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The Human and Mouse Homologs of the Yeast RAD52 Gene: cDNA Cloning, Sequence Analysis, Assignment to Human Chromosome 12p12.2–p13, and mRNA Expression in Mouse Tissues

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The yeast *Saccharomyces cerevisiae* RAD52 gene is involved in DNA double-strand break repair and mitotic/meiotic recombination. The N-terminal amino acid sequence of yeast *S. cerevisiae, Schizosaccharomyces pombe,* and *Kluyveromyces lactis* and chicken is highly conserved. Using the technology of mixed oligonucleotide primed amplification of cDNA (MOPAC), two mouse RAD52 homologous cDNA fragments were amplified and sequenced. Subsequently, we have cloned the cDNA of the human and mouse homologs of yeast RAD52 gene by screening cDNA libraries using the identified mouse cDNA fragments. Sequence analysis of cDNA derived amino acid revealed a highly conserved N-terminus among human, mouse, chicken, and yeast RAD52 genes. The human RAD52 gene was assigned to chromosome 12p12.2–p13 by fluorescence *in situ* hybridization, R-banding, and DNA analysis of somatic cell hybrids. Unlike chicken RAD52 and mouse RAD51, no significant difference in mouse RAD52 mRNA level was found among mouse heart, brain, spleen, lung, liver, skeletal muscle, kidney, and testis. In addition to an ~1.9-kb RAD52 mRNA band that is present in all of the tested tissues, an extra mRNA species of ~0.85 kb was detectable in mouse testis.

INTRODUCTION

Ionizing radiation causes different types of DNA damage. Misrepair or nonrepair of this damage may cause cell death, mutation, and neoplastic transformation. Distinctive pathways of DNA repair that respond to different DNA damage may exist. In the yeast *Saccharomyces cerevisiae,* three epistasis groups of DNA damage-repair genes have been identified (Friedberg *et al.,* 1991). The RAD52 epistasis group, which is mainly responsible for DNA double-strand break (DSB) repair, contains 8 genes: RAD50–RAD57. Several yeast genes of the RAD52 group have been cloned, including RAD51 (Basile *et al.,* 1992; Shinohara *et al.,* 1992), RAD52 (Schild *et al.,* 1983; Adzuma *et al.,* 1984), RAD54 (Emery *et al.,* 1991), RAD55 (Lovett, 1994), and RAD57 (Kans *et al.,* 1991). Yeast, chicken, mouse, and human RAD51 protein have a certain level of homology to the bacterial Rec-A protein, which is required for homologous recombination (Yoshimura *et al.,* 1993; Morita *et al.,* 1993; Shinohara *et al.,* 1992; Bezzubova *et al.,* 1993a), as well as to a yeast meiosis-specific gene DMC-1 (Bishop *et al.,* 1992). Yeast RAD51, RAD55, and RAD57 are also homologous to each other in certain regions, especially in their nucleotide binding regions (Lovett, 1994; Kans *et al.,* 1991).

Yeast RAD51, RAD52, and RAD54 genes are induced during meiosis (Cole *et al.,* 1989; Shinohara *et al.,* 1992). Yeast RAD54 is also inducible by DNA damage agents (Cole *et al.,* 1987; Emery *et al.,* 1991). Chicken RAD51/RAD52 (Bezzubova *et al.,* 1993a,b) and mouse RAD51 are highly expressed in reproductive tissues such as testis and ovary. Mutation of the RAD52 gene results in reduced repair of double-strand breaks and abnormal DNA recombination (Boudy-Mills and Livingston, 1993). The yeast RAD52 gene is also able to substitute for the phage T4 gene 46 in carrying out DNA repair and recombination (Chen and Bernstein, 1988). All of these studies suggest that genes in the RAD52 epistasis group are important not only for DNA damage repair but also for meiotic and mitotic recombination.

Although yeast RAD52 mRNA is not induced by X rays (Emery *et al.,* 1991), and little or no RAD52–LacZ fusion protein induction by DNA damage can be detected (Cole *et al.,* 1987), RAD52 is highly expressed in yeast meiosis (Cole *et al.,* 1989). Chicken RAD52 mRNA is highly transcribed in reproductive tissue (Bezzubova *et al.,* 1993b). Yeast RAD52 protein also physically interacts with RAD51 protein (Shinohara *et al.,* 1992; Milne and Weaver, 1993), which is involved in ATP-dependent homologous DNA pairing and strand exchange (Sung, 1994). A third component may also be involved with the RAD51 and RAD52 complex (Milne and Weaver, 1993). Mutations in different regions of

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the RAD52 gene often result in different phenotypes (Boudy-Mills and Livingston, 1993). All of these features make RAD52 an interesting component in the DNA DSB repair pathway. It is predicted that RAD52 gene products play an essential role in the repair of DNA DBS and DNA recombination. All of the cloned *S. cerevisiae* homologous RAD52 genes, including chicken RAD52 (Bezzubova *et al.,* 1993b), fission yeast *Schizosaccharomyces pombe* RAD22 (Ostermann *et al.,* 1993), and *Kluyveromyces lactis* RAD52 (Milne and Weaver, 1993) genes, show a highly conserved N-terminal amino acid sequence and a less homologous C-terminal sequence. However, the cloning of human and mouse homologs of the yeast RAD52 gene has not been reported.

In this report, we identified two mouse cDNA fragments by mixed oligonucleotide primed amplification of cDNA (MOPAC) (Lee and Caskey, 1990; Shen *et al.,* 1993). We cloned and sequenced the mouse and human cDNA homologs of yeast RAD52 genes using the identified mouse fragment. The human RAD52 gene is assigned to chromosome 12p12.2–p13 by fluorescence *in situ* hybridization (FISH). The mouse RAD52 mRNA level was also measured in several tissues.

MATERIALS AND METHODS

Materials. A human T-cell cDNA library in Lambda-ZAP Express Vector was purchased from Stratagene (La Jolla, CA). C3H 10T1/2 cell cDNA library in Lambda-ZAP II vector has been described (Shen *et al.,* 1994). Mouse A9 cells and a panel of A9 cell hybrids that each contain a single human chromosome 1–8, 11, 12, 14–22, and Y (Koi *et al.,* 1989) were kindly provided by Oshimura (Kanagawa Cancer Research Institute, Japan). Other CHO cell hybrids containing human chromosomes 9, 10, and 13 were purchased from the NIGMS Human Genetic Mutant Cell Respository (National Institutes of Health). Human skin fibroblasts were established in this laboratory (Tsuboi *et al.,* 1992). A mouse multiple tissue Northern blot was purchased from Clontech Laboratories, Inc. (Palo Alto, CA). A gel-extraction kit and plasmid mini- and midi-kits were purchased from Qiagen (Chatsworth, CA). [α-^{32}P]dCTP (3000 Ci/mmol) was purchased from Amersham Corp. (Arlington Heights, IL).

Mixed oligonucleotide primed amplification of cDNA. To amplify the RAD52 cDNA, an approach similar to that of Shen *et al.* (1993) was used. Two consecutive rounds of 35-cycle PCR were performed. The PCR reaction mix contained 0.1 vol (i.e., 2 μl for 20 μl of total reaction volume) of cDNA template; 0.2 μM dNTP; 1× PCR reaction buffer (100 mM Tris·HCl, pH 8.3; 500 mM KCl; 15 mM $MgCl_2$); 0.5 u/μl *Taq* DNA polymerase (Perkin–Elmer Cetus Corp., Norwalk, CT); and 5 μM of both of the sense degenerate primers (S1) and one of the antisense degenerate primers (either A1 or A2) (see Results for more information about the degenerate primers). PCR was performed by annealing at moderate stringency at 52°C. For the first round of PCR, 0.1 vol of a Lambda-ZAP II cDNA library made from C3H mouse embryo fibroblast (titering: 1×10^{10} plaque forming units (PFU)/ml) (Shen *et al.,* 1994) was used directly as the template cDNA. For the second round of PCR, 0.1 vol of the resultant first-round PCR products was used as template.

The amplified mouse RAD52 cDNA fragment was cut from an agarose gel, purified with a Qiagen gel-extraction kit, and confirmed by direct DNA sequencing (see Results). This DNA was used as probe for the first round of screening of cDNA libraries (see below).

Screening of cDNA libraries. One round of hybridization-based screening and two rounds of PCR-based screening were used to isolate the human and mouse RAD52 cDNA clones from two separate cDNA libraries.

A human T-cell cDNA library in Lambda-ZAP Express (purchased from Stratagene) and a Lambda-ZAP II mouse cDNA library that was constructed from C3H embryo fibroblast 10T1/2 cells (Shen *et al.,* 1994) were screened with a Duralon-UV membrane (Stratagene) according to the manufacturer's manual. The probe used in the hybridization was an ~350-bp purified PCR fragment (see Results) of mouse RAD52 cDNA that was labeled with [α-^{32}P]dCTP by PCR as described previously (Shen *et al.,* 1993). The temperature of the hybridization screen for the mouse library was at high stringency (42°C) and for human at moderate stringency (37°C), with 50% formamide.

For the first round of PCR screening, plugs containing putative RAD52 clones that had been identified from the above radioactive screen were plated at about 30–50 PFU per 100-mm agar plate. The phages grown on each plate were pooled by eluting them with SM buffer (0.58% NaCl, 0.2% $MgSO_4 \cdot H_2O$, 50 mM Tris·HCl (pH 7.5), 0.01% gelatin) and stored with 0.3% chloroform at 4°C. Two microliters of the pooled phage was subjected to PCR with two independent sets of PCR primers (see Results for primer information in PCR screen).

For the second round of PCR screening, pooled phage from plates with positive PCR results from the first PCR screening were plated on 100-mm agar plates at a density of 30–50 PFU per plate. About 30 plaques per agar plate were individually transferred into SM buffer as described above. Two microliters from each plaque was subjected to PCR. Positive phage clones were therefore isolated.

Positive phage clones from the second-round PCR screening were subcloned into pBluescript plasmid (for Lambda-ZAP II vector) or pBK-CMV plasmid (for Lambda-ZAP Express vector) by employing the Exassist/SOLR *in vivo* excision system or the Exassist/XLOLR *in vivo* excision system (Stratagene) according to the manufacturer's manual.

DNA sequencing. A primer-walking strategy was used to sequence cloned plasmid cDNA insert. Sequencing was performed with the *Taq* DyeDeoxy Terminator Cycle Sequencing Kit and ABI 373A automatic DNA sequencer (Applied Biosystems Inc., Foster City, CA) according to the protocols provided by the manufacturer. Both strands of the DNA were sequenced at least once. Sequence editing was carried out with the SeqEd software (Applied Biosystems) on a Macintosh computer. Further sequence analysis was performed on a VAX computer using the GCG sequence analysis software package (University of Wisconsin, Madison, WI).

Fluorescence in situ hybridization and R-banding. Fluorescence *in situ* hybridization was performed essentially as described elsewhere (Korenberg *et al.,* 1993). A biotinylated human RAD52 cDNA clone in pBK-CMV was mixed with the human C_0t-1 fraction and salmon sperm DNA to suppress repetitive sequences or block nonspecific hybridization. Hybridization was detected using successive rounds of fluorescein (FITC)–avidin D and biotinylated goat anti-avidin D (Vector Laboratories, Burlingame, CA). Three applications of FITC–avidin were required for signal visualization. Chromosomes were R-banded using chromomycin A3 and distamycin (Sigma Chemical Co., St. Louis, MO) following hybridization and detection (Donlon and Magenis, 1983). Scoring was performed on a Zeiss Axioplan with 100X plan NEOFluar objective and a long bandpass filter (Zeiss filter pack 09: 450–490 nm excitation/FT 510-nm dichroic mirror/LP 520-nm barrier filter) to visualize FITC signals and R-bands simultaneously.

Northern hybridizations. A multiple-tissue Northern blot containing mouse heart, brain, spleen, lung, liver, skeletal muscle, kidney, and testis was purchased from Clontech Laboratories, Inc. This membrane was sequentially hybridized with mouse RAD52 cDNA, RAD51 cDNA, and human β-actin cDNA (provided with the blot). After autoradiography, hybridized probes were stripped off before the next hybridization. The probe for RAD51 cDNA was prepared by PCR amplification of the human Lambda-ZAP cDNA library (see Results for explanation of why RAD51 is also used for hybridization). Hybridization probes were labeled with a random labeling kit (Amersham).

Other molecular methods. DNA purification from agarose gel and mini- and midi-plasmid isolation were performed using the Gel-ex-

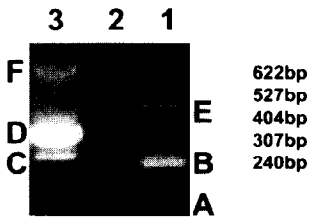

FIG. 1. Amplification of mouse RAD52 cDNA. C3H10T1/2 cell cDNA in Lambda-ZAP II phages was directly amplified with the degenerate primers (see text). A total of two rounds of 35 cycles were performed. Lane **1,** amplification by primers A1 and S1; lane **2,** DNA ladder; lane **3,** amplification by S1 and A2. A total of 6 major bands were visible. Bands B and D have the predicted sizes for the corresponding primer sets and therefore were subjected to further analysis (see text).

traction and plasmid isolation kits (Qiagen). Primers were synthesized with an Applied Biosystems Model 394 synthesizer. DNA isolation from cultured cells was performed as described (Sambrook *et al.,* 1989). All PCR reactions were performed as described in MOPAC. However, only 2 μM primers were used in PCR when nondegenerate primers were used.

RESULTS

Identification of Mouse RAD52 Gene

Based on the conserved N-terminal amino acid of chicken (Bezzubova *et al.,* 1993b), *S. cerevisiae* (Adzuma *et al.,* 1984), and *K. lactis* (Milne and Weaver, 1993) RAD52 peptide, the following degenerate primes were synthesized: S1, AA(A/G)(T/C)TIGGICCIGA(G/A)TA(C/T)AT; A1, (G/A)(T/C)ICC(G/A)TAICCIA(T/C)(G/A)TC(T/C)TC; and A2, CA(G/A)TTICC(G/A/T/C)A(A/G)IGC(G/A)TTICC. S1 is a sense primer; A1 and A2 are antisense primers. "I" indicates inosine.

After two rounds of PCR amplification (see Materials and Methods), six major bands (three by pairing S1 with A1, three by pairing S1 and A2) were amplified (Fig. 1). The band lengths of B and D (Fig. 1) were close to the predicted sizes for the chicken and yeast RAD52 cDNAs for the corresponding primer sets. Therefore, bands B and D were purified for DNA sequence analysis.

We used the degenerate primers (S1, A1, and A2) to sequence the purified bands B and D. The 5′ end sequence of band D (Fig. 2, bases 273–591) overlapped the whole sequence of band B (Fig. 2, bases 273–480) as expected. High sequence homology between band D (excluding the primer region) and the chicken and yeast RAD52 genes led to the conclusion that bands D and B are amplified from a mouse RAD52 cDNA homolog of the yeast and chicken genes. Sequences in the primer regions were not used for this comparison because mismatches could occur using the degenerate primers at moderate stringency in PCR amplification (Shen *et al.,* 1993, 1994; Lee and Caskey, 1990).

Specific primers were synthesized according to the sequence of band D and subsequently used for PCR screening of libraries in combination with the degenerate primers. Preliminary experiments showed that these specific primers designed from the mouse cDNA and the degenerate primers were also able to amplify human cDNA under moderate PCR annealing stringency (data not shown). Presumably, this is because of the high sequence homology between human and mouse cDNA in this region, as will be discussed later. The mouse cDNA-specific primers were used for PCR screening of both mouse and human phage cDNA library, as described under Materials and Methods.

Cloning and Sequencing of Mouse RAD52 cDNA

After one round of ^{32}P hybridization screening and two rounds of PCR screening, two positive clones from $\sim 1.0 \times 10^6$ PFU of C3H mouse Lambda-ZAP II cDNA library (see Materials and Methods) were purified and subcloned into pBluescript plasmid. DNA sequencing revealed that the longer mouse clone had a RAD52 insert of 1664 bp, as shown in Fig. 2. It had an open reading frame (bases 115–1374 in Fig. 2) that codes for 419 amino acids. Codon 115–117 was assigned as the translation start codon because it was the first ATG codon in the same frame and it had the characteristic A/G at the −3 position relative to ATG (Kozak, 1984). The shorter mouse clone had a 975-bp RAD52 insert corresponding to bases 51–1022 at the 5′ end (Fig. 2). We compared the two clones and found that the second

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   1 GGCGAGGACTCTGAGCTTGCAGAGTCTCCATTCCTTCTGCGAGGCCCTCTCCGGCTTCCC
  61 GGCAGGCACCCGGACCGAGCTCCTGCGTGCACCGCGTTCCGAAGCGCGGTCAACATGGCT
                                                              M  A
 121 GGGCCTGAAGAAGCAGTCCACAGAGGGTGTGACAACCATCCTCCCTTTGTTGGTGGGAAG
      G  P  E  E  A  V  H  R  G  C  D  N  H  P  P  F  V  G  G  K
 181 TCTGTGCTCCTCTTTGGACAGAGCCAGTATACAGCGGATGAATACCAGGCCATCCAGAAA
      S  V  L  L  F  G  Q  S  Q  Y  T  A  D  E  Y  Q  A  I  Q  K
 241 GCTCTGAGACAGACACTGGGTCCAGAGTACATTAGCAGCCGCATGGCTGGAGGAGGTCAG
      A  L  R  Q  B  I  G  P  E  Y  I  S  S  R  M  A  G  G  G  Q
 301 AAGGTGTGTTATATTGAAGGTCATCGGGTAATTAATCTGGCCAATGAGATGTTTGGTTAC
      K  V  C  Y  I  E  G  H  R  V  I  N  L  A  N  E  M  F  G  Y
 361 AATGGCTGGGCACACTCCATCACCCAGCAAAATGTGGATTTTGTTGACCTCAACAATGGC
      N  G  W  A  H  S  I  T  Q  Q  N  V  D  F  V  D  L  N  N  G
 421 AAGTTCTACGTGGGAGTCTGTGCATTTGTAAAGGTGCAGTTAAAGGATGGTTCCTATCAT
      K  F  Y  V  G  V  C  A  F  V  K  V  Q  L  K  D  G  S  Y  H
 481 GAGGACGTGGGCTATGGAGTTAGTGAGGGCCTCCGTTCAAAGGCCTTGTCACTGGAGAAG
      E  D  V  G  Y  G  V  S  E  G  L  R  S  K  A  L  S  L  E  K
 541 GCCAGGAAGGAGGCTGTGACTGATGGGCTGAAGCGAGCACTCAGGAGTTTTGGGAATGCA
      A  R  K  E  A  V  T  D  G  L  K  R  A  L  R  S  F  G  N  A
 601 CTTGGAAACTGTATTCTGGACAAAGACTATCTGAGGTCACTAAATAAGCTTCCACGACAG
      L  G  N  C  I  L  D  K  D  Y  L  R  S  L  N  K  L  P  R  Q
 661 CTCCCTCTTGATGTGGATTTAACTAAAACAAAGAGAAAAGATTTTGAACCATCTGTAGAA
      L  P  L  D  V  D  L  T  K  T  K  R  K  D  F  E  P  S  V  E
 721 CAGGCAAGATACAACAGCTGCCGACAGAATGAAGCATTGGGACTCCCCAAACCACAGGAA
      Q  A  R  Y  N  S  C  R  Q  N  E  A  L  G  L  P  K  P  Q  E
 781 GTGACTTCCCCTTGCAGATCAAGCCCCCCACATGACTCGAACATTAAGCTTCAGGGGGCT
      V  T  S  P  C  R  S  S  P  P  H  D  S  N  I  K  L  Q  G  A
 841 AAGGACATCAGCAGCTCCTGCAGTCTGGCCGCCACCTTGGAGAGTGACGCCACTCACCAG
      K  D  I  S  S  S  C  S  L  A  A  T  L  E  S  D  A  T  H  Q
 901 AGGAAGCTCCGGAAGCTCCGGCAGAAGCAGCTCCAGCAGCAGTTCCGGGAACAGATGGAG
      R  K  L  R  K  L  R  Q  K  Q  L  Q  Q  Q  F  R  E  Q  M  E
 961 ACTCGCCGGCAGACGCCATGCACCTGCCGAAGAGGTGGCAGCCAAGCATGCGGTACTTCCA
      T  R  R  Q  S  H  A  P  A  E  E  V  A  A  K  H  A  V  L  P
1021 GCCCCTCCAAAACACAGCACCCCTGTAACTGCAGCCTCAGAACTCCTCCAGGAGAAAGTC
      A  P  P  K  H  S  T  P  V  T  A  A  S  E  L  L  Q  E  K  V
1081 GTCTTTCCAGATAACCTTGAAGAGAACCTTGAAATGTGGGACCTTACTCCAGACTTAGAG
      V  F  P  D  N  L  E  E  N  L  E  M  W  D  L  T  P  D  L  E
1141 GACATCATTAAGCCCTTGTGTAGAGCAGAACCAGCCCAAACTTCTGCCACTCGAACCTTC
      D  I  I  K  P  L  C  R  A  E  P  A  Q  T  S  A  T  R  T  F
1201 AACAACCAGGACAGCGTCCCACATATCCATTGCCATCAGAAACCACAAGAAAAGCCTGGA
      N  N  Q  D  S  V  P  H  I  H  C  H  Q  K  P  Q  E  K  P  G
1261 CCTGGGCACCTGCAGACCTGCAACACCAACCAGCATGTTCTAGGTAGCAGAGAAGACTCT
      P  G  H  L  Q  T  C  N  T  N  Q  H  V  L  G  S  R  E  D  S
1321 GAACCTCATAGGAAGAGCCAGGACCTGAAGAAAAGGAAACTAGATCCATCCTGAGACTCA
      E  P  H  R  K  S  Q  D  L  K  K  R  K  L  D  P  S  *
1381 AGATGTCACTAGACCGTCACAAAAGGACTTTGGAAAACTGCAGTTTGGTCACAAAATTGT
1441 TCCACCTTGGCTCATGCTGAACTCTTTAGAGGACTATAGAGGAAGCTAACTACTTAAAAA
1501 GGTTGTCATACCCCATGATGGGAAGGAGGCCAACAGATAAACTTTGTTCAGTATTACTCA
1561 CTCCTAAGCTGATTCTAGGACCTTGCAGGCTGCCTTTCATTTAACGATGTTGCCCACCAA
1621 GCCCCAGCATGACTGTTACCTACATTAAATTATGTGTGACCTCA
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FIG. 2. cDNA sequence and derived amino acid sequence of the mouse RAD52 gene. The amino acid sequence is presented in single-letter code directly below the first nucleotide of the codon. The putative poly(A) addition signal and addition site according to Birnstiel *et al.* (1985) are underlined. Positions used for degenerate primers are boxed.

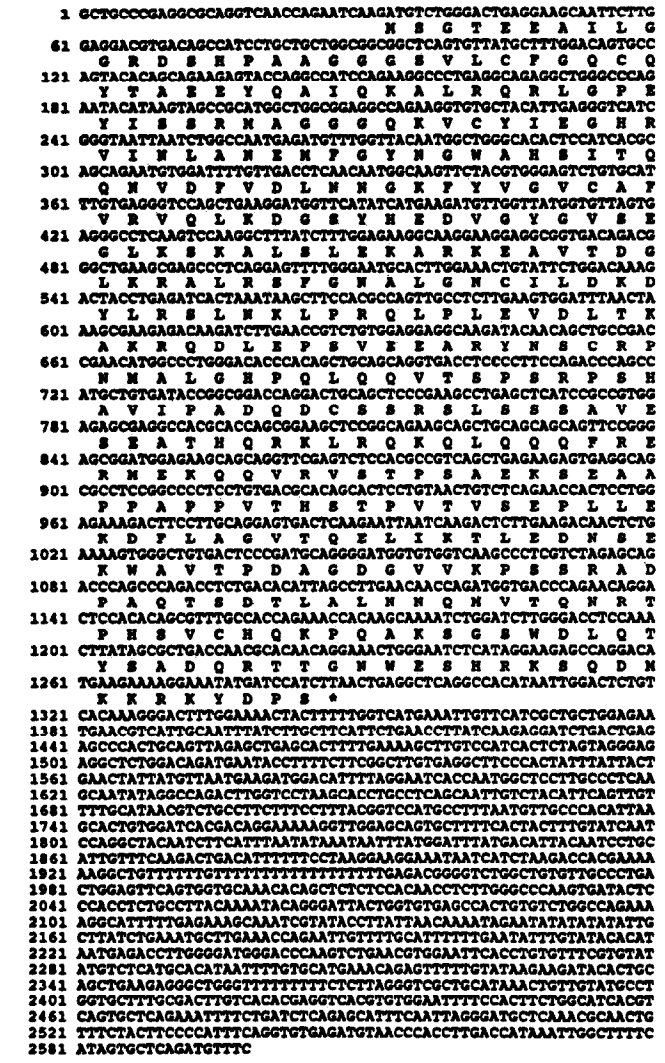

FIG. 3. cDNA sequence and derived amino acid sequence for the human RAD52 gene. Symbols as in Fig. 2.

clone has an A → G (resulting in Lys → Glu) transition at base 697 (Fig. 2) and an insertion GCA (coding for Ala) between bases 1011 and 1012 (Fig. 2). These differences may represent polymorphisms of the RAD52 gene in C3H 10T1/2 cells. We compared the cDNA sequences with the degenerate primers used to amplify mouse cDNA and found only one mismatch in the degenerate primer region of primer S1.

Cloning and Sequencing of Human RAD52 cDNA

Two positive clones were isolated from $\sim 1.0 \times 10^6$ PFU of the human T-cell cDNA library. The longer clone had an insert of 2598 bp (Fig. 3). The shorter clone had a RAD52 insert of 654 bp, with an identical sequence of bases 0–641 in Fig. 3, plus an extra sequence of GTGTGCACCGCGC at the 5′ end. Based on the same standard of Kozak (1984), codon 33–35 was assigned as the translation start codon. An open reading frame (bases 33–1289 in Fig. 3) that codes for 418 amino acids was identified.

Comparison of RAD52 Amino Acid Sequences

We used the BESTFIT program in the GCG package to list the overall amino acid sequence similarity between human and other RAD52 polypeptides (Table 1). It is evident that mouse and human RAD52 have the best similarity and identity. All of the available RAD52 peptide sequences were aligned using the PILEUP program in the GCG software package (Fig. 4). It is evident that all of the RAD52 peptides show a highly conserved N-terminal amino acid sequence, especially from position 61 to position 210 (Fig. 4). At the C-terminus, little homology between yeast and mammalian RAD52 was found. A noticeable R(K/R)xxxxKKR(K/R) sequence at the C-terminus is present in human, mouse, and chicken, but not in yeast RAD52. This sequence is similar to some of the reported nuclear localization signal (Roberts, 1989).

Human RAD52 Gene Is Located at Chromosome 12p12.2–p13

Simultaneous R-banding and fluorescence *in situ* hybridization show that the human RAD52 gene is located at chromosome 12p12.2–p13 (Fig. 5). One hundred metaphases were scored for FITC signal on both chromatids of one chromosome at a single band position. Hybridization of cDNA clones is less efficient compared with hybridization of cosmids or YACs, but more specific. Therefore, the presence of incompletely homologous sequences may not be detected by this method. Higher background is associated with the repeated amplification that is necessary for signal detection. Therefore, individual FITC grains were disregarded in scoring cells. Of the 100 metaphases scored, paired signals were observed in only 20 metaphases, presumably due to the low efficiency of FISH with cDNA probe. Seventeen paired signals occurred at the 12p locus, and the remaining signals each occurred once at three different loci. The signal on 12p occurred most frequently in the R-positive terminal band (12p13), sometimes appearing quite telomeric. However, because the chromosomes of some metaphases were more condensed, or banding less observable, signals could be interpreted as abutting the positive–negative interface with 12p12.2. The localization is therefore reported here as a range. A second hybridization on metaphases from an unrelated donor was used to confirm the localization.

TABLE 1

Amino Acid Comparison of Human RAD52 with Others

	Overall length	Gaps	Similarity (%)	Identity (%)
Mouse	431	9	80.5	71.7
Chicken	432	7	73.0	57.8
S. cerevisiae	501	12	51.0	30.8
K. lactice	438	11	51.8	30.5
S. pombe	472	8	49.6	30.7

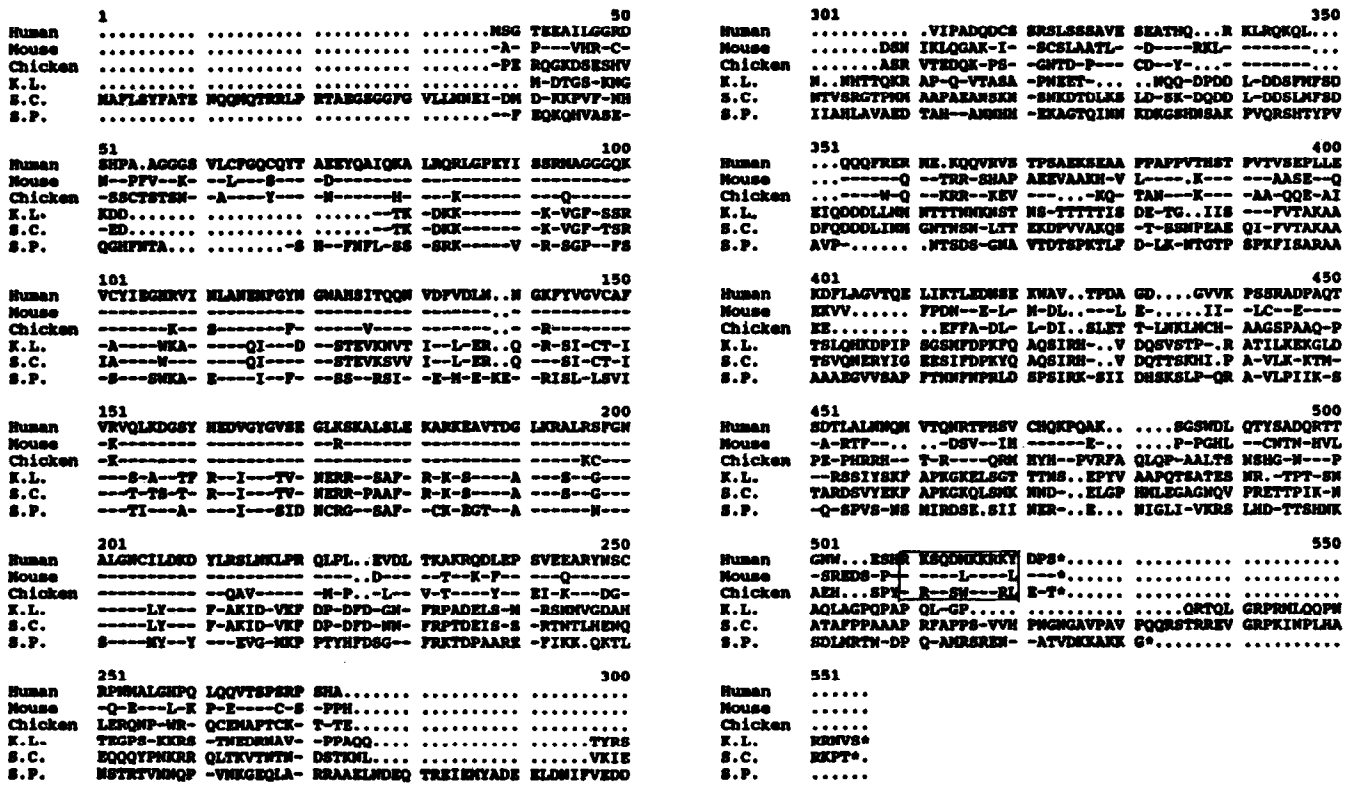

FIG. 4. Alignment of other RAD52 peptides with human RAD52 peptide. A dash indicates amino acids identical to human RAD52. A dot indicates a gap. K. L., *K. lactice* RAD52; S.C., *S. cerevisiae* RAD52; S.P., *S. pombe* RAD22. The putative nuclear localization signal for human, mouse, and chicken is boxed.

To confirm the localization of the RAD52 gene on chromosome 12, DNA from mouse A9 cell or CHO cell hybrids that each contain one human chromosome 1–22 or Y was used for PCR amplification of the human RAD52 gene. As shown in Fig. 4, there is a high degree of homology between human and mouse RAD52 in the N-terminal peptides. Therefore, two sets of independent primers outside the N-terminus conserved region were used to PCR amplify the human DNA in these hybrids (see Fig. 6 legend for details of the regions). Among all of the tested hybrids, PCR products identical to the total human DNA (positive control) were amplified only from the mouse cell line that contains human chromosome 12. Figure 6 shows the agarose gel of the PCR product of some cell hybrids. Therefore, the PCR results confirmed that the RAD52 gene is located on human chromosome 12.

Mouse mRNA Expression in Tissues

RAD52 is highly expressed in chicken testis. To test whether this is the case in mouse, a mouse multiple Northern blot, which contained 2 μg of mRNA from mouse heart, brain, spleen, lung, liver, skeletal muscle, kidney, and testis, was hybridized with the mouse RAD52 cDNA probe (bases 135–1486 of Fig. 2). Hybridization results showed the following (Fig. 7): (1) A RAD52 mRNA species of ~1.9 kb is present in all of the tested tissues. Considering that the average poly(A) size of mammalian mRNA is 200–300 bp, this result suggests that the mouse cDNA clone (Fig. 2) is a full-length (or at least close to full-length) copy of mouse RAD52 mRNA. (2) By normalizing with the β-actin mRNA level, the level of RAD52 in mouse testis is not significantly higher than others as detected in chicken RAD52 mRNA (Bezzubova *et al.*, 1993b), nor is it higher in spleen/testis as detected in mouse RAD51 mRNA (Morita *et al.*, 1993). This observation is different from what has been seen in chicken RAD52. However, (3) an extra band of ~0.85 kb is present in mouse testis tissue. Since only one mouse tissue membrane was used and no independent mouse tissue blots were tested, we used RAD51 to test whether this membrane is representative, because RAD51 is highly expressed in mouse spleen and testis (Morita *et al.*, 1993). Hybridization with RAD51 confirmed that it is highly expressed in mouse spleen and testis (autoradiography not shown). Therefore, we believe that Northern hybridization results from this membrane are representative of the RAD52 mRNA expression in these mouse tissues.

DISCUSSION

We cloned the mouse and human homologs of the yeast RAD52 gene using a PCR-based technique. The human and mouse RAD52 peptides share a significant degree of homology in their N-terminal region, suggesting the functional importance of this region. Mouse

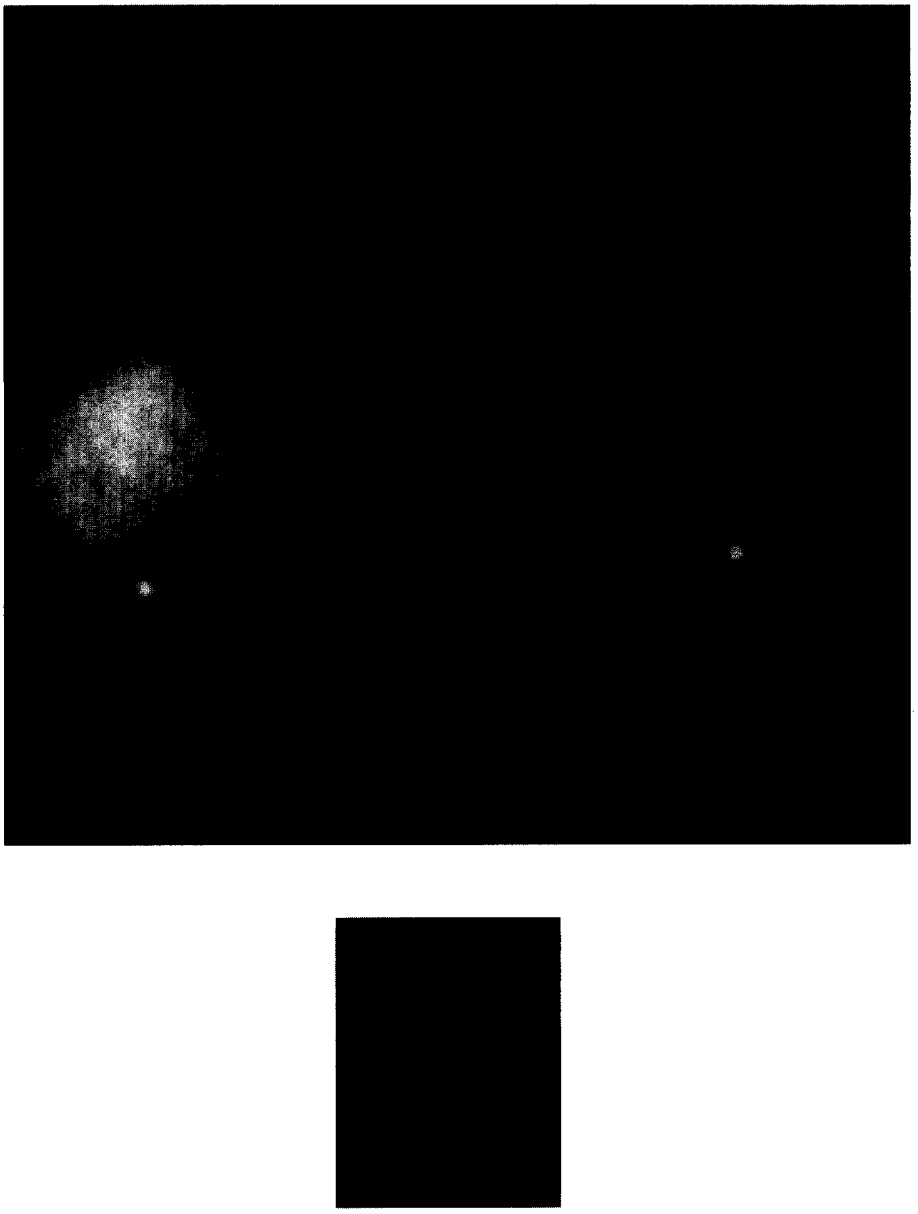

FIG. 5. Mapping of RAD52 to human chromosome 12p12.2–p13 by fluorescence *in situ* hybridization (FISH). Peripheral lymphocyte metaphases were hybridized with the biotinylated human RAD52 cDNA clone. Detection with fluorescein was followed by chromomycin A3–distamycin R-banding (see Materials and Methods). (**Top**) Partial metaphase spread showing hybridization to both chromatids of one homologue (indicated by arrow) and a single chromatid of the second homologue. This cell was scored as a positive localization based on the presence of the paired signal. Single FITC grains coincident with chromosomes were disregarded because of the background associated with three applications of fluorescein (see Results for details). (**Bottom**) Single chromosome 12 from another metaphase exhibiting telomeric positioning of FITC on both chromatids.

testis expresses roughly the same level of 1.9-kb RAD52 mRNA, plus an extra species of 0.85 kb. The RAD52 gene was assigned to human chromosome 12p12.2–p13. Other genes located around this region include G-protein β_3-subunit (Levine *et al.,* 1990), lymphocyte antigen CD69 (Lopez-Cabrera *et al.,* 1993), and T4 (CD4, Isobe *et al.,* 1986).

In yeast, the RAD52 gene is involved in DNA DSB repair and mitotic/meiotic recombination. The exact functions of mammalian RAD52 have not been studied directly. Studies with yeast *S. cerevisiae* RAD52 mutants may provide clues to the function of mammalian RAD52. Currently available mutants of yeast RAD52 include: (1) *Rad51-1,* which has a mutation resulting in one amino acid change A→V at position 113 in Fig. 4; (2) *Rad52-2,* which has a single amino acid change of P→L at position 87 in Fig. 4; (3) *Rad52-327,* which expresses a truncated peptide with only the N-terminal 327 amino acids (i.e., truncated at position 372 in Fig. 4); and (4) *Rad52-169,* which has only the N-terminal 167 amino acids (position 192 in Fig. 4) plus two extra amino acids at the end that have been introduced by construction of the mutant.

While *Rad52-1* and *Rad52-169* have a deletion phe-

notype, i.e., increased X-ray sensitivity and no mitotic/meiotic recombination, *Rad52-2* and *Rad52-327* retain partial DNA-repair capability and considerable ability for mitotic recombination (Boundy-Mills and Livingston, 1993; Malone *et al.,* 1988). However, *Rad42-1* still has some nonhomologous DNA-repair ability, which can rejoin broken DNA but results in deletions in the broken ends (Kramer *et al.,* 1994). One interesting observation is that although neither *Rad52-2* nor *Rad52-327* confers a high level of resistance to DNA damage independently, together they can confer wildtype resistance to DNA damage (Boundy-Mills and Livingston, 1993). These studies in yeast suggest that the RAD52 protein may have different functional domains and that each has relatively independent functions. Dornfeld and Livingston (1991) have shown that RAD52 protein and mRNA have a short half-life in yeast, indicating an unstable RAD52 mRNA. We noticed that there are five ATTTA elements in the 3′-end nontranslated region of human RAD52 cDNA and one ATTTA element in mouse. The ATTTA element in the 3′-end nontranslated region has been recognized as a sequence determinant controlling mRNA decay in some of the early-response genes, such as c-*fos,* c-*myc, GM-CSF,* and β-*IFN* (for review, see Greenberg and Belasco, 1993). Bezzubova *et al.* (1993b) have observed that chicken RAD52 expression is significantly higher in testis than in other tissues. In this report, we did not observe this phenomenon.

Many proteins may be involved in the RAD52 repair and recombination pathway, including genes RAD50–RAD57. Yeast XRS-2 may also have been involved in the RAD52 repair pathway (Ivanov *et al.,* 1992). Interaction between RAD51 and RAD52 and perhaps other proteins may be involved in converting DSBs to the next intermediate in the recombination pathway (Shinohara *et al.,* 1992; Milne and Weaver, 1993). From yeast studies, it is known that normal RAD52 function is essential for yeast meiosis. Genes involved in meiosis can be classified into three groups (Ajimura *et al.,* 1992). Mutations of the first group of genes (RAD51, RAD52, RAD54, RAD57) result in deficient DNA damage repair and mitotic/meiotic recombination. Mutations in the second group (RAD50, MRE11) result in deficient DNA damage repair but are able to confer some mitotic recombination. Mutations in the third group of genes (SPo11, HOP1, MERE2, REC114, and MRE4/MEK1) result only in deficient meiotic recombination. These studies suggest that genes in RAD52 groups may be involved in a common pathway for DNA repair and mitotic/meiotic recombination.

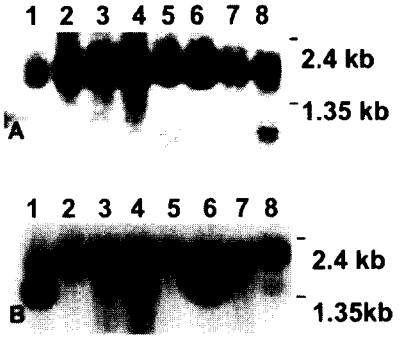

FIG. 7. Northern hybridization of mouse tissues with RAD52 cDNA. A mouse multiple tissue mRNA blot was hybridized with mouse RAD52 cDNA (**A**), stripped off, and then hybridized with human β-actin cDNA probe (**B**). The numbers to the side indicate the mRNA size. In mouse heart and skeletal muscle, two β-actin mRNA bands are present as expected. From left to right, the lanes represent heart (lane **1**), brain (lane **2**), spleen (lane **3**), lung (lane **4**), liver (**5**), skeletal muscle (lane **6**), kidney (lane **7**), and testis (lane **8**). While all of the tested mouse tissues expressed a major mRNA species of ~1.9 kb, an extra band of ~0.85 kb mRNA in testis is observed.

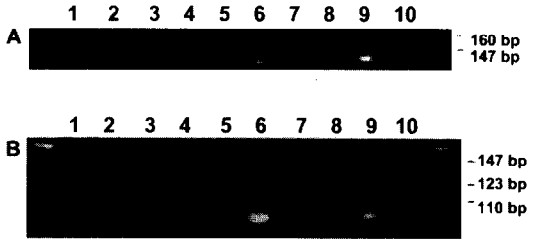

FIG. 6. PCR confirmation of RAD52 localization on chromosome 12. Fifty nanograms of genomic DNA from HSF cells, A9 cells, and A9 cells that contain a single human chromosome 3, 4, 11, 12, 19, or 20 was subjected to PCR amplification using two independent sets of primers. The resultant PCR products were run on a 2.4% agarose gel. The left- and rightmost lanes are DNA markers (number to the right indicates the DNA size). The DNAs used as PCR template in other lanes are lane **1,** no DNA (TE buffer only) as negative control; and lane **2,** A9 cells containing no human chromosome as negative control. Lanes **3, 4, 5, 6, 7,** and **8** are A9 cells containing human chromosomes 3, 4, 11, 12, 19, and 20, respectively. Lane **9** is total human DNA from HSF (see Materials and Methods) as positive control, and lane **10** is HSF lysate prepared as in Shen *et al.* (1993), as another positive control. (**A**) PCR from a primer set in the coding regions of 1088–1105 and 1211–1228. (**B**) PCR from a primer set in 3′ noncoding regions of 1400–1419 and 1480–1497.

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