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A mouse homolog of the *Escherichia coli recA* and *Saccharomyces cerevisiae RAD51* genes

(double-strand-break repair/homologous recombination)

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ABSTRACT **Analysis of mitotic and meiotic recombination in mammalian cells has been hampered by the complexity of the reactions involved as well as lack of mutants. Furthermore, none of the genes involved in the process has yet been identified. In budding yeast, *Saccharomyces cerevisiae*, the *RAD51* gene is essential along with other genes of the *RAD52* epistasis group for mitotic and meiotic recombination and DNA repair. The Rad51 protein is structurally similar to *Escherichia coli* RecA protein, which is required in homologous recombination and SOS responses in bacteria. Here we report the isolation of a mouse homolog of the yeast *RAD51* gene. The amino acid sequence predicted from the gene shows 83% and 55% homology with those of the yeast *RAD51* and the *E. coli recA* product, respectively. The mouse gene complemented a *rad51* mutation of *S. cerevisiae* with sensitivity to methylmethanesulfonate, which produces double-strand breaks of DNA. This gene is expressed in the thymus, testis, ovary, spleen, and intestine, suggesting that its product is involved in mitotic and meiotic recombination in addition to DNA repair.**

The molecular mechanisms of the recombination process have been studied extensively in prokaryotes and fungi. In *Escherichia coli* and many other prokaryotes, the RecA protein or RecA-like protein plays an essential role in homologous recombination and in a variety of SOS responses to DNA damage. It participates in the initiation of pairing and strand exchange leading to genetic recombination (1, 2).

In cells of yeast, a lower eukaryote, homologous recombination predominates and is used for gene manipulation in genetic studies. A double-strand-break repair model has been proposed for yeast recombination (3). It initiates from a double-strand break and involves gap formation. The 3′ ends invade a homologous duplex and form repair synthesis. This generates a region of double-strand gene conversion. It is bound on both sides by Holliday junctions. These junctions can branch-migrate to elongate regions of heteroduplex DNA. The Holliday junctions are then resolved to produce a crossover event. In these processes, genes of the *RAD52* epistasis group (*RAD50–RAD57*) are involved (4, 5). They were initially identified by mutants deficient in repair of DNA damage by ionizing radiation but are also impaired for mitotic and meiotic recombination and spore formation. The *RAD51* gene has recently been cloned and its product shown to be structurally similar to *E. coli* RecA protein with ATP-dependent DNA binding activity (6–8). In meiosis the null mutant of *rad51* accumulates double-strand breaks at meiotic recombination hot spots. Then the Rad51 protein may play a role in a step that converts double-strand breaks into the next intermediate in recombination (6).

In mammalian cells, gene targeting by homologous recombination is an effective way of generating mutant genes in embryonic stem cells in order to analyze the function of cloned genes. However, the mechanisms of mitotic and meiotic recombination in mammalian cells are still unclear. An enzymatic activity has been detected that catalyzes homologous recombination in mammalian somatic cells and a double-strand break improves the efficiency of introduction of exogenous DNA. But none of the genes involved in general recombination events has yet been identified in mammalian cells (9). Here, we report the isolation of a mouse *Rad51* homolog that functionally complements a yeast *rad51* mutation, suggesting a common mechanism of mitotic and meiotic recombination between yeast and mammalian cells.†

MATERIALS AND METHODS

Isolation of Mouse *Rad51* cDNA Clone and DNA Sequencing Analysis. Two oligonucleotides (5′-AGTTGGATCCGAA/GTTC/TA/CGIACIGGIAAA/G-3′ and 5′-AAGGAAGCT-TGCIAA/GA/GTGCATT/CTGICT/GA/G/C/TGC-3′, where I refers to inosine) were designed to code for two peptides in the conserved regions of *Saccharomyces cerevisiae, RAD51*, and its *Schizosaccharomyces pombe* homolog. The amino acid sequences used were EFRTGKS, corresponding to residues 186–192, and ARQMHLA, corresponding to residues 298–304 of the *RAD51* gene of *S. cerevisiae* (6), encompassing the two ATP binding motifs. The oligonucleotides were used in a reverse transcriptase polymerase chain reaction with total RNA from mouse testis as a template. The 360-bp fragment generated was subcloned and used as a probe to screen a λZAP cDNA library from 129/Sv mouse testes. A clone, pMR51, was isolated and both strands were sequenced.

Complementation of the Methylmethanesulfonate (MMS) Sensitivity of the Yeast *rad51-1* Mutant with Cloned Mouse *Rad51* cDNA. The mouse *Rad51* cDNA was inserted into the pYES2 shuttle vector under the *GAL1* promoter. Transformants were selected with *URA3* marker and expression of the cDNA products was induced with 5% galactose. The transformants were spread on synthetic complete medium plates containing various concentrations of MMS. The fraction of viable cells was calculated by counting colonies after 4 days at 30°C, setting the viability of untreated cells at time 0 as 1. Values were determined in parallel and each point represents the average of three determinations that typically varied by <10%.

RNA Blot Analysis. Total RNAs were isolated from adult mouse tissues with guanidinium thiocyanate by the single-step method (10). Total RNAs (35 μg) were separated in 1.2% agarose/2.2 M formaldehyde gel and blotted onto a nitrocellulose membrane filter (0.45 μm, Micron Separations Inc.).

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Abbreviation: MMS, methylmethanesulfonate.

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†The sequence reported in this paper has been deposited in the GenBank data base (accession no. D13473).

The filter was prehybridized for 1 hr and then hybridized at 42°C for 16 hr in 6× SSC (1× SSC = 0.15 M NaCl/15 mM sodium citrate), 50% formamide, 5× Denhardt's solution, 120 μg of sheared salmon sperm DNA per ml, and 0.1% SDS with an [α-^{32}P]dCTP-labeled cDNA containing nucleotides 1–1755 of pMR51. The filter was washed at 50°C in 0.1× SSC buffer containing 0.1% SDS and exposed to Kodak XAR-5 film for 7 days at −70°C.

RESULTS AND DISCUSSION

Cloning and Sequence Analysis of Mouse *Rad51* cDNA. Mouse genomic DNA gives a hybridization signal with yeast *RAD51* DNA as a probe, suggesting the existence of the homolog. However, it could not be isolated by screening mouse cDNA libraries by hybridization with the yeast probe at low stringency. To clone a mouse *Rad51* homolog, we designed oligonucleotides to code for two peptides present in highly conserved regions between the two *RAD51* genes of *S. cerevisiae* and *S. pombe* (unpublished data). These oligonucleotides were used in a reverse transcriptase PCR with cDNA from mouse (129/Sv) testis RNA as template (11). The 360-bp PCR product had a nucleotide sequence coding for an amino acid sequence homologous to that of the yeast *RAD51* gene and was then used as a probe to isolate cDNA clones from a mouse (129/Sv) testis cDNA library (12).

a

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   1                                                                                                GATCTGCGCAAAGCCGAGGCGCTCCCACAGGTGTGGCCAGGAAGACTGAAATTTT
  56 TCGTAGAAAACCTGACAGAGGAGCAGCGAAGCGCGTTCGAGCCGGGTGAAGTGGGGGGAGTGTGGTGTGTGCAGAGCCGGCTTCCCGGAGGAGCGAGCGGCCCGTGGCCGCGGGACAGTC
 176 ATGGCTATGCAAATGCAGCTTGAAGCAAGTGCAGATACTTCAGTGGAAGAAGAAAGTTTTGGTCCACAGCCTATTTCACGGTTAGAGCAGTGTGGCATAAATGCCAATGATGTGAAGAAA
   1 MetAlaMetGlnMetGlnLeuGluAlaSerAlaAspThrSerValGluGluGluSerPheGlyProGlnProIleSerArgLeuGluGlnCysGlyIleAsnAlaAsnAspValLysLys

 296 TTAGAAGAAGCCGGTTACCATACAGTGGAGGCTGTTGCTTATGCACCGAAGAAGGAACTAATAAATATTAAGGGAATTAGTGAAGCCAAAGCTGACAAAATTCTGACTGAGGCAGCAAAA
  41 LeuGluGluAlaGlyTyrHisThrValGluAlaValAlaTyrAlaProLysLysGluLeuIleAsnIleLysGlyIleSerGluAlaLysAlaAspLysIleLeuThrGluAlaAlaLys

 416 TTGGTTCCAATGGGTTTCACCACGGCAACTGAGTTTCACCAGCGCCGGTCAGAGATCATACAGATAACTACTGGCTCCAAAGAGCTGGACAAGCTGCTTCAAGGTGGAATTGAGACTGGA
  81 LeuValProMetGlyPheThrThrAlaThrGluPheHisGlnArgArgSerGluIleIleGlnIleThrThrGlySerLysGluLeuAspLysLeuLeuGlnGlyGlyIleGluThrGly

 536 TCTATCACAGAGATGTTTGGAGAATTCCGAACTGGGAAGACACAGATCTGTCATACGTTGGCTGTTACATGCCAGCTCCCCATTGACCGTGGTGGAGGTGAAGGGAAGGCCATGTACATT
 121 SerIleThrGluMetPheGlyGluPheArgThrGlyLysThrGlnIleCysHisThrLeuAlaValThrCysGlnLeuProIleAspArgGlyGlyGlyGluGlyLysAlaMetTyrIle

 656 GACACCGAGGGCACCTTTAGGCCGGAGCGGCTGCTAGCAGTAGCTGAGAGATACGGTCTCTCTGGCAGCGATGTCCTAGATAATGTAGCATATGCGCGAGGGTTCAACACAGACCACCAG
 161 AspThrGluGlyThrPheArgProGluArgLeuLeuAlaValAlaGluArgTyrGlyLeuSerGlySerAspValLeuAspAsnValAlaTyrAlaArgGlyPheAsnThrAspHisGln

 776 ACCCAGCTCCTTTACCAAGCGTCAGCCATGATGGTAGAATCCAGGTATGCACTGCTTATTGTAGACAGTGCTACTGCCCTTTACAGAACAGACTACTCAGGGCGGGGAGAGCTTTCAGCC
 201 ThrGlnLeuLeuTyrGlnAlaSerAlaMetMetValGluSerArgTyrAlaLeuLeuIleValAspSerAlaThrAlaLeuTyrArgThrAspTyrSerGlyArgGlyGluLeuSerAla

 896 AGGCAAATGCATTTGGCCAGATTTCTGAGGATGCTGCTTCGACTTGCTGATGAGTTTGGTGTCGCAGTGGTAATCACCAACCAGGTAGTAGCCCAAGTAGATGGAGCAGCCATGTTCGCT
 241 ArgGlnMetHisLeuAlaArgPheLeuArgMetLeuLeuArgLeuAlaAspGluPheGlyValAlaValValIleThrAsnGlnValValAlaGlnValAspGlyAlaAlaMetPheAla

1016 GCAGATCCCAAAAAACCCATTGGAGGGAACATCATCGCTCATGCGTCAACCACCAGGCTGTACCTGAGAAAAGGAAGAGGGGAGACCAGAATCTGCAAAATCTATGACTCTCCCTGTCTT
 281 AlaAspProLysLysProIleGlyGlyAsnIleIleAlaHisAlaSerThrThrArgLeuTyrLeuArgLysGlyArgGlyGluThrArgIleCysLysIleTyrAspSerProCysLeu

1136 CCTGAAGCTGAAGCTATGTTTGCCATTAATGCAGATGGAGTGGGCGATGCCAAAGACTGACTCCTTAGGCTTCTTTCCTGTGATGCTATACGGCTTTTCTAAATGCTCTACAGCTAATGG
 321 ProGluAlaGluAlaMetPheAlaIleAsnAlaAspGlyValGlyAspAlaLysAsp *

1256 AGAGGACTGTGTACTGGGCTTCTTCACAGGCTCATGTTGTGACTGCCCCGCATGGCTTCCGGGAAAACAACTATTGTATCAGCTCTCTGGTAGCAGAAACACATCTGATCTGAAAGGTTT
1376 CCTGTGGAGTCTATTAATAACTGATTTCTTTGGTGTTTAGAGGAGAAGTATGAAATAGCAGGTGTGGGGCAGTCTTTTGTCTTCAGGAGAAGTAAACTGGAAAGATCCGAGTACTCTCAC
1496 TTCTCAAGGATGGTAAACTAAATGCCTGAGGATCTGTAAGTGGGAATGGGTGGTTTACACATTCTTTTCTCTGTAAGACAGATGCAAACTTAGTTATATGCCTGAACTGTCACATCATTT
1616 TAAAATTTATTTTTGATTAAGGAGTCTTACATGCAGCATGCTAGACTACGATCCCTGCATGCTTGTTCTCTAAATTACTAAGACTTCACTTAAGGAAATTTGTTCTGCTGCTAATAAAGT
1736 AGTTGTGTGCAAAAAAAAAA
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b

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Mouse Rad51              2 AMQMQLEASADTSVEEESFGPQ......PISRLEQCGINANDVKKLEEAGYHTVEAVAYAPKKELINIKGISEAKADKILTEAAKLVPMGFTTATEFH
                           : .:| :|.|:..:|:|.::..      ||.:|:  ||. .||||| |.|.||.|||||||:|:|::|||||||||||:|.|||:|||||.||.:||
S.cereviciae Rad51      54 GGGLQEQAEAQGEMEDEAYDEAALGSFVPIEKLQVNGITMADVKKLRESGLHTAEAVAYAPRKDLLEIKGISEAKADKLLNEAARLVPMGFVTAADFH

 94 QRRSEIIQITTGSKELDKLLQGGIETGSITEMFGEFRTGKTQICHTLAVTCQLPIDRGGGEGKAMYIDTEGTFRPERLLAVAERYGLSGSDVLDNVAYARGFNTDHQTQLLYQASAMMVE
    ||||:| :|||||:||.||.||:|||||||:||||||||.|:|||||||||:|:| ||||||.:|||||||||| ||:.:|:|:||...|.|:|||||::|.||| .|| .|..|| |
152 MRRSELICLTTGSKNLDTLLGGGVETGSITELFGEFRTGKSQLCHTLAVTCQIPLDIGGGEGKCLYIDTEGTFRPVRLVSIAQRFGLDPDDALNNVAYARAYNADHQLRLLDAAAQMMSE
                                                A

214 SRYALLIVDSATALYRTDYSGRGELSARQMHLARFLRMLLRLADEFGVAVVITNQVVAQVDGAANFAADPKKPIGGNIIAHASTTRLYLRKGRGETRICKIYDSPCLPEAEAMFAINADG
    ||:.|::|||. ||||||:|||||||||||||||:|:| | ||||:|||||:|||||||||: .|.:|||||||||:||.||||| ::||:|  |:||: |||||||||.:||| .||
272 SRFSLIVVDSVMALYRTDFSGRGELSARQMHLAKFMRALQRLADQFGVAVVVTNQVVAQVDGGMAFNPDPKKPIGGNIMAHSSTTRLGFKKGKGCQRLCKVVDSPCLPEAECVFAIYEDG
          B

334 VGDAKD 339
    |||:::
392 VGDPRE 397

Mouse Rad51     65 GISEAKADKILTEAAKLVPMGFTTATEFH...QRRSEIIQITTGSKELDKLL.QGGIETGSITEMFGEFRTGKTQICHTLAVTCQLPIDRGGGEGKA.MYIDTEG
                   :|.|.| :| |..|   :...|..:. ::   :|. ::  |.||| .|| | .||:. |.|.|::|. ..||| :  ||.|    |. :. |||.  :||.|
E.coli RecA      1 AIDENK.QKALAAALGQIEKQFGKGSIMRLGEDRSMDVETISTGSLSLDIALGAGGLPMGRIVEIYGPESSGKTTL..TLQV.....IAAAQREGKTCAFIDAEH
                                                                                A

165 TFRP..ERLLAVAERYGLSGSDVLDNVAYARGFNTDHQTQLLYQASAMMVESRYALLIVDSATALYRTDYSGRGELSARQMHL.ARFLRMLLR.LADEF...GVAVVITNQVVAQVDGAA
    .: | .| |:|.         :||: :... :|:.|.  :::| |.  .:  .:::|||..|| ...  : ||::..:| | ||::.  :| ||:::   .. :::.||:  .::.
 98 ALDPIYARKLGVD.........IDNLLCSQP.DTGEQALEICDALAR..SGAVDVIVVDSVAALTPKAEIE.GEIGDSHMGLAARMMSQAMRKLAGNLKQSNTLLIFINQIRMKIGV..
                                                          B

278 NFAADPKKPIGGNIIAHASTTRLYLRK.........GRGETRICKIYDSPCLP.............................EAEAMFAINADGVGDAKD 339
    : ::|....||| :   ...|| :|:          . :|||:  : :. . |                              .|:| :...:: :|::|.
202 .MFGNPETTTGGNALKFYASVRLDIRRIGAVKEGENVVGSETRVKVVKNKIAAPFKQAEFQILYGEGINFYGELVDLGVKEKLIEKAGAWYSYKGEKIGQGKA 303
```

FIG. 1. Nucleotide and predicted amino acid sequence of mouse *Rad51* cDNA and sequence similarities between mouse RAD51 and other proteins. (*a*) One in-frame stop codon precedes the single long open reading frame starting with the first ATG in the sequence. A possible poly(A) signal is underlined. (*b*) Protein sequences were aligned using the BESTFIT program. Dashes between amino acids indicate identical sequences, and double dots signify conservative changes grouped as follows: D, E; S, T; I, L, V, M; A, G; F, W, T; N, Q; H, R, K; C; P. Two consensus sequences of purine nucleotide binding sites are designated as A and B in bold letters.

The nucleotide sequence of one positive clone obtained, pMR51, had a nucleotide sequence of 1755 bp containing an open reading frame (176–1192 bp), which encodes a 339-amino acid polypeptide with a predicted M_r of 36,902 (Fig. 1*a*). The predicted amino acid sequence of the mouse RAD51 protein showed significant homology with its yeast counterpart: optimized alignment revealed 67% sequence identity along the entire length of the protein (amino acids 2–339) and 83% overall similarity when conservative substitutions were included. The mouse RAD51 protein, like the yeast Rad51 protein, has significant homology with the bacterial RecA protein in the regions from amino acid residues 65–339 of the mouse RAD51 protein and residues 1–303 of the RecA protein: these regions showed 30% identity and 55% similarity of amino acids. This region includes domains of the RecA protein responsible for recombination, UV resistance, formation of active oligomer, and ATP binding (13). The consensus sequences for purine nucleotide binding (sequence A and sequence B in Fig. 1*b*) are conserved (14). The major difference between mouse RAD51 and yeast Rad51 is in the amino-terminal region—i.e., 53 residues at the amino terminus of yeast Rad51 are missing in mouse RAD51. The yeast Rad51 protein also shows close similarity to the yeast Dmc1 protein (15), which is expressed only in meiosis and is required for chromosome synapsis as well as for meiotic recombination. The identity of mouse RAD51 with the yeast Dmc1 protein is 48% and the similarity is 65%, indicating lower homology to Dmc1 than to Rad51.

Complementation by Mouse *Rad51* for MMS Resistance of Yeast Mutant. To examine the ability of the mouse *Rad51* product to complement the function of the yeast Rad51 protein, we transformed the yeast *rad51-1* mutant, XS3672-3C, with plasmids of yeast *RAD51* gene and mouse *Rad51* cDNAs (Fig. 2). The mouse *Rad51* cDNAs and yeast *RAD51* gene were joined to the inducible *GAL1* promoter and MMS

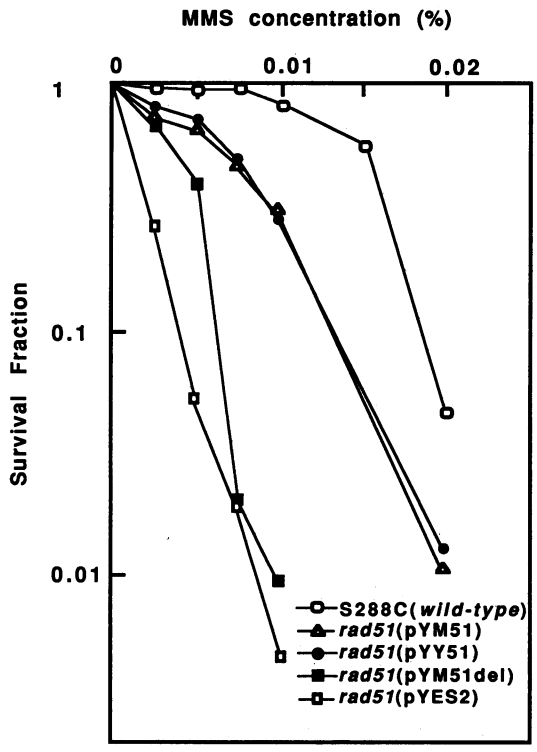

FIG. 2. Complementation of MMS sensitivity of the yeast *rad51-1* mutant with the cloned mouse homolog. Cell survival of *S. cerevisiae* was determined on MMS plates. Curves are shown for the mutants (X3672-3C; a *rad51-1 ura3 leu2 trp1 his4 lys2 ade2*) carrying plasmid with the *S. cerevisiae RAD51* gene (pYY51), mouse *Rad51* cDNA (pYM51), disrupted mouse *Rad51* cDNA (pYM51del; amino acids 129–339 deleted), or the shuttle vector (pYES2) only and for *RAD*$^+$ cells, S288C.

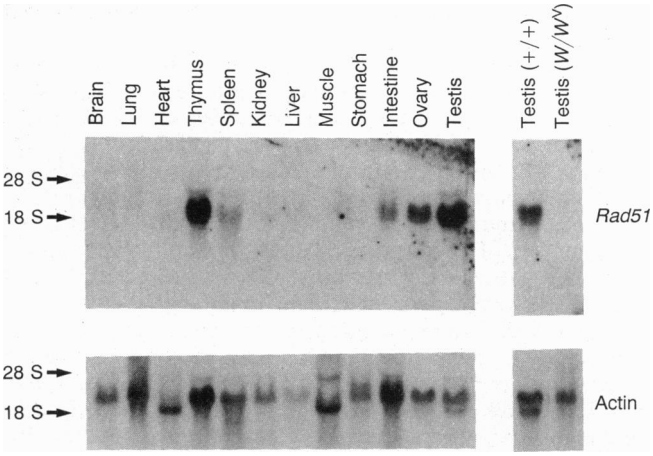

FIG. 3. Northern blot analysis of mRNA from mouse tissues with mouse *Rad51* cDNA as a probe. Lanes contained 35 μg of total RNA from mouse brain, lung, heart, thymus, spleen, kidney, liver, muscle, stomach, small intestine, ovary, and testis (129/Sv, +/+) or mutant testis (W/W^V). β-Actin and α-actin (in muscle and heart) transcripts were hybridized with human β-actin cDNA.

resistance was measured as the fraction of cells surviving on plates containing galactose and various amounts of MMS. The haploid yeast *rad51-1* mutant carrying the plasmid of the yeast *RAD51* gene (pYY51) was found to be resistant to MMS. The MMS sensitivity of the mutant carrying the mouse *Rad51* cDNA (pYM51) decreased to the same extent as that of the mutant with the yeast *RAD51* gene: both formed 30-fold more colonies on plates containing 0.01% MMS than either of the mutants carrying a truncated mouse *Rad51* cDNA (pYMdel) resulting in deletion of the carboxyl-terminal half of the peptide (residues 129–339) or carrying the vector (pYES2) only. The transformants were kept on Ura$^-$ plates, and plasmids were maintained stably (pYY, 89%; pYM51, 87%; pYM51del, 81%; pYES2, 85%). But the wild-type strain (S288C) showed more resistance than those complementing strains, indicating these complementations by pYY51 and pYM51 plasmids were partial, possibly caused by different expression levels of Rad51 proteins or some

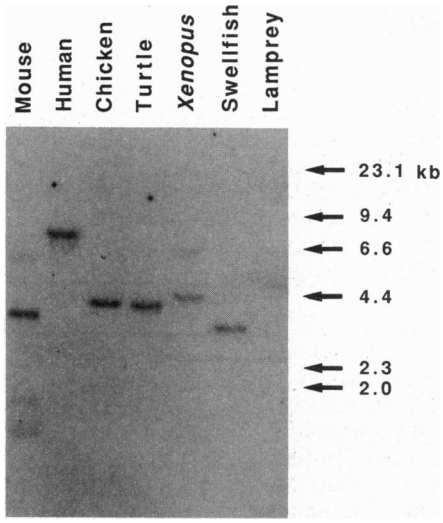

FIG. 4. Autoradiograph of a Southern blot of DNAs from various vertebrates hybridized with the mouse *Rad51* cDNA, pMR51. Genomic DNAs were cut with *Hin*dIII and separated in a 0.7% agarose gel. The DNA was blotted onto a nitrocellulose filter and the filter was baked and hybridized at 65°C with cDNA containing nucleotides 558–1755 of pMR51 as described (22). Then the filter was washed at 60°C in 0.5× SSC.

interactions of the Rad51 proteins with the Rad51-1 mutant polypeptide.

Expression of *Rad51* in Mouse Cells. RNA blot analysis with mouse *Rad51* cDNA as probe gave a hybridization band with an estimated mRNA size of 1.9 kb. Results showed that mouse *Rad51* mRNA is present at highest levels in the thymus and testis and at lower levels in the ovary, spleen, and small intestine, where cells were proliferating rapidly (Fig. 3). The spleen and thymus contain mitotically dividing B and T lymphocytes. The intestine contains intraepithelial lymphocytes with the $\gamma\delta$ T-cell receptor. At least two genes (RAG-1 and RAG-2) are known to activate immunoglobulin or T-cell receptor V(D)J recombination in the thymus (16, 17). Another genetic locus, *scid*, is also known to be involved in lymphocyte development and double-strand-break repair (18, 19). These observations on expression of *Rad51* in cells of the immune system are consistent with the notion that the *Rad51* product is involved in some step(s) of these immunological recombination events in addition to mitotic recombination and repair in these cells. We also detected the *Rad51* mRNA in the reproductive system. The germ cells in reproductive organs divide mitotically and meiotically. In the testis, undifferentiated spermatogonia undergo repeated mitotic divisions. After their growth, each resulting spermatocyte enlarges and then divides mitotically and meiotically to produce haploid spermatids. Fertility is severely impaired in the testis of adult W/W^V (c-*kit*) mutant mice (20), due to the almost complete absence of germ cells in the gonads. We were unable to detect the mouse *Rad51* transcript in the testis of this mutant, indicating that it is expressed in germ cells, not in the supporting cells of the adult testis. After cell separation of spermatogenic cells by unit gravity sedimentation, the hybridization signal was observed in fraction including spermatogonia and in that of pachytene spermatocytes (data not shown), which represent an important stage of meiotic recombination to produce the synaptonemal complex. Thus, the mouse *Rad51* gene is expressed in meiotically dividing cells as well as in mitotically dividing cells. We did not detect a hybridization signal in brain, where somatic recombination has been reported (21).

Molecular Properties of the *Rad51* Gene. Hybridization of the mouse *Rad51* cDNA probe to DNAs from several vertebrates (human, mouse, chicken, turtle, *Xenopus*, swellfish, and lamprey) at moderate stringency showed a high degree of conservation of the *Rad51* gene sequence (Fig. 4). One band, with or without additional faint bands, was observed, showing that *Rad51* may be a single gene. The result indicates that the *Rad51* gene is highly conserved from yeast to vertebrates, suggesting that its function is also required in other higher eukaryotes. The cloning and sequence analysis of a human homolog cDNA of *RAD51* have been reported (25).

The double-strand break and gap repair model has been proposed for homologous recombination in yeast (3). The Rad51 and Rad52 protein complex may be involved in the step of conversion of DNAs after the introduction of a double-strand break (5). In mammalian cells also, an increase in the frequency of homologous recombinations was seen when a double-strand break or gap was present in the homology of the substrate (23, 24). These findings suggest that the double-strand-break model is shared by yeast and mammalian cells. Our isolation of the mouse homolog of the yeast *RAD51* supports this view. In higher eukaryotes, in addition to *RAD51*, homologous genes of the *RAD52* epistasis group of yeast may also be present and they would have essentially the same function as in yeast in double-strand break repair and mitotic and meiotic recombination. This would be similar to the *RecA* system, which is common among prokaryotes.

Genes of the *RAD52* epistasis group are also required in recombination events other than those coupled to meiosis and mitosis in yeast. Mating-type switching, which is a gene conversion event that replaces a block of DNA, is also initiated from a double-strand break followed by functions of Rad51, Rad52, and Rad54 proteins (4). Similarly, the mouse homolog of the *Rad51* gene may contribute to other recombination events, such as V(D)J recombination of immunoglobulin and T-cell receptor genes. Although DNA rearrangement and presence of RAG-1 transcript have been described (16), we did not detect the *Rad51* transcript in the brain.

Molecular investigations of homologous recombination in mammalian cells will provide insights not only into mechanisms for general recombination but also techniques for more precise and effective manipulation of the mammalian genome for gene targeting and gene therapy.

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