pGBT52T(287–409)	−	++
pGBT52F(287–418)	−	++
pGBT52M(334–418)	−	−
pGBT52L(387–418)	−	−

[a] Numbers in parentheses denote the human RAD52 amino acids contained in the construct.

RAD52 does not share homology with the yeast RAD52 protein, the interaction between RAD51 and RAD52 appears to be species-specific. This specificity does not appear to be determined by the RAD51 protein, because RAD51 and its homologs

are well conserved. The RAD52 self-association region has been mapped to its amino-terminal region,[3] which is independent of the RAD51 interaction region. Therefore, we have demonstrated that the human RAD52 protein has at least two domains responsible for protein-protein interactions.

Self-association of human RAD51 is consistent with the results seen in yeast (12). This self-association may be required for self-assembly of RAD51 on DNA to form nucleofilaments with a characteristic regularity that has been observed previously (27). The homotypic interaction domain of human RAD51 appears to be present near its amino terminus[4] as seen with yeast RAD51 protein (12).

The yeast RAD51 protein has been shown to exhibit the catalytic activities of strand exchange and homologous pairing (26). More recently, the human RAD51 has been shown to bind to DNA and also form RecA-like nucleofilaments on DNA (27), suggesting the possibility that the human RAD51 also possesses either or both homologous pairing and strand exchange activities (29). The yeast RAD52 protein can bind to both single- and double-stranded DNAs in the absence of ATP and carries out annealing of homologous single-stranded DNA (40). It can also promote the strand transfer reaction (40). However, the reaction was ATP-independent and had an efficiency only 5% of that of the RecA protein (40). Besides the heterotypic and homotypic interactions of RAD52, there is no direct information about the biochemical activities of the mammalian RAD52 protein to date. Although it can be speculated that the RAD52 protein might modulate the catalytic activities of RAD51 based upon the information presented in this study showing the direct association of RAD52 and RAD51 *in vivo* and *in vitro*. The catalytic activity of yeast RAD51 can be exhibited in the absence of RAD52 protein *in vitro*.[2] However, a finer tuning of these processes *in vivo* may require other proteins such as RAD52. Overexpression of the RAD52 protein in monkey cells provides a positive effect on homologous recombination and protection from ionizing radiation (29). These results may further support the notion of the RAD52 protein as a positive modulator of RAD51 activities for DSB repair in mammalian cells. However, more direct evidence for this hypothesis needs to be drawn from biochemical experiments in the presence of both proteins *in vitro*.

REFERENCES

1. Haynes, R. H., and Kunz, B. A. (1981) in *the Molecular Biology of the Yeast Saccharomyces cerevisiae: Life Cycle and Inheritance* (Strathern, J. N., Jones, E. W., and Broach, J. M. eds) pp. 371–414, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York
2. Game, J. C. (1983) in *Yeast Genetics: Fundamental and Applied Aspects* (Spencer, J. F. T., Spencer, D. H., and Smith, A. R. W., eds) pp. 109–137, Springer-Verlag, New York
3. Resnick, M. A. (1987) in *Meiosis* (Moens, P. B., ed) pp. 157–210, Academic Press, New York
4. Petes, T. D., Malone, R. E., and Symington, L. S. (1991) in *The Molecular and Cellular Biology of the Yeast Saccharomyces: Genomic Dynamics, Protein Synthesis, and Energetics* (Broach, J., Pringle, J., and Jones, E., eds) pp. 407–521, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
5. Friedberg, E. C., Siede, W., and Cooper, A. J. (1991) in *The Molecular and Cellular Biology of the Yeast Saccharomyces: Genomic Dynamics, Protein Synthesis, and Energetics* (Broach, J., Pringle, J., and Jones, E., eds) pp. 147–192, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
6. Sancar, A. (1994) *Science* **266,** 1953–1958
7. Aboussekhra, A., Biggerstaff, M., Shivji, M. K., K., Vilpo, J. A., Moncollin, V., Podust, V. N., Protic, M., Hubscher, U., Egly, J.-M., and Wood, R. D. (1995) *Cell* **80,** 859–868
8. Modrich, P. (1994) *Science* **266,** 1959–1960
9. Jessberger, R., Podust, V., Hubscher, U., and Berg, P. (1993) *J. Biol. Chem* **268,** 15070–15079
10. Shinohara, A., Ogawa, H., and Ogawa, T. (1992) *Cell* **69,** 457–470
11. Milne, G. T., and Weaver, D. T. (1993) *Genes & Dev.* **7,** 1755–1765
12. Donovan, J. W., Milne, G. T., and Weaver, D. T. (1994) *Genes & Dev.* **8,** 2552–2562
13. Ajimura, M., Leem, S. H., and Ogawa, H. (1993) *Genetics* **133,** 51–66
14. Aboussekhra, A., Chanet, A. R., Adim, A., and Fabre, F. (1992) *Mol. Cell. Biol.* **12,** 3224–3234
15. Basile, G., Aker, M., and Mortimer, R. (1992) *Mol. Cell. Biol.* **12,** 3235–3246
16. Bezzubova, O., Shinohara, A., Mueller, R. G., Ogawa, H., and Buerstedde, J. M. (1993) *Nucleic Acids Res.* **7,** 1577–1580
17. Morita, T., Yoshimura, Y., Yamamoto, A., Murata, K., Mori, M., Yamamoto, H., and Matsushiro, A. (1993) *Proc. Natl. Acad. Sci. U. S. A.* **90,** 6577–6580
18. Muris, D. F., Vreeken, K., Carr, A. M., Broughton, B. C., Lehman, A., and Pastink, A. (1993) *Nucleic Acids Res.* **21,** 4586–4591
19. Yoshimura, Y., Morita, T., Yamamoto, A., and Matsushiro, A. (1993) *Nucleic Acids Res.* **21,** 1655
20. Shinohara, A., Ogawa, H., Matsuda, Y., Ushio, N. Ikeo, K., and Ogawa, T. (1993) *Nature Genet.* **4,** 239–243
21. Jang, Y. K., Jin, Y. H., Kim, E. M., Fabre, F., Hong, S. H., and Park, S. D. (1994) *Gene (Amst.)* **142,** 207–211
22. Roca, A. I., and Cox, M. M. (1990) *CRC Crit. Rev. Biochem.* **25,** 415–456
23. Kowalczykowski, S. C. (1991) *Biochimie (Paris)* **73,** 289–304
24. Radding, C. M. (1991) *J. Biol. Chem.* **266,** 5355–5358
25. Ogawa, T., Yu, X., Shinohara, A., and Egelman, E. (1993) *Science* **259,** 1896–1899
26. Sung, P. (1994) *Science* **265,** 1241–1243
27. Benson, F. E., Stasiak, A., and West, S. C. (1994) *EMBO J.* **13,** 5764–5771
28. Haaf, T., Golub, E. I., Reddy, G., Radding, C. M., and Ward, D. C. (1995) *Proc. Natl. Acad. Sci. U. S. A.* **92,** 2298–2342
29. Park, M. S. (1995) *J. Biol. Chem.* **270,** 15467–15470
30. Shen, Z., Denison, K., Lobb, R., Gatewood, J. M., and Chen, D. J. (1995) *Genomics* **25,** 199–206
31. Muris, D. F. R., Bezzubova, O., Buerstedde, J.-M., Vreeken, K., Balajee, A. S., Osgood, C. J., Troelstra, C., Hoeijmakers, J. H. J., Ostermann, K., Schmidt, H., Natarajan, A. T., Eeken, J. C. J., Lohman, P. H. M., and Pastink, A. (1994) *Mutat. Res.* **315,** 295–305
32. Bartel, P., Chien, C. T., Strenglanz, R., and Fields, S. (1993) *BioTechniques* **14,** 920–924
33. Bartel, P., Chien, C. T., Strenglanz, R., and Fields, S. (1993) in *Cellular Interactions in Development: A Practical Approach* (Hartley, D. A., ed) pp. 153–179, Oxford University Press, Oxford
34. Gietz, D., St. Jean, A., Woods, R. A., and Schiestl, R. H. (1992) *Nucleic Acids Res.* **20,** 1425
35. Studier, F. W., Rosenberg, A. H., Dunn, J. J., and Dubendorff, J. W. (1990) *Methods Enzymol.* **185,** 60–89
36. Summers, M. D., and Smith, G. E. (1987) *Texas Agric. Exp. Station Bull.* **1555**
37. O'Reilly, D. R., Miller, A. K., and Luckow, V. A. (1992) *Baculovirus Expression Vectors. A Laboratory Manual*, W. H. Freeman and Company, New York
38. Blake, M. S., Johnston, K. H., Russell-Jones, G. J., and Gotschlich, E. C. (1984) *Anal. Biochem.* **136,** 175–179
39. Henikoff, S. (1987) *Methods Enzymol.* **155,** 156–165
40. Ogawa, T., Shinohara, A., Nabetani, A., Ikeya, T., Yu, X., Egelman, E. H., and Ogawa, H. (1993) *Cold Spring Harbor Symp. Quant. Biol.* **58,** 567–576

[4] Z. Shen, K. G. Cloud, D. J. Chen, and M. S. Park, unpublished data.