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Mutation Research, DNA Repair 315 (1994) 295–305

DNA Repair

Cloning of human and mouse genes homologous to *RAD52*, a yeast gene involved in DNA repair and recombination

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Received 25 July 1994; accepted 1 August 1994

Abstract

The *RAD52* gene of *Saccharomyces cerevisiae* is required for recombinational repair of double-strand breaks. Using degenerate oligonucleotides based on conserved amino acid sequences of RAD52 and rad22, its counterpart from *Schizosaccharomyces pombe*, *RAD52* homologs from man and mouse were cloned by the polymerase chain reaction. DNA sequence analysis revealed an open reading frame of 418 amino acids for the human RAD52 homolog and of 420 amino acid residues for the mouse counterpart. The identity between the two proteins is 69% and the overall similarity 80%. The homology of the mammalian proteins with their counterparts from yeast is primarily concentrated in the N-terminal region. Low amounts of *RAD52* RNA were observed in adult mouse tissues. A relatively high level of gene expression was observed in testis and thymus, suggesting that the mammalian RAD52 protein, like its homolog from yeast, plays a role in recombination. The mouse *RAD52* gene is located near the tip of chromosome 6 in region G3. The human equivalent maps to region p13.3 of chromosome 12. Until now, this human chromosome has not been implicated in any of the rodent mutants with a defect in the repair of double-strand breaks.

Keywords: Human *RAD52* homologue; Mouse *RAD52* homologue

1. Introduction

Double-strand breaks (DSBs) in DNA arise as intermediates in several cellular processes including gene rearrangement during lymphocyte differ-

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SSDI 0921-8777(94)00039-5

entiation and recombination, or may be induced by DNA-damaging agents such as ionizing radiation. Numerous studies have implicated radiation-induced DSBs in the induction of chromosomal aberrations and in cytotoxicity. Direct evidence that DSBs can lead to chromosomal aberrations and intragenic alterations has come from studies showing that restriction enzymes induce chromosomal rearrangements and mutations when introduced into mammalian cells (Bryant, 1984; Natarajan and Obe, 1984; Winegar et al., 1989; Winegar et al., 1992).

To study the repair of DSBs in mammalian cells, several X-ray-sensitive rodent cell lines have been isolated. To date, at least nine complementation groups are known (for a review, see Collins, 1993). Among them, three groups are defective in the repair of DSBs: xrs (Kemp et al., 1984; Weibezahn et al., 1985), XR-1 (Giaccia et al., 1985), and V-3 (Whitmore et al., 1989). The identification of complementing human chromosomes by microcell-mediated chromosome transfer is a first step towards the identification of human genes involved in DSB repair. X-ray repair cross-complementing (*XRCC*) gene *4*, which complements XR-1 cells, is located on chromosome 5 (Giaccia et al., 1990). *XRCC5* is located on chromosome 2 and complements xrs cells (Chen et al., 1992; Jeggo et al., 1992). Cell lines derived from mice affected by the severe combined immune deficient (*scid*) mutation also have a reduced capacity of DSB repair and are sensitive to X-ray radiation (Biedermann et al., 1991; Hendrickson et al., 1991). The *scid* mice are characterized by a defect in one of the terminal steps of immunoglobulin rearrangement (Bosma and Carroll, 1991). The human gene complementing the *scid* defect is located on chromosome 8 (Itoh et al., 1993; Komatsu et al., 1993; Kirchgessner et al., 1993; Kurimasa et al., 1994). Recently, it has been shown that *scid* cells belong to the same complementation group as the Chinese hamster CHO mutant V-3 (Taccioli et al., 1994; Zdzienicka, personal communication). Cell lines derived from patients suffering from ataxia telangiectasia (AT) or Nijmegen Breakage Syndrome (NBS) are characterized by radiation sensitivity. However, the repair of DSBs is normal in AT cells, though the fidelity of repair appears not to be optimal (Fornace and Little, 1980; Hariharan et al., 1981; Van der Schans et al., 1980; Jaspers et al., 1982; North et al., 1990). No data have been reported on the repair of breaks in NBS cells.

In each organism several mechanisms are present to repair DSBs in DNA. In addition to end-to-end rejoining, DSBs can be repaired by resection-annealing or by recombinational repair (Price, 1993). The first step in the resection-annealing process (also called single-strand annealing) is an exonucleolytic degradation of the 5′ termini leaving 3′ single-strand tails. If repeats are present on both sites of the break, annealing of the single strands may occur. After further exonucleolytic processing of the branched intermediates, the remaining strands are rejoined by ligation. As a consequence of this type of repair, deletions are introduced in the DNA. The recombinational repair mechanism (also called double-strand break repair or gap repair) requires a second, undamaged homologous sequence. After pairing and strand invasion, information of the homologous sequence is used to repair the break.

In the yeast *Saccharomyces cerevisiae*, genes belonging to the *RAD52* epistasis group (*RAD50-57*, *XRS2* and probably also *MRE11*) are required for the recombinational repair of DSBs (Friedberg, 1988; Game, 1993; Ivanov et al., 1992; Ajimura et al., 1993). Mutants of this epistasis group have been isolated on the basis of their sensitivity towards ionizing radiation and methyl methanesulfonate. The most extreme sensitivity to X-rays is shown by *rad51*, *rad52* and *rad54* mutants. Thus far, only for these three mutants has direct molecular evidence been substantiated for a defect in DSB repair (Game, 1993). The *rad51*, *52* and *54* mutants are also defective in induced mitotic recombination and in mating-type switching. Meiotic recombination is affected in *rad51* and *rad52* mutants, as manifested by the formation of inviable spores. Several of the *RAD52* group genes have been isolated and characterized (Alani et al., 1989; Aboussekhra et al., 1992; Basile et al., 1992; Shinohara et al., 1992; Adzuma et al., 1984; Emery et al., 1991; Kans and Mortimer, 1991). Little is known about the

precise function of the products encoded by these genes and their role in DSB repair. Evidence has been presented that the RAD52 protein binds directly to the RAD51 gene product, which is homologous to the RecA protein from *E. coli* (Shinohara et al., 1992). This observation has been substantiated by *GAL4* two-hybrid studies, which revealed a physical interaction between the C-terminal one-third of RAD52 and the RAD51 protein (Milne and Weaver, 1993). Genetic studies suggest that the RAD52 protein is not involved in the first steps of recombination. The formation of inviable spores in *rad52* null mutants is not suppressed by a *spo13* mutation, which eliminates the first meiotic division (Klapholz and Esposito, 1980a,b). Possible recombination intermediates have also been detected in *rad52* mutant strains (Nag and Petes, 1993). Therefore, the RAD52 protein is presumably required for an intermediate or late step in recombination (Petes et al., 1991).

Homologs of *RAD51*, *RAD52* and *RAD54* have been identified in the distantly related yeast strain *Schizosaccharomyces pombe* (Muris et al., 1993; Ostermann et al., 1993; Muris et al., unpublished). Based on conserved amino acid domains, degenerate primers can be designed and used for the identification of mammalian homologs. In this way, *RAD51* and *RAD52* genes from chicken have been isolated (Bezzubova et al., 1993a,b). Recently, the mouse and human homologs of *RAD51* have also been cloned (Shinohara et al., 1993; Morita et al., 1993). The structural similarity of the yeast and the mammalian homologs indicates that certain steps in the recombinational repair pathway are conserved during evolution.

Here, we report the isolation of the human and mouse *RAD52* homologs. The protein sequences predicted by the two mammalian genes share extensive homology and strongly resemble the chicken and yeast homologs.

2. Materials and methods

Gene isolation

Two conserved stretches of six amino acids shared by the *Saccharomyces cerevisiae* RAD52 protein and its homolog from *Schizosaccharomyces pombe*, rad22, were used to design degenerate primers to clone the mouse *RAD52* homolog by amplification in vitro. The sequences of the upstream and downstream oligonucleotides are 5′-GGAATTCAA(A/G)CTIGGICCIGA(A/G)TA-3′ and 5′-GGAATTCC(A/G)TAICCIAT-(A/G)TC(T/C)TC-3′. I is the symbol used for inosine and alternative nucleotides are given in brackets. Restriction enzyme sites for *Eco*RI were included at the 5′ end of the primers to facilitate subcloning. Poly A^+ RNA from mouse testis was reverse transcribed by random priming according to the GeneAmp^R RNA PCR kit (Perkin-Elmer). The reaction mixture was heated for 2 min at 94°C and used in a PCR reaction with 50 pmol of each primer. The samples were cycled for 30 times through three temperatures: 94°C (5 s), 42°C (15 s) and 72°C (1 min). Amplification products were purified on 4% Nusieve^R agarose (FMC Bioproducts) and subcloned in M13 vectors for sequence analysis.

cDNA isolation and sequencing

A 252-bp PCR-derived fragment was used to screen a mouse thymus cDNA library (kindly provided by M. van Lohuizen) and a human testis cDNA library (Clontech). Hybridization was performed for 16 h at 62°C in 1 M NaCl, 1% SDS, 5% dextran sulfate, 50 mM Tris-HCl (pH 7.5), 1 mM EDTA and 20 μg/ml salmon sperm DNA. Filters were washed to a final stringency of 1 × SSC at 62°C. After subcloning into pUC119 and pUC120 and into M13mp18 and M13mp19 vectors (Maniatis et al., 1989; Yanisch-Perron et al., 1985), the nucleotide sequence of both strands was determined using T7 polymerase according to the manufacturer (Pharmacia/LKB). In some cases sequencing reactions were carried out using an AutoRead sequencing kit (Pharmacia/LKB) and the products were analyzed on an ALF Automatic Sequencer (Parmacia/LKB). Deduced amino acid sequences were aligned according to the Boxshade program (Corpet, 1988).

Northern analysis

RNA was isolated from different adult mouse tissues by homogenization of frozen material in

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ScRAD52    1 MAFLSYFATENQQMQTRRLPRTAEGSGGFGVLLMNEIMDMDEKKPVFGNHSEDIQTKLDK
SpRAD22    1 MSF.....EQKQHV.......ASEDQGHFNTAYSHEEFN.......F......LQSSLSR
ChRAD52    1 MP......ERQGKDSESHVSSSCTSTSNSVACFGQYQYTANE....Y....QAIQHALRQ
MmRAD52    1 MA......GPEEAVHRGCDNHPPFVGGKSVLLFGQSQYTADE....Y....QAIQKALRQ
HsRAD52    1 MS......GTEEAILGGRDSHPA.AGGGSVLCFGQCQYTAEE....Y....QAIQKALRQ

ScRAD52   61 KLGPEYISKRVGFGTSRIAYIEGWRVINLANQIFGYNGWSTEVKSVVIDFLDERQ..GKF
SpRAD22   36 KLGPEYVSRRSGPGGFSVSYIESWKAIELANEIFGFNGWSSSIRSINVEFMDENKENGRI
ChRAD52   47 KLGPEYISSRQAGGGQKVCYIEGHKVISLANEMFGFNGWAHSVTQQNVDFVDLNN..GRF
MmRAD52   47 RLGPEYISSRMAGGGQKVCYIEGHRVINLANEMFGYNGWAHSITQQNVDFVDLNN..GKF
HsRAD52   46 RLGPEYISSRMAGGGQKVCYIEGHRVINLANEMFGYNGWAHSITQQNVDFVDLNN..GKF

ScRAD52  119 SIGCTAIVRVTLTSGTYREDIGYGTVENERRKPAAFERAKKSAVTDALKRSLRGFGNALG
SpRAD22   96 SLGLSVIVRVTIKDGAYHEDIGYGSIDNCRGKASAFEKCKKEGTTDALKRALRNFGNSLG
ChRAD52  105 YVGVCAFVKVQLKDGSYHEDVGYGVSEGLKSKALSLEKARKEAVTDGLKRALKCFGNALG
MmRAD52  105 YVGVCAFVKVQLKDGSYHEDVGYGVSEGLRSKALSLEKARKEAVTDGLKRALRSFGNALG
HsRAD52  104 YVGVCAFVRVQLKDGSYHEDVGYGVSEGLKSKALSLEKARKEAVTDGLKRALRSFGNALG

ScRAD52  179 NCLYDKDFLAKIDKVKFDPPDFDENNLFRPTDEISESSRTNTLHENQEQQQYPNKRRQLT
SpRAD22  156 NCMYDKYYLREVGKMKPPTYHFDSGDLFRKTDPAARESFIKK.QKTLNSTRTVNNQPLVN
ChRAD52  165 NCILDKDYLQAVNKLPRQMPP..ELDLVKTK....RQDYEPEIEKARYDGCLERQNPGWR
MmRAD52  165 NCILDKDYLRSLNKLPRQLPL..DVDLTKTK....REDFEPSVEQARYNSCRQNEALGLP
HsRAD52  164 NCILDKDYLRSLNKLPRQLPL..EVDLTKAK....RQDLEPSVEEARYNSCRPNMALGHP

ScRAD52  239 KVTNTNPDSTKNLVKIENTVSRGTPMMAAPAEANSKNSSNKDTDLKSLDASKQDQDDLLD
SpRAD22  215 KGEQLAPRRAAEL...NDEQTREIEMYADEELDNIFVEDDIIAHLAVAEDTAHPAANN.H
ChRAD52  219 QQCEMAPTC........KPTHTEASRVTEDQKQPSSSGNTDSPAVEC.DATYQRKLR...
MmRAD52  219 KPQEVTSPC........RSSPPHDSNIKLQGAKDISSSCSLAATLES.DATHQRKLRKLR
HsRAD52  218 QLQQVTSPS........R..PSHAV...IPADQDCSSRSLSSSAVES.EATHQRKLR...

ScRAD52  299 DSLMFSDDFQDDDLINMGNTNSNVLTTEKDPVVAKQSPTASSNPEAE...QITFVTAKAA
SpRAD22  271 HSEKAGTQINNKDKGSHNSAKPVQRSHTYPVAVPQNTSDSVGNAVTDTSPKTLFDPLKPN
ChRAD52  267 QKQL.QQQFWEQ.MEKRRQVKEVTPS...SKQATANPPVKHSTPAA............VQ
MmRAD52  270 QKQL.QQQFREQ.METRRQSHAPAEEVAAKHAAVLPAPPKHSTPVT............AA
HsRAD52  261 QKQL.QQQFRER.ME.KQQVRVSTPSAEKSEAAPPAPPVTHSTPVT............VS

ScRAD52  356 TSVQNERYIGEESIFDPKYQAQSIRHTVDQTTSKHIPASVLKDKTMTTARDSVYEKFAPK
SpRAD22  331 TGTPSPKFISARA...AAAAEGVVSAPFTNNFNPRLDSPSIRKTSIIDHSKSLP...VQR
ChRAD52  310 QELAIEE..................EFFADDLELWDISLETTDLNKLMCHKAA.....GS
MmRAD52  316 SELLQEKVV..............FPDNLEENLEMWDLTPDLED...II..KPL.....CR
HsRAD52  306 EPLLEKDFLAG........VTQELIKTLEDNSEKWAVTPDAGD..GVV..KPS.....SR

ScRAD52  416 GKQLSMKNNDKELGPHMLEGAGNQVPRETTPIKTNATAFPPAAAPRFAPPSKVVHPNGNG
SpRAD22  385 ASVLPIIKQSSQTSPV...SNNSMIRDSESIINERKENIGLIGVKRSLHDSTTSHNKSDL
ChRAD52  347 PAA....QQPPETPHR....RHQMTTRNRTPQRMHYHKPPVRFAQLQPSAALTSNSHGAN
MmRAD52  352 AEP....AQTSATRTF....NNQ....DSVPHIHCHQKPQEKPGPGHLQTC.NTNQHVLG
HsRAD52  349 ADP....AQTSDTLAL....NNQMVTQNRTPHSVCHQKPQAKSGSWDLQTY.SADQRTTG

ScRAD52  476 AVPAVPQQRSTRREVGRPKINPLHARKPT
SpRAD22  442 MRTNSDPQSAMRSRENYDATVDKKAKKG
ChRAD52  399 QRTPAEHSPYRRSQSWKKRRLEPT
MmRAD52  399 SREDSE..PHRKSQDLKKRKLNPS
HsRAD52  400 NWE.....SHRKSQDMKKRKYDPS
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Fig. 1. Amino acid alignment of *S. cerevisiae* (*Sc*) RAD52, *S. pombe* (*Sp*) rad22, chicken (*Ch*) RAD52, mouse (*Mm*) RAD52 and human (*Hs*) RAD52 proteins. Alignments were determined using the Boxshade program (Corpet, 1988). Amino acids which are identical in at least two RAD52 homologs are highlighted in black. Highlighting in gray indicates functionally conserved amino acid residues. Conserved amino acids were taken as follows: V, I, L and M; D, E, Q and N; F, Y and W; G, S, T, P and A; K, R and H. The accession numbers of the human and mouse *RAD52* nucleotide sequences are L33262 and Z32767, respectively.

10 vol 3 M LiCl, 8 M urea. After 3 h on ice, RNA was isolated by centrifugation and further purified by proteinase K treatment and phenol-chloroform-isoamyl alcohol (25:24:1) extraction. Total RNAs (approximately 30 μg) were separated on 1% agarose, 0.6 M formaldehyde gels, transferred to Hybond-N^+ (Amersham) and hybridized for 16 h at 42°C (Maniatis et al., 1989).

In situ hybridization

Mouse and human metaphase chromosomes were obtained from splenocytes and whole blood samples, respectively, as described before (Boei et al., 1994; Natarajan et al., 1991). Slides were prepared and processed for hybridization according to standard procedures (Pinkel et al., 1986). The mouse *RAD52* cDNA, or a genomic *RAD52* clone isolated by screening a genomic lambda library (a kind gift of G. Weeda), was used to determine the chromosomal location. To map the human *RAD52* gene, the human cDNA clone was used. Specific human and mouse chromosomes were identified using a human chromosome 12 specific centromeric probe (provided by P. Devilee) and the mouse pRAF1 (Kolch et al., 1991), respectively. DNAs were labeled with a mixture of digoxigenin-11-dUTP and dTTP (1:3) in case of the centromeric specific probe and pRAF1 or with biotin-16-dUTP for the *RAD52* specific probes using a nick translation kit (Promega Biotech). After labeling, the DNA was dissolved in hybridization buffer (50% formamide, 2 × SSC and 10% dextran sulfate) at a final concentration of 2.5–5 ng/μl. To reduce background hybridization, mouse genomic probes were mixed with 5 μg mouse Cot1 DNA (Gibco), denatured at 80°C for 10 min, chilled on ice and incubated for 2 h at 37°C prior to use. The slides were incubated with 20 μl hybridization buffer for 16 h in a humidified chamber at 42°C. After hybridization, slides were washed with 50% formamide, 2 × SSC at 42°C (three times 5 min) followed by three washes of 5 min in 0.1 × SSC at 60°C. Immunological detection was carried out as described (Pinkel et al., 1988). Chromosomes were counterstained with a Vectashield antifade solution containing 1 μg/ml propidium iodide or 0.15 μg/ml 4′,6-diamidino-2-phenylindole · 2HCl (DAPI).

3. Results

Cloning of mouse and human RAD52 homologs

The RAD52 protein from *Saccharomyces cerevisiae* shares two stretches of six identical amino acids at positions 61–66 and 137–142 with its counterpart from the distantly related yeast strain *Schizosaccharomyces pombe*, rad22 (see Fig. 1). These regions were used to design degenerate primers to clone part of the mouse *RAD52* cDNA by amplification. Using Poly A^+ RNA from mouse testis as a source, a fragment of approximately 250 bp was obtained after RT-PCR. Sequence analysis revealed an open reading frame (ORF) of 252 bp with strong homology to the yeast RAD52 and rad22 proteins. Using the 252-bp fragment as a probe, a cDNA clone was isolated from a mouse thymus cDNA library. The same fragment was also used to isolate a human clone from a testis cDNA library. The mouse clone contains an insert of 1719 nucleotides, which is only slightly shorter than the size of the RNA species identified by Northern hybridization (see below). The first ATG at position 140 determines the start of an ORF coding for a putative polypeptide of 420 amino acids. The sequence context of the ATG codon (*G*CG*G*TC*A*ACATG*G*) matches perfectly to the eukaryotic consensus sequence for translation initiation (a G at positions −9, −6 and + 4 and a purine at −3) (Kozak, 1989). A presumed polyadenylation signal, ATTAAA, is present beginning at nucleotide 1672. The poly A tail starts at position 1692. The human cDNA clone contains an insert of 2371 bp. From position 270 to 1523 an ORF can be identified coding for a putative protein of 418 amino acid residues. In the 5′-untranslated region in-frame stop codons are present at positions 90, 192, 213 and 237. The sequence context of the start codon (*A*GA*A*TC*A*AGATG*T*) does not match very well to the consensus sequence for start codons (Gs at positions −9, −6 and + 4 and a purine at −3) (Kozak, 1989). Since the homology with the mouse RAD52 protein is very

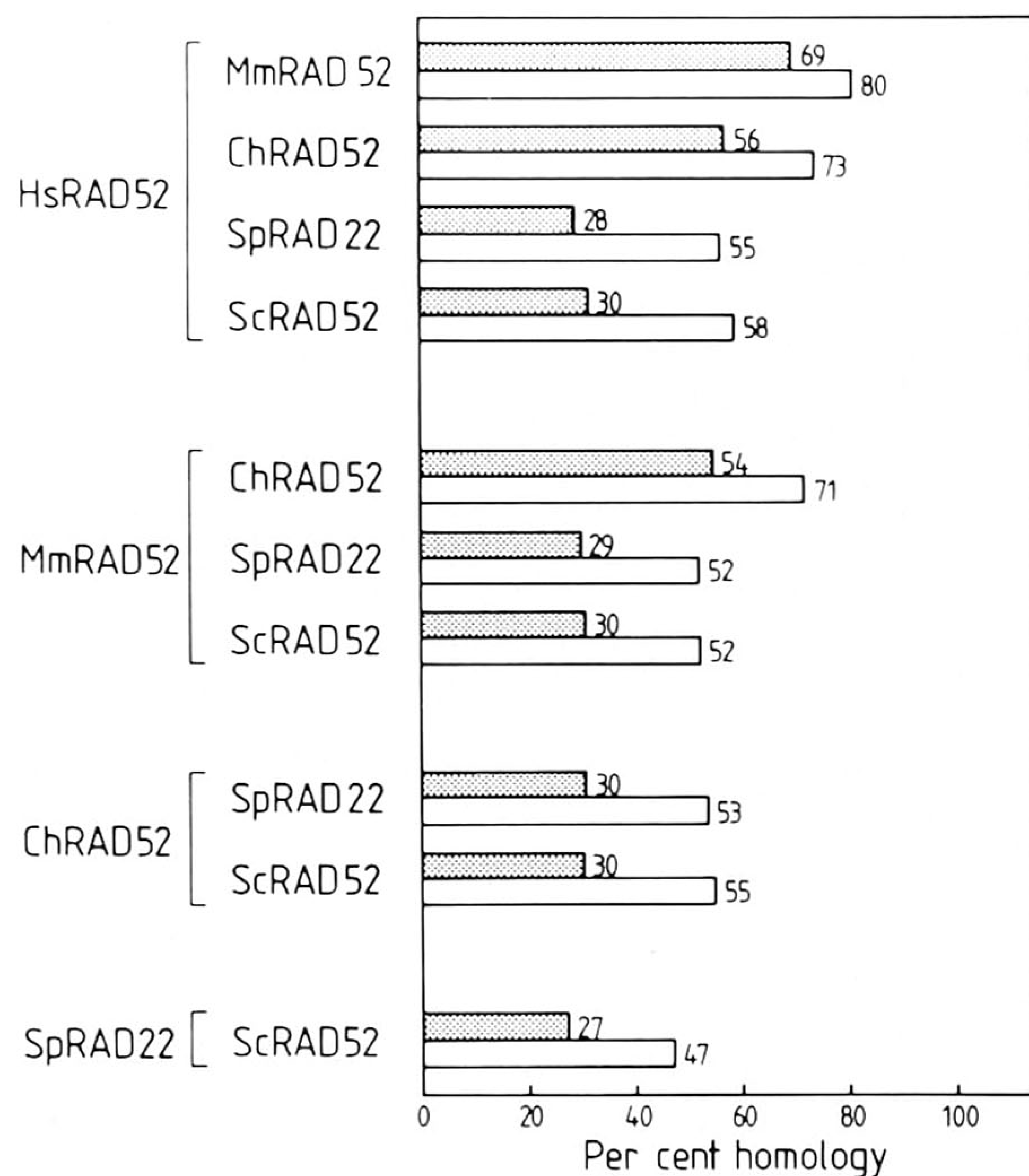

Fig. 2. Homology between pairs of RAD52 proteins. The extent of identity (dotted bars) or similarity (open bars) is given in percentages. The level of similarity includes identical and functionally conserved amino acid residues. The results are based on a pairwise comparison and may slightly deviate from the alignment of the five proteins shown in Fig. 1.

extensive in the N-terminal end (see Fig. 1), the use of a downstream ATG as a start codon is very unlikely. A potential polyadenylation signal could not be recognized and a poly A tail is not present at the end of the insert.

Sequence comparison

The alignment of amino acid sequences of *S. cerevisiae* RAD52 (Adzuma et al., 1984), *S. pombe* rad22 (Ostermann et al., 1993), chicken RAD52 (Bezzubova et al., 1993b), mouse RAD52 and human RAD52 proteins is shown in Fig. 1. The results of pairwise comparison of the various proteins are summarized in Fig. 2. The human RAD52 protein strongly resembles its counterparts from mouse and chicken. The identity with the mouse homolog is 69% and the overall similarity is 80%. With the chicken RAD52 protein the human protein displays 56% amino acid sequence identity and 73% similarity. The homology between *S. cerevisiae* RAD52 and *S. pombe* rad22 is primarily concentrated in the N-terminal region of both proteins (Ostermann et al., 1993). The region of homology between the yeast proteins and their counterparts from higher eukaryotes is also concentrated in the N-terminal part (see Fig. 1). The stretches of conserved amino acid residues within the N-terminal part of RAD52 proteins suggest functional importance of this region.

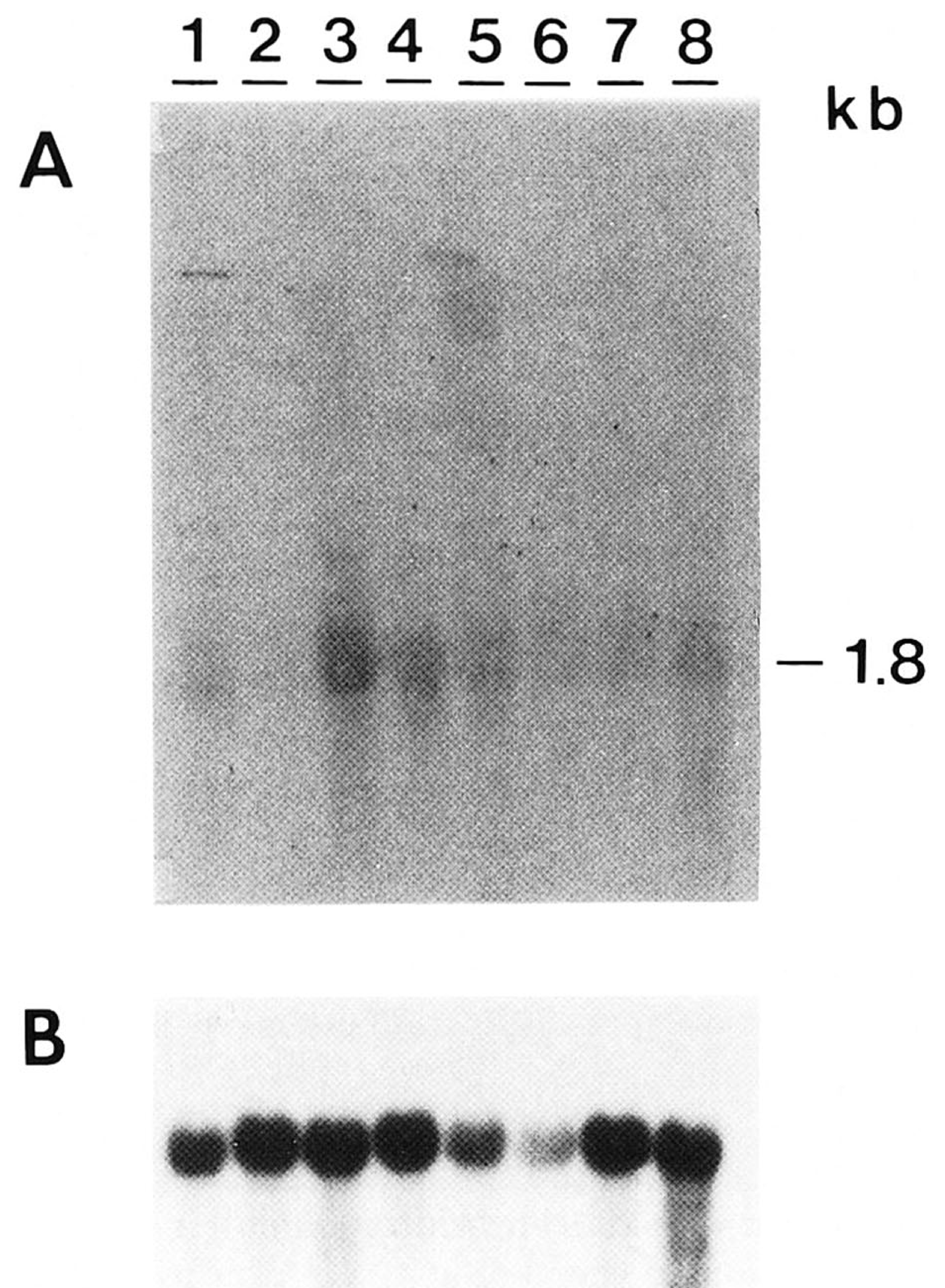

Fig. 3. Northern blot analysis of *RAD52* expression in different adult tissues of the mouse. Lane 1: lung; lane 2: brain; lane 3: thymus; lane 4: testis; lane 5: kidney; lane 6: heart; lane 7: liver; lane 8: spleen. (A) The filter was hybridized with the insert of the mouse *RAD52* cDNA clone. (B) As a control for the amount of RNA loaded, the filter was rehybridized with a HEF1 cDNA probe, encoding the human elongation factor 1 (Brands et al., 1986).

Expression of RAD52 in mouse tissues

The expression of the mouse *RAD52* gene in different tissues from adult mice was studied by Northern blot hybridization (Fig. 3). The cDNA insert hybridizes to a RNA species of about 1.8 kb, which is in agreement with the size of the cDNA insert. The amount of RNA loaded in each lane (30 μg) and the exposure time (2 weeks) indicate that the *RAD52* gene is expressed at a very low level. The relatively highest expression levels were observed in thymus and testis.

In situ hybridization

To determine the chromosomal location of the human and mouse *RAD52* genes, in situ hybridization was carried out using biotinylated probes. In case of the human gene a distinctive hybridization signal was observed on the telomeric end of the short arm of chromosome 12, as detected by the morphology of the chromosome. To confirm the identity of this chromosome a specific centromeric probe was used (Fig. 4). Based on the known banding pattern of chromo-

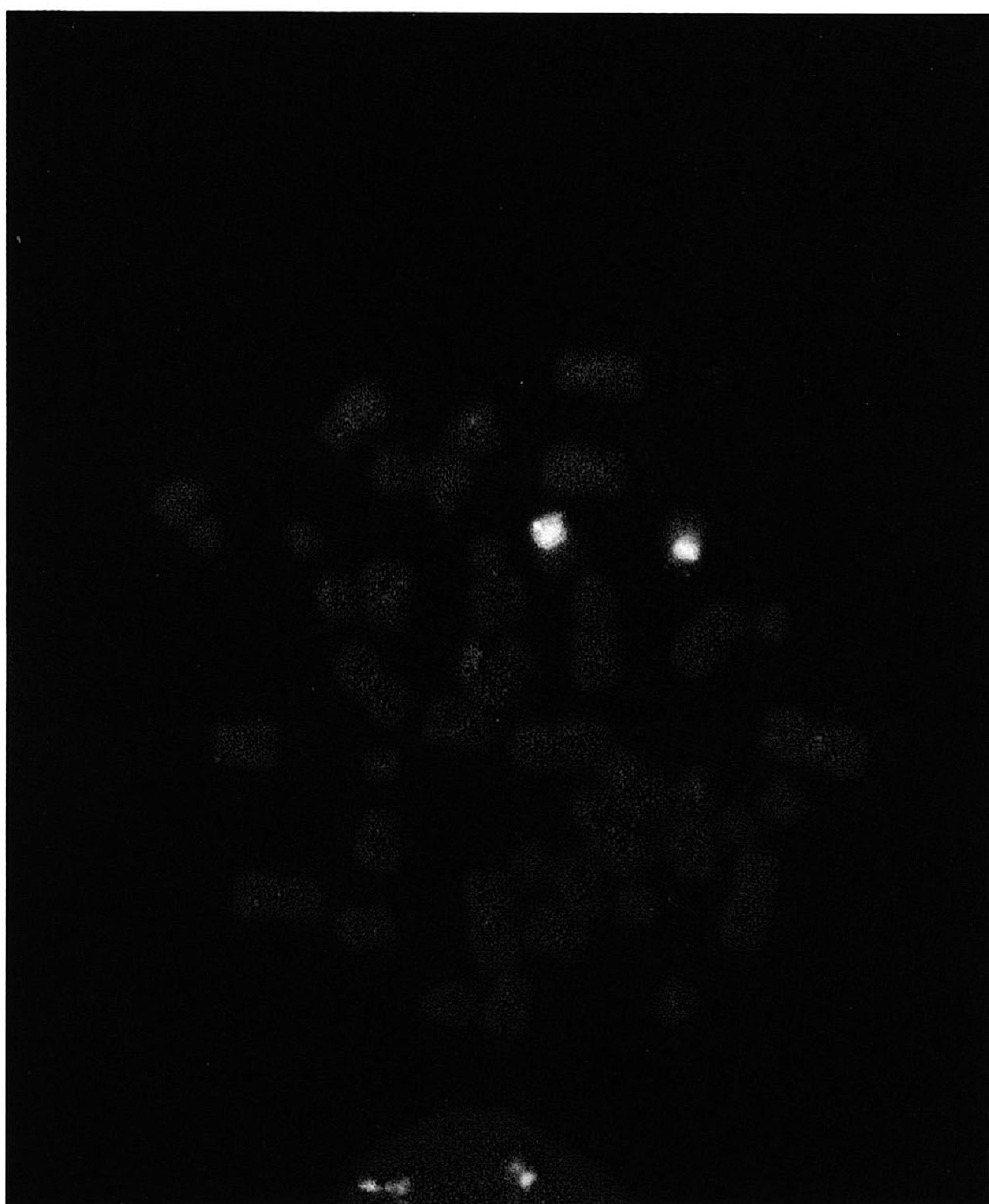

Fig. 4. Localization of the human *RAD52* gene by in situ hybridization. Metaphase spreads of human chromosomes were hybridized with a biotinylated *RAD52* cDNA and a digoxigenin labeled chromosome 12 specific centromeric probe. Chromosome spreads were counterstained with DAPI. The biotinylated probe gives rise to a green signal and the digoxigenin labeled probe to a red signal.

some 12, the *RAD52* gene is localized to band p13.3. The mouse *RAD52* gene is located on the tip of chromosome 6, region G3. The identity of this chromosome was confirmed by hybridization with pRAF1 (Kolch et al., 1991), a chromosome 6 specific probe (results not shown).

4. Discussion

The isolation of human and mouse *RAD52* cDNA clones using degenerate primers based on conserved amino acid sequences of *S. cerevisiae* RAD52 and *S. pombe* rad22 is presented here. The human and mouse clones specify highly homologous proteins (69% identity and 80% similarity). The mammalian proteins also strongly resemble the chicken RAD52 protein. The homology of the mammalian RAD52 proteins with their counterparts from *S. cerevisiae* and *S. pombe* is somewhat less. The human protein displays 58% similarity (30% identity) with the *S. cerevisiae* RAD52 protein and 55% similarity (28% identity) with the *S. pombe* rad22 protein. The level of conservation with the *S. cerevisiae* and *S. pombe* proteins is about the same as that between the two yeasts (27% identity, 47% similarity). The homology between the *S. cerevisiae* RAD52 protein and its homolog from the more closely related yeast *Kluyveromyces lactis* is more extensive (49% identity) (Milne and Weaver, 1993).

The conservation of RAD51 homologs in evolution is much stronger. The human and mouse RAD51 proteins are almost identical and are very similar (83%) to their counterparts from *S. cerevisiae* and *S. pombe* (Shinohara et al., 1993; Morita et al., 1993). The two yeast proteins display 69% identity and 83% similarity (Muris et al., 1993). Phylogenetic studies based on 5S rRNA homologies suggest that *S. pombe* is evolutionarily as far removed from *S. cerevisiae* as from man (Huysmans et al., 1983). The conservation of RAD52 and RAD51 homologs is in agreement with this observation.

The highly conserved N-terminal region of RAD52 proteins suggests an important role in the function of this protein. This is supported by the analysis of two known *S. cerevisiae* mutant *rad52* alleles. These mutations are due to basepair changes at codons 64 and 90, respectively (Adzuma et al., 1984; Boundy-Mills and Livingston, 1993). Moreover, a *rad52* allele encoding only the N-terminal 65% of the protein still retains part of its repair activities (Boundy-Mills and Livingston, 1993). The C-terminal one-third of the RAD52 protein, which is required for the physical interaction with RAD51 (Milne and Weaver, 1993), is less well conserved.

The mouse *RAD52* gene is expressed in all tissues analyzed, although the level is very low (Fig. 3). The relatively highest expression was observed in thymus and testis material. These results suggest the participation of the *RAD52* gene product in meiotic recombination during germ cell development and in immunoglobulin rearrangement in lymphoid tissues. Similar observations have been made in case of the chicken *RAD51* and *RAD52* genes and for the mouse *RAD51* gene (Bezzubova et al., 1993a,b; Shinohara et al., 1993; Morita et al., 1993). The expression pattern of these genes in higher eukaryotes is compatible with the role of the *RAD52* epistasis group genes in mitotic and meiotic recombination in yeast.

The human *RAD52* gene is localized on chromosome 12p13.3. The mouse counterpart was assigned to region G3 of chromosome 6, which shows homology with the short arm of human chromosome 12 (Searle et al., 1989). The chromosomal location excludes the possibility that the human *RAD52* gene is identical with *XRCC4*, *XRCC5* or the *scid* gene, which are located on chromosomes 5, 2 and 8, respectively (Giaccia et al., 1990; Chen et al., 1992; Jeggo et al., 1992; Itoh et al., 1993; Komatsu et al., 1993; Kirchgessner et al., 1993; Kurimasa et al., 1994). Therefore, the *RAD52* gene may be considered another mammalian gene possibly involved in DSB repair.

The analysis of genes involved in the nucleotide excision repair pathway has shown a strong resemblance between genes from yeast and man, suggesting a functional conservation of this mode of DNA repair (Hoeijmakers, 1993a,b). The isolation and characterization of mammalian homologs of yeast genes involved in recombina-

tional repair indicate that this repair pathway is also functionally conserved in evolution.

Acknowledgements

This work was supported by the J.A. Cohen Institute, Interuniversity Research Institute for Radiopathology and Radiation Protection (IRS; project 4.4.12) and by the Medical Genetics Centre (project MGC2). We wish to thank C. van Sluis, A. van Gool and P. van der Spek for their assistance in the alignment of the amino acid sequences and M. Zdzienicka, R. Jessberger and S. Takeda for critical reading of the manuscript. The Basel Institute for Immunology was founded and is supported by F. Hoffmann-LaRoche & Co. Ltd. The mouse *RAD52* gene has also been isolated recently by C. Bendixen and R. Rothstein. We would like to thank them for sharing unpublished data with us.

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