

Isolation and characterization of *RAD51C*, a new human member of the *RAD51* family of related genes

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Received December 10, 1997; Revised and Accepted January 20, 1998

DDBJ/EMBL/GenBank accession nos AF029669 and AF029670

ABSTRACT

The yeast and human *RAD51* genes encode strand-transfer proteins that are thought to be involved in both recombinational repair of DNA damage and meiotic recombination. In yeast, the Rad51 family of related proteins also includes Rad55, Rad57 and Dmc1. In mammalian cells, five genes in this family have been identified (*HsRAD51*, *XRCC2*, *XRCC3*, *RAD51B/hREC2* and *HsDMC1*), and here we report the isolation of the sixth member, *RAD51C*. *RAD51C* was originally identified by a computer screen of the EST database. A full-length ~1.3 kb cDNA clone has been isolated that encodes a protein of 376 aa, having a 18–26% aa identity with other human Rad51 family members. *RAD51C* includes a previously mapped sequenced-tagged site location near the end of chromosome 17q. The *RAD51C* transcript is expressed in various human tissues, with highest level of expression in testis, followed by heart muscle, spleen and prostate. Yeast two-hybrid experiments indicate that the Rad51C protein binds to two other members of the Rad51 protein family (*Xrcc3* and *Rad51B*) but not to itself. These findings suggest that Rad51C may function similarly to the yeast Rad55 or Rad57 proteins, rather than as a Rad51 functional homolog.

INTRODUCTION

In the yeast *Saccharomyces cerevisiae*, the *RAD50* to *RAD57* genes are involved in the recombinational repair of DNA damage, including DNA double-strand breaks, as well as playing a role in meiotic recombination (reviewed in 1). The yeast Rad51 protein has been demonstrated to encode a strand transfer protein (2), and the *RAD55* and *RAD57* genes encode proteins that share sequence homology with the Rad51 protein, as does *DMC1*, a gene only expressed during meiosis. There is considerable evidence that protein–protein interactions are important in recombinational repair in yeast. In support of this hypothesis are biochemical studies that have demonstrated that the yeast Rad51 and Rad52 proteins bind to each other (3), and this binding has been confirmed by use of the yeast two-hybrid method (4). Reports

using the two-hybrid system indicate that the yeast Rad55 protein binds to both the Rad51 and Rad57 proteins (5,6). Biochemical confirmation of these interactions has recently been reported by Sung (7), who observed a strong interaction between Rad55 and Rad57 and a much weaker interaction between the Rad51 protein and the Rad55–Rad57 heterodimer. Sung (7) also presented evidence that the role of the Rad55–Rad57 dimer may be to facilitate the displacement of single-strand DNA binding protein RPA from single-stranded DNA, allowing the entry of Rad51 onto this DNA and the initiation of strand exchange. Recently, the yeast Rad54 protein has also been shown to interact with the Rad51 protein (8,9). These results suggest that recombinational repair in yeast involves a series of protein interactions, but it is not clear if these interactions occur simultaneously. The Rad51 associated proteins may form a complex, sometimes referred to as a ‘recombinosome’ (5). If so, it may contain three members of the Rad51 family of proteins (Rad51, 55 and 57), and the Rad52 and Rad54 proteins.

In mammalian cells, one functional homolog of the *RAD51* gene and one of the *DMC1* gene have already been isolated (10–12). The mammalian *RAD51* gene, like yeast *RAD51*, encodes a strand transfer protein (13), but unlike its yeast counterpart, the mammalian *RAD51* is an essential gene (reviewed in 14). The human Rad51 protein, like yeast Rad51, can interact with itself and with the human Rad52 and Rad54 proteins (15,16), but the human Rad51 protein can additionally interact with p53, Brca1, Brca2, Ube2I, Ubl1 and other proteins (17–23). Recently, three additional human members of the *RAD51* family of related genes have been identified, including *XRCC2* and *XRCC3* (24,25; N. Liu, R. S. Tebbs and L. H. Thompson, personal communication) and *RAD51B* (also named *hREC2*) (26,27). Although the three new proteins clearly share homology with other members of the Rad51 family, they are less similar to the yeast and mammalian Rad51 proteins than these two functional homologs are with each other. One possibility is that these three Rad51-like proteins are functional homologs of the yeast Rad55 and Rad57 proteins.

Here we report the isolation and characterization of *RAD51C*, the sixth member of the mammalian family of *RAD51*-related genes. Since in yeast all three of the mitotically expressed members of the *RAD51* family show protein–protein interactions

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among themselves, the Rad51C protein was tested for interaction with HsRad51 and other mammalian Rad51-like proteins. Recently, the yeast two-hybrid system has been used to show that the Xrcc3 protein interacts with the HsRad51 protein, and this interaction has been confirmed by co-immunoprecipitation experiments (D. Schild, K. W. Brookman and L. H. Thompson, unpublished result). (For consistency, all proteins names here are in mixed upper and lower cases, although the human Xrcc2 and Xrcc3 proteins are normally in all upper case to distinguish them from the mouse proteins.)

MATERIALS AND METHODS

GeneTrapper system

The GeneTrapper system (Life Technologies) was used to isolate the full length *RAD51C* cDNA. PCR primers derived from the first third of *RAD51C* were used in PCR reactions with eight different human SuperScript cDNA libraries (Life Technologies) in order to determine which libraries showed high levels of expression of *RAD51C*. Although five libraries showed relatively high expression levels (see Results), the leukocyte (mixed population) was chosen for the screen since it had been successfully used in a screen for the *RAD51B* cDNA (27).

Northern analysis

Multiple tissue northern blots with each lane containing 2 µg of purified polyA+ RNA from specific tissues were obtained from Clontech Laboratories. The blots were pre-hybridized for 1 h and then hybridized with a randomly primed radioactive *RAD51C* probe at 42°C for 16 h in 5× SSC, 50% formamide, 5× Denhardt's solution, 1% SDS and 100 µg/ml sheared salmon sperm DNA. A 2 ng/ml sample of the purified probe was used for hybridization. Each membrane was washed twice with 2× SSC/0.1% SDS, twice with 0.2× SSC/0.1% SDS at room temperature and at 50°C in 0.2× SSC buffer containing 0.1% SDS and exposed to either Kodak XAR-5 film or to a phosphorimaging screen. The human GAPDH (glyceraldehyde-3-phosphate dehydrogenase) cDNA (Clontech) was used to probe the membranes as a loading control.

Construction of plasmids for two-hybrid experiments

The *RAD51B* and *RAD51C* ORFs were cloned into the Gal4 DNA-binding domain vector pGBT9 and the transcriptional-activation domain vector pGAD424 (28) or closely related vectors (29). For *RAD51B*, a *Bgl*III linker was first ligated into an *EcoRV* site 3' to the ORF. A *Pvu*II-*Bgl*III fragment containing the entire ORF was then subcloned into the *Pvu*II-*Bgl*III sites of pGBD-C3 (29), resulting in plasmid pDS151. Since the *Pvu*II site in *RAD51B* is 10 bp prior to the ATG start codon, the resulting fusion protein encodes three additional amino acids not present in either the vector or *RAD51B*. The pDS151 plasmid contains two *EcoRI* sites, one in the pGBD-C3 polylinker 5' to the *Pvu*II site, and a second one in the 3' UTR of *RAD51B*. This *EcoRI* fragment was subcloned into pGAD424 to create pDS154.

For *RAD51C*, a *Sal*I-*Bam*HI fragment from clone RAD51C-B3 (see Results) was cloned into the *Sal*I-*Bgl*III sites of vector pGBD-C3, resulting in plasmid pDS152. After a 24 bp *Mlu*I fragment was removed, the final construct fused the complete Rad51C protein to the Gal4 binding domain, with an 18 aa linker between them derived from the 5'-UTR of *RAD51C*. An *EcoRI*-

*Nae*I fragment from pDS152- Δ *Mlu*I was subcloned into the *EcoRI*-*Sma*I sites of pGAD424, to produce pDS157. The truncated *RAD51C* cDNA encoded by IMAGE clone 281643 (see Results) was cloned into pGAD-C2 (29) to produce pDS161, using a *Hin*PII site (adjacent to *RAD51C*'s initiation codon) to clone into an in-frame polylinker *Cl*aI site. The encoded protein fusion deletes only the first Rad51C amino acid. A very similar plasmid was also constructed (pDS162) that contained the non-truncated *RAD51C* ORF, as a control for the truncated *RAD51C* plasmid fusion. Since the fusion protein encoded by pDS162 interacted with both the Xrcc3 and Rad51B DNA binding-domain fusion proteins (data not shown), this plasmid also acted as a control to ensure that the 18 aa linker in our original constructs were not the source of the observed interactions. The DNA sequences of the fusion regions of all two-hybrid plasmid constructions were determined in order to confirm that all plasmids encoded in-frame fusions. The construction of plasmids fusing the human *RAD51* and *RAD52* ORFs to the *GAL4* domains has already been published (15) and the *XRCC3* fusion constructs will be published elsewhere.

Yeast two-hybrid system

Yeast strains Y190 (30) and PJ69-4A (29) were used for most two-hybrid experiments. In addition, some experiments were carried out using a derivative of PJ69-4A, PJ69-4A-*rad51* Δ ::*ura3*, in which over half of the yeast *RAD51* gene had been deleted by replacement with the *URA3* gene. A *rad51*::*URA3* plasmid (31) was used to make this *RAD51* deletion and the resulting strain was sensitive to 0.01% MMS, as expected for a *rad51* Δ strain. Y-190, PJ69-4A and PJ69-4A-*rad51* Δ ::*ura3*, were each co-transformed with both a DNA-binding domain and a transcription-activating domain fusion plasmid and transformants were recovered on selective media (synthetic complete media lacking leucine and tryptophan, SC-Leu, Trp).

In order to test for reporter gene activity, patches from isolated colonies of the PJ69-4A transformants were replica-plated to SC-Ade and -His + 50 mM aminotriazol, and scored for growth. The *RAD51C* transformants were also retested on SC-Ade using strain PJ69-4A-*rad51* Δ ::*ura3* with the exception of *Rad51C*-binding domain/*Rad51C*-activation domain. Transformants of strain Y190 were assayed qualitatively for β -galactosidase activity using X-gal filter assays (28) and scored after 24 h as + (blue color), +/- (very slight blue color) or - (no blue). With all positive combinations, tests were carried out to ensure that the apparent interaction was dependent on the presence of both fusion plasmids.

Quantification of β -galactosidase activity

β -Galactosidase activity was quantified using transformants of PJ69-4A-*rad51* Δ ::*ura3* or PJ69-4A and the chemiluminescent detection method with Galacton-Star substrate and Sapphire-II enhancer (32). Luminescent reaction buffer and positive control β -galactosidase were obtained from Clontech. Cell lysates were prepared and reactions carried out according to manufacturer's instructions. Luminescent reactions were performed in triplicate and light signals were integrated over 5 s using a Berthold Lumat LB 9501 luminometer and averaged. A β -galactosidase control was used to determine the linear range of the assay. 10^{-5} U of β -galactosidase corresponded to 9500 RLU. The signals were normalized to 50 µl of cells at OD₆₀₀ = 2.5. Each experiment was repeated a total of two or three times on different days and results

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                                M R G K T F
GTCCGGAGTTTGGCTCCGCCGGTGTAGCAGTGGACCTGCGATGCGCGGGAAGACGTT 60
R F E M Q R D L V S F P L S P A V R V K
CGCTTTGAAATGCAGCGGGAATTTGGTGAATTTCCCGCTGTCTCCAGCGGTGCGGGTGAAG 120
L V S A G F P Q T A E E L L E V L K P S E L
CTGGTGTCTCCGGGGTCCAGACTGCTGAGGAACCTCTAGAGGTGAAACCCCTCCAGGCTT 180
S K E V G I S K A E A L E T L Q I I R R
AGCAAAGAAGTTGGGATATCTTAAAGCAGAAGCCTTAGAAACTCTGCAAAATATCAGAAGA 240
E C L T N K P R Y A G T S E S H K K C T
GAATGTCTCACAAATAAACCAAGATATGCTGGTACATCTGAGTCACACAAGAAGTGTACA 300
A L E L L L E Q E H T Q G F I I T F C S A
GCACGGAACTTCTTGAGCAGGACATACCAGGGCTTCAATAACCTCTCTGTTCAGCA 360
L D D I L G G G V P L M K T T E I C G A
CTAGATGATAATCTTGGGGGTGGAGTGCCTTAATGAAAACAACAGAAATTTGTGGTGCA 420
P G V Q K T Q L C M Q L A V D V Q I P E
CCAGGTGTGGAAAAACAATAATGATGTCAGTGGCAGTAGATGTGCAGATACCAGAA 480
C F G G V A G E A V F I D T E G S F M V
TGTTTGGAGGATGGCAGGTGAAGCAGTTTATTATTGATACAGAGGAAAGTTTATGGTT 540
D R V V D L A T A C I Q H L Q L I A E K
GATAGAGTGTAGACCTTGTACTGCTCCATCAGCAGCTTACAGCTTATAGCAGAAAAA 600
H K G E E H R K A L E D F T L D N I L S
CACAAAGGAGGAAACCCGAAAAGCTTTGGAGGATTTCACTCTGTGATAATATCTTTCT 660
H I Y Y F R C R D Y T E L L A Q V Y L L
CATATTTATATTTCTCGTCTGCTGACTACAGAGTTACTGGCACAAGTTTATCTTCTT 720
P D F L S E H S K V R L V T V D G I A F
CCAGATTTCTCTCAGAACACTCAAGGTTTCGACTAGTATAGTGGATGATGCTTTT 780
P F R H D L D D L S L R T R L L N G L A
CCATTTCTCATGACCTAGATGACCTGTCTCTCTGTACTCGGTTATTAAATGGCCTAGCC 840
Q Q M I S L A N N H R L A V I L T N Q M
CAGCAATGATCAGCCCTTCAAAATAACACAGATFAGCTGTAAATTTTAAACCAATCAGATG 900
T T K I D R N Q A L L V P A L G E S W G
ACAACAAAGATTGATAGAAATCAGGCTTGTCTTCTGCTGATAGGGGAAAGTTGGGGA 960
H A A T I R L I F H W D R K Q R L A T L
CATGCTGTACAATACGGCTAATCTTTCAITGGGACCCGAAAGAGGTTGGCAACATTG 1020
Y K S P S Q K E C T V L F Q I K P Q G F
TACAAGTCACCCAGCAGAAGAACTGACAGTACTGTTTCAAAATCAAACTCAGGAGTTT 1080
R D T V V T S A C S L T E G S L S T R
AGAGATCTGTGTACTTCTGCATTTCAATTTGCAACAGAAAGGTTCTCTGAGCACCCGG 1140
K R S R D P E E E L *
AAACGGTCCAGAGACCCAGAGGAAGAAATATAACCCAGAAACAAATCTCAAAGTGTACAA 1200
ATTTATGATGTGTGAAATCAATGTGTACAAGTGGACTTGTACCTTAAAGTATAAATA 1260
AACACACTATGGCATGAATGAAAAAATAAAAAA 1295

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Figure 1. The DNA and predicted amino acid sequence of *RAD51C*. The two highly conserved nucleotide binding regions are highlighted. The underlined bases appear to be the sites of introns, but the exact site of each could not be determined from our data.

sequence analysis of these PCR products demonstrated that cDNAs were present that did not contain the in-frame stop codon shortly after the GKT region and that the predicted amino acid sequence homology with the RAD51 family continued past the GKT region (data not shown). Using the GeneTrapper System (BRL), a clone (RAD51C-B3) was isolated from a human leukocyte SuperScript cDNA library. This ~1.3 kb cDNA was completely sequenced on both strands, revealing an ORF of 376 aa (Fig. 1) (GenBank AF029669). Although the context of neither the first nor second ATG is ideal for translation initiation (34), this cDNA is likely to be full length or near full length, since a slightly extended 5'-PCR product that was subsequently isolated has an in-frame stop codon before the first ATG (sequence not shown). The Rad51C protein shares significant sequence homology with other members of the human Rad51 family (Fig. 2). In separate pairwise analyses, Rad51C shares considerably more sequence identity to Rad51B, Xrcc3 and HsRad51 (26.2, 25.6 and 26.9%, respectively) than with Xrcc2 (18.2%).

In addition to the RAD51C-B3 clone, the GeneTrapper screen of the leukocyte cDNA library resulted in the isolation of several other cDNA clones, and some encoded alternatively spliced products. One clone encoded a 27 bp insert, with an in-frame stop codon, in the same location as the alternately spliced site in the original IMAGE clone 281643. A second clone contained a 145 bp insert after bases 746 or 747 in the *RAD51C* sequence, underlined in Figure 1; a clone from the 3'-end of *RAD51C* present in the EST database (locus W86736, from fetal liver spleen) also contains the identical 145 bp insertion. Since neither the 27 bp or the 145 bp introns conformed exactly to the GT-AG rule for intron splicing, they probably represent only parts of the normal introns that were not properly excised, rather than complete introns that were missed.

averaged, with the exception of Rad51C-binding domain/pGAD424, done once.

RESULTS

Cloning and sequencing of *RAD51C*, a new member of the human *RAD51* family

In order to determine whether there might be additional members of the human *RAD51* family lurking in one of the sequence databases, TBLASTN was used to query each database with the amino acid sequences of the human Xrcc3 and yeast Rad51 proteins. The EST database revealed several clones, apparently from the same gene, that shared significant amino acid sequence similarities to the N-terminal end of Xrcc3 and Rad51. The DNA sequence (dbEST locus #s N30816, N41590 and N53986) from these clones encoded what appeared to be short proteins and each encoded a stop codon 4 aa distal to Gly Lys Thr (or GKT), part of the well-conserved nucleotide-binding motif. IMAGE clone 281643 (containing the N53986 sequence) was obtained from Genome Systems, and the complete DNA sequence from both strands was determined (GenBank AF029670). This sequence showed that this clone encoded an ORF of 135 aa and confirmed that this clone encoded a stop codon shortly after the GKT region. It seemed likely that these cDNA clones might be alternately or aberrantly spliced forms of a larger ORF encoded by this gene.

Partial cDNA clones from the 5'- and the 3'-ends of this gene were isolated from a HeLa cDNA library (33) using anchored PCR. DNA

***RAD51C* is expressed in a wide variety of human tissues**

Northern blot analysis with the *RAD51C* cDNA probe showed the presence of an ~1.3 kb mRNA species in all tissues examined, except peripheral leukocytes, where no signal was observed (Fig. 3A). Lung did show an extremely weak, but visually detectable signal, but the weakness of this signal is at least partially due to low levels of total mRNA loaded in this lane (see *GAPDH* loading control). The ~1.3 kb mRNA band appears to be highly expressed in testis (~20-fold) as might be predicted if this gene plays a role in meiotic recombination, followed by heart muscle, spleen and prostate (~3-fold) (Fig. 3B). As an initial step in the GeneTrapper screen, different tissue-specific human SuperScript cDNA libraries were tested using semi-quantitative PCR with primers from *RAD51C*. These results showed that *RAD51C* is expressed at relatively high levels in brain, heart, leukocytes, spleen and testis, but at very low, but detectable levels in kidney, liver and lung. These results are consistent with our northern analysis, with the exception of leukocytes, and this difference may be due to the northern blot containing mRNA from peripheral blood leukocytes, while the cDNA library was constructed using mixed population leukocytes that presumably include many more dividing cells.

Although the EST database contained several entries with ~580 bp cDNAs, this shorter transcript was not visible on our northern blots. Thus, either this transcript is rare or it might have run off the bottom of these gels. From size standard markings

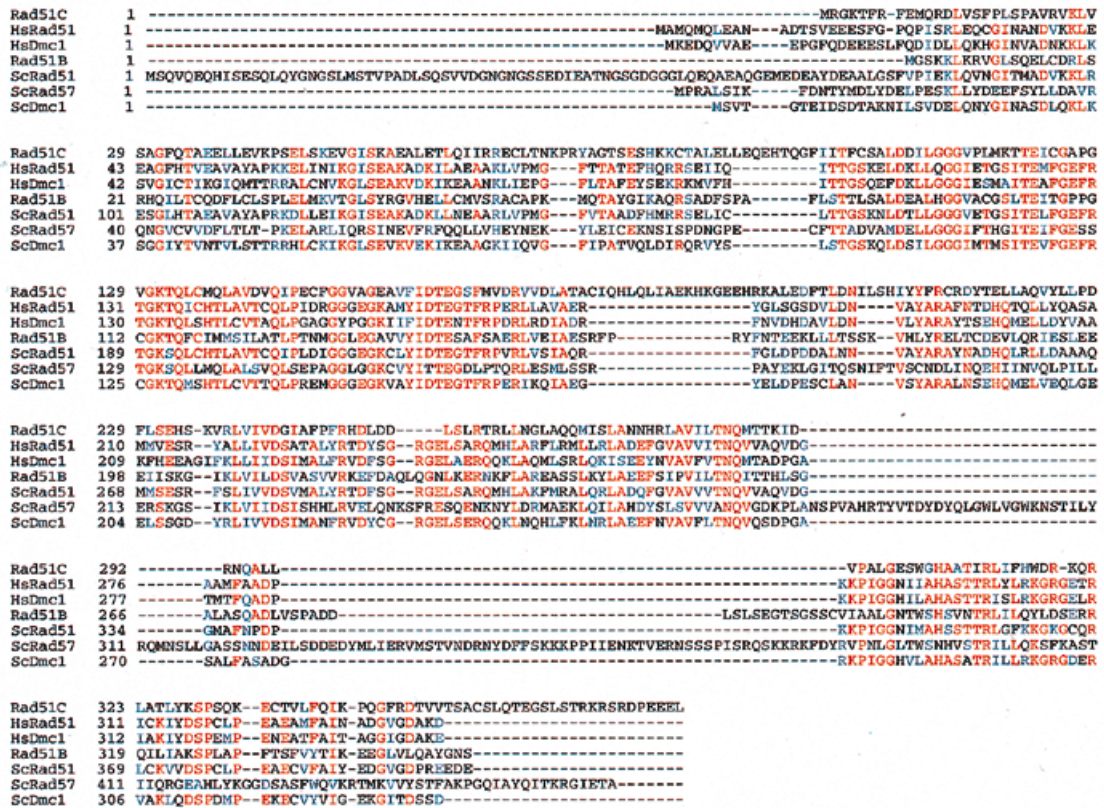


Figure 2. Alignment of the Rad51C protein with other human and *S.cerevisiae* members of the Rad51 family of related proteins using ClustalW. Yeast Rad55 was excluded from this analysis due to its distant relationship. Red indicates identical amino acids in over half of the proteins and blue indicates conserved amino acid replacements.

supplied by Clontech, it appears that the size cut off for these gels is close to 0.6 kb.

Rad51C interactions with Xrcc3 and Rad51B/hREC2 in the yeast two-hybrid system

Rad51C was tested for potential protein–protein interactions with other human members of the Rad51 family using the yeast two-hybrid system. As shown in Figure 4, Rad51C appears to interact strongly with Rad51B, and moderately with Xrcc3, using the HsRad51–HsRad51 interaction as a positive control. The interaction of Rad51C with Xrcc3 is asymmetrical, similar to what was observed with the interaction of HsRad51 with Xrcc3 (D. Schild and L. H. Thompson, unpublished data), and with published two-hybrid interactions involving the human and yeast Rad51 fusion proteins (8,15,16). These asymmetries are probably due to interference of the Gal4–fusion. When Rad51C was tested for interaction with HsRad51, no indication of any interaction was observed in strain PJ69-4A, nor a *rad51*-deletion derivative of this strain, either qualitatively by testing for growth on plates lacking adenine, or quantitatively by assaying for β-galactosidase activity. The *rad51*-deletion strain was used in some of these studies because the yeast Rad51 protein can interact with both HsRad51 and Xrcc3 in the two-hybrid system (T. Tsomondo, D. Collins and D. Schild, unpublished result). This raised concerns that these heterologous interactions might compete with the interactions we were testing, but no differences were observed in interactions in the *rad51*-deletion strain versus the RAD51 strain. In strain Y190, when Rad51C was in the DNA binding domain and HsRad51 was in the activation

domain, a very weak signal was repeatedly observed on X-gal plates, but no growth was observed on SC-his plates supplemented with 25 or 50 mM aminotriazol. This result might indicate a very weak or transient interaction, or might represent an artifact. In addition to the positive and potentially positive interactions observed, many pairwise combinations failed to give any indication of an interaction. These included Rad51B and Rad51C with themselves, and Rad51B with either Xrcc3 or HsRad51 (Fig. 4). No interaction was observed between HsRad52 and either Rad51B or Rad51C (data not shown).

The truncated Rad51C protein encoded by clone 281643 was also tested in the two-hybrid system. When fused to the Gal4 activation domain, it did not interact with Rad51B, Xrcc3 or HsRad51 fused to the Gal4 DNA-binding domain (data not shown).

DISCUSSION

There is increasing evidence in recent years that mammalian cells utilize a recombinational-repair pathway, although it appears not to be as critical for the repair of DNA double-strand breaks as it is in yeast cells (reviewed in 25). One strong line of evidence for recombinational repair in mammalian cells comes from the human *XRCC2* and *XRCC3* genes. These genes have recently been cloned (24; N. Liu and L. H. Thompson, personal communication) by complementation of the mutant CHO cell lines *irs1* and *irs1SF*, respectively. These cell lines exhibit some (~2-fold) X-ray and UV sensitivity, but much greater sensitivity (~40-fold) to DNA cross-linking agents such as mitomycin C and psoralens, and also show a high level of spontaneous chromosomal aberrations. Since the mutant CHO cell lines are defective in DNA

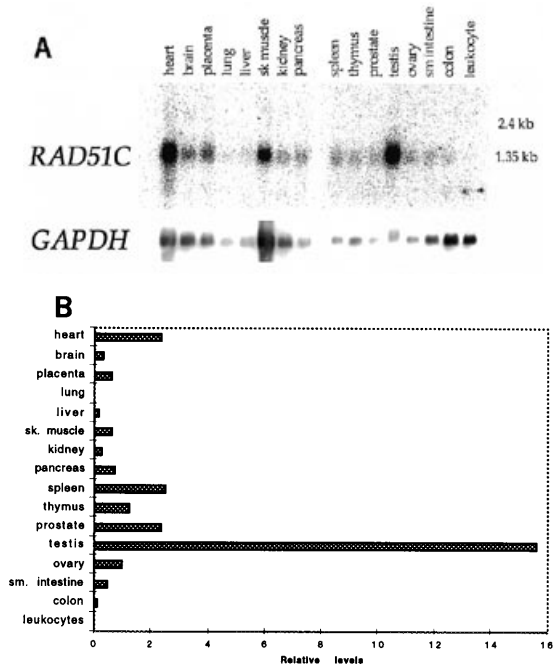


Figure 3. Northern analysis of *RAD51C*. (A) Human tissue blots were first probed with part of *RAD51C*, then by *GAPDH*, for use as a loading control. (B) The relative levels of *RAD51C* transcripts in different human tissues tested, after first controlling for loading differences. The average of the median two samples was arbitrarily set as 1.0.

repair, the *Xrcc2* and *Xrcc3* proteins are clearly involved in DNA repair, and probably recombinational repair specifically. The *XRCC2* and *XRCC3* cDNAs have recently been sequenced, and their predicted amino acid sequences share significant homologies with the human and yeast Rad51 proteins and other members of this family (N. Liu, J. E. Lamerdin and L. H. Thompson, personal communication). In addition, the *Xrcc3* protein appears to physically interact with the HsRad51 protein (D. Schild, K. W. Brookman and L. H. Thompson, unpublished data), a protein known to be involved in both recombination and DNA repair, and therefore, probably recombinational repair. If *Xrcc2* and *Xrcc3* are recombinational-repair proteins, as seems likely, then *irs1* and *irs1SF* are defective in a recombinational-repair pathway. If so, this pathway is unlikely to be the major one for UV and X-ray induced damage, but it may be for the repair of DNA cross-links.

In contrast to *XRCC2* and *XRCC3*, we currently have no direct evidence that *RAD51B* and *RAD51C* are repair genes, since no cell lines exist with known mutations in either of these new genes. It does seem likely though that they are involved in recombination and/or recombinational-repair of DNA damage, since their encoded proteins share sequence homology to the *RAD51* family and appear to physically interact with known repair proteins (Fig. 4). Although some evidence for a weak interaction between Rad51C and HsRad51 was found, we did find convincing evidence that Rad51C interacts with the DNA repair protein *Xrcc3*, which in turn interacts with HsRad51. Results presented here also suggest that the Rad51B protein is probably a recombinational-repair protein, since in the two-hybrid system it strongly interacts with the Rad51C protein. Furthermore, Rice *et al.* (26) have reported that *hREC2*, which is identical to *RAD51B*, is transcriptionally induced by DNA damage, and this also suggests a role in DNA repair. Cell lines and/or transgenic mice deficient for *RAD51B* and *RAD51C* will have to be

Fusions:				β-galactosidase assay
DBD	AD	X-Gal	SC-Ade	
HsRad51	HsRad51	+	+	31.3
Rad51C	Rad51B	+	+	112.7
Rad51B	Rad51C	+	+	115.0
<i>Xrcc3</i>	Rad51C	+	+	17.8
Rad51C	<i>Xrcc3</i>	-	-	1.9
Rad51B	<i>Xrcc3</i>	-	-	7.7
<i>Xrcc3</i>	Rad51B	-	-	5.6
Rad51C	HsRad51	+/-	-	3.8
HsRad51	Rad51C	-	-	3.4
Rad51B	HsRad51	-	-	6.9
HsRad51	Rad51B	-	-	7.7
Rad51C	Rad51C	-	-	4.9
Rad51B	Rad51B	-	-	7.2
Rad51C	pGAD424	-	-	3.7
pGBT9	pGAD424	-	-	2.2

Relative Light Units (x1000)

Figure 4. Two hybrid results. DBD fusions are fusions of the protein listed to the DNA-binding domain of the yeast Gal4 protein, and the AD fusions to the activation domain of Gal4. The X-gal results are from strain Y190, and the ability to grow on media lacking adenine (SC-ade) and the quantitative β-galactosidase activity are from strain PJ69-4A and a *rad51Δ* derivative of this strain (see Materials and Methods).

studied to definitively determine if these genes encode DNA repair functions; such experiments are currently underway in a number of different laboratories. It is also possible that already existing DNA repair deficient cell lines may have a defect in *RAD51B* or *RAD51C*, but both have been mapped and neither maps with any known repair gene. *RAD51B* has been mapped to chromosome 14q23-24 (26,27), and *RAD51C* is located near the end of chromosome 17q (413.6 cR from the top), since this gene contains a previously mapped sequenced-tagged site (STS WI-18519) (T. Hudson, GenBank accession G20939).

If *HsRAD51*, *XRCC2*, *XRCC3*, *RAD51B* and *RAD51C* are all involved in recombination and recombinational repair, the question arises why there are so many genes in mammalian cells, when yeast can undergo both processes in mitotic cells with only three members of this family (*RAD51*, *RAD55* and *RAD57*). Since the entire DNA sequence of *Saccharomyces cerevisiae* has been determined, it is known that there are no other members of this family in this yeast, other than *DMC1*, which encodes a meiosis-specific function. One possibility is that in mammalian cells some of the *RAD51*-related genes encode duplicated functions, but this seems unlikely at least for some of these genes. Since *RAD51* is an essential gene in mice, no other genes can substitute for its function. In addition, CHO cell lines with mutations in their homologs of *XRCC2* and *XRCC3* (i.e. *irs1* and *irs1SF*) are defective in DNA repair, indicating that these gene functions are probably not duplicated in CHO cells. Until mutants lacking *RAD51B* and *RAD51C* are isolated and characterized, it is still possible that these genes are functional duplications of each other or of some other genes in this family. However, our two-hybrid results indicate that this is unlikely, since these two genes each show a different pattern of protein interaction from each other and from *XRCC2*, *XRCC3* and *HsRAD51*. Unlike HsRad51, none of the other mitotically expressed members of the Rad51 protein family appear to interact with themselves. In this respect they seem to more closely resemble the yeast Rad55 and Rad57 proteins that do not interact with themselves. These do form a tight dimer that weakly interacts with the yeast Rad51 protein via an interaction with Rad55 (5-7). Our two-hybrid results indicate that Rad51C can bind to both *Xrcc3* and Rad51B, but it is not clear whether Rad51C can bind to both simultaneously, or if these interactions are mutually exclusive.

The two-hybrid system was also used to test a hypothesis regarding the original truncated *RAD51C* transcript. Alternately spliced transcripts frequently have been shown to have biological significance, but others have not been demonstrated to have any importance. Since this truncated protein lacks most of the nucleotide-binding motifs, one hypothesis was that this protein might have sites for interacting with other proteins, but lack other activities. In two-hybrid experiments, the truncated protein was not able to interact with Rad51B, Xrcc3 or HsRad51. This result does not exclude the possibility that it interacts with other, yet unidentified proteins, but it makes this hypothesis considerably less likely.

Since it seems unlikely that the human RAD51-related genes will turn out to be duplicated functions, there are at least two competing hypotheses that could explain the larger number of gene products involved compared to yeast. One hypothesis is that the mammalian recombination pathway contains many more proteins, and the other is that there are several related recombination pathways, each with some unique proteins and some common proteins such as HsRad51. A combination of these hypotheses is also possible, where there is more than one recombination pathway and each contains more proteins than in yeast. If there are multiple recombination pathways, they might either function in different tissues or cell types, or function in all cell types but only in response to different types of DNA damage or recombinational signals.

Our northern analysis of *RAD51C* indicates that this gene is widely expressed in different human tissues, but at very different levels. The highest level was observed in testis, which is consistent with a role for this gene in meiotic recombination. The transcript level was not very high in adult ovaries, but this does not rule out a role in female meiotic recombination, since in females the early stages of meiosis, including most steps in recombination, occur during fetal ovarian development. Among somatic tissue, the highest levels of expression were observed in heart muscle, spleen and prostate. Unlike *MmRAD51* and *RAD51B* (10,26,27), *RAD51C* is not particularly highly expressed in thymus, where T-lymphocyte differentiation occurs by a recombinational mechanism. Using virtually identical mRNA blots purchased from Clontech, Rice *et al.* (26) and Albala *et al.* (27) found different levels of expression of *RAD51B* in different tissues than we have observed with *RAD51C*, but the significance of these differences is not yet clear. Since both of these genes, as well as *XRCC2* and *XRCC3* (N. Liu, C. A. Walter and L. H. Thompson, personal communication), are widely expressed in different tissues, although at different levels, it seems unlikely that some of these genes are functional in only certain tissues.

Our general understanding of recombinational repair in mammalian cells is still very rudimentary. There is now a seventh member of the mammalian Rad51 family that is currently being characterized (D. Pittman and J. Schimenti, personal communication; Schild, unpublished data). In addition, none of the current members of this family shares as much sequence similarity with the yeast Rad55 protein as they do with Rad51 and Rad57. One possibility is that mammalian cells do not contain any proteins more similar to Rad55, and that one or more of the already isolated Rad51 family members is a functional Rad55 homolog. Another possibility is that there are still more genes in this family to be discovered in mammalian cells and that one or more of them will resemble *RAD55*. With regards to *RAD51C*, characterization of mutant cell lines and of transgenic knockout mice will certainly help elucidate the role of this gene, unless it turns out to be an essential function.

ACKNOWLEDGEMENTS

We wish to thank Larry H. Thompson for extensive and extremely useful comments and encouragement. This work was primarily supported by National Institutes of Health grant GM30990 to D.S. W.F., G.G.L. and J.S.A. were supported by the US Department of Energy under contract No. W-7405-ENG-48 to LLNL, and Z.S. was supported by the US Department of Energy (grant to LANL) and NIH grant ES08353.

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