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# Sequence, chromosomal location and expression analysis of the murine homologue of human *RAD51L2/RAD51C*

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**Abstract**

The Rad51 protein has been shown to play a vital role in the DNA repair process. In humans, its interaction with proteins like BRCA1 and BRCA2 has provided an insight into the mechanism of how these molecules function as tumor suppressors. Several members of the Rad51-like family have been recently identified, including *RAD51L2*. This gene has been found to be amplified in breast tumors suggesting its role in tumor progression. Here, we describe the cloning of the murine homologue of the human *RAD51L2/RAD51C* gene. Sequence analysis has revealed that the murine Rad51l2 protein is 86% identical and 93% similar to its human homologue. In spite of such high sequence conservation, the murine protein lacks the first nine amino acids present in the human protein. We have cloned and confirmed the sequence of the 5′ end of the murine *Rad51l2* cDNA using 5′ RACE technique as well as by sequencing the genomic region flanking the first exon of the murine *Rad51l2* gene. Northern analysis shows that *Rad51l2* is expressed in several adult tissues as well as in embryos at various developmental stages. The murine *Rad51l2* gene maps to chromosome 11 and is located in the syntenic region of human chromosome 17q22-23, where the human *RAD51L2* is present. Published by Elsevier Science B.V.

*Keywords*: TestCode; Genscan; 5′ RACE; DNA repair; Bacterial artificial chromosome (BAC)

## 1. Introduction

A potential biological role of the human breast cancer susceptibility genes, *BRCA1* and *BRCA2* in the maintenance of genomic integrity was demonstrated based on their interaction with the Rad51 protein (Scully et al., 1997; Sharan et al., 1997). Mammalian Rad51 is a homologue of the yeast Rad51 and bacterial RecA proteins (Shinohara et al., 1992, 1993). All these proteins are known to play a key role in the recombinational repair process, including double strand break repair and mitotic as well as meiotic recombination (Raddling, 1991; Shinohara et al., 1992, 1993; Bishop, 1994; Rockmille et al., 1995; Sung and Robberson, 1995; Malkova et al., 1996; Lim and Hasty, 1996; Baumann et al., 1996). Mouse embryos lacking Rad51 die soon after implantation (Lim and Hasty, 1996; Tsuzuki et al., 1996). Rad51 deficient cells show chromosomal loss, decreased cell proliferation and increased radiation sensitivity (Lim and Hasty, 1996). It has been hypothesized that the early embryonic lethality of mice lacking Brca1 or Brca2 proteins may be due to defects in the Rad51-mediated DNA repair process (Scully et al., 1997; Sharan et al., 1997).

In spite of its critical role in mammalian cells, in yeast Rad51 is not essential for viability (Shinohara et al., 1992). This may reflect a functional divergence. Alternatively, this may suggest that it may have evolved to perform other functions in higher eukaryotes. The yeast *RAD51* is a member of the *RAD52* epistasis group that includes *RAD52, RAD54, RAD55* and *RAD57* (reviewed by Game, 1983). Proteins encoded by each of these genes are known to have a distinct function in the homologous recombination process and may form a multimeric-complex on the damaged DNA during the repair process. In mammals, in

Abbreviations: BAC, bacterial artificial chromosome; bp, base pair(s); *BRCA1*, breast cancer susceptibility gene1; *BRCA2,* breast cancer susceptibility gene2; cDNA, DNA complementary to RNA; EST, expressed sequence tag; kb, kilobase(s) 1000 bp; MMS, methylmethansulfonate; PCR, polymerase chain reaction; RACE, rapid amplification of cDNA ends; RT-PCR, reverse transcription – polymerase chain reaction; SDS, sodium dodecyl sulfate; SSC, 0.15 M NaCl/0.015 M Na3·citrate pH 7.6; UTR, untranslated region(s)

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addition to Rad51, six other Rad51-like proteins have been identified (reviewed by Thacker, 1999; Thompson and Schild, 1999). These include DMC1, XRCC2, XRCC3, RAD51L1 (previously named RAD51B or REC2), RAD51L2 (previously named RAD51C), and RAD51L3 (previously named RAD51D). Dmc1 is a meiosis-specific protein that is not essential for viability (Pittman et al., 1998; Yoshida et al., 1998). However, *Dmc1* mutant mice are infertile and show arrest of germ cells at the prophase stage of meiosis I. *Xrcc2* and *Xrcc3* were identified from hamster cell lines based on their increased radiation sensitivity (Fuller and Painter, 1988; Johnson et al., 1999; Pierce et al., 1999). The human and murine homologues of *Rad51l1* and *Rad51l3* have been cloned and mutations in mice have been shown to result in embryonic lethality (Shu et al., 1999; Pittman and Schimenti, 2000). *Rad51l3* deficient cells show defects in cell proliferation but do not show increased sensitivity to methylmethansulfonate (MMS) and γ-radiation suggesting that it may not play a vital role in the double-strand-break repair process (Pittman and Schimenti, 2000).

The human RAD51L2 was identified from the EST database, based on sequence homology to human *XRCC3* and *RAD51* genes (Dosanjh et al., 1998). The RAD51L2 protein has been shown by yeast two-hybrid analysis to directly interact with XRCC2 and RAD51L1 (Dosanjh et al., 1998). It has also been shown to enhance the interaction between Rad51l1 and Rad51l3 proteins (Schild et al., 2000). In recent studies, *RAD51L2* has been found to be amplified in primary breast tumors and cell lines which suggests that it could play a role in breast cancer development and progression (Barlund et al., 2000; Wu et al., 2000). The murine homologues of all the members of the *Rad51-like* family have been cloned with the exception of murine *Rad51l2*. We describe here the cloning of the murine *Rad51l2* gene. The murine cDNA encodes a protein that is 86% identical to its human homologue. We have also determined the chromosomal location of the gene, studied its expression pattern in embryos and multiple adult tissues.

## 2. Materials and methods

### 2.1. Cloning of human RAD51L2 cDNA

A 464 bp fragment of the human *RAD51L2* cDNA was amplified from the total RNA extracted from a human ovarian cell line using RT-PCR. The RT-PCR reaction was performed using Superscript Preamplification system (Life-Technologies) as per the protocol provided by the manufacturers. The PCR reaction was performed using primers sks-74 (5′-TTGGCAGTAGATGTGCAGAT-3′) and sks-75 (5′-CTATCAATCTTTGTTGTCAT-3′). The PCR product was cloned into pGEM T-vector (Promega) and sequenced to confirm that it was human *RAD51L2* cDNA.

### 2.2. Screening murine cDNA library

The human *RAD51L2* probe was used to screen a 12.5 day mouse cDNA library (Stratagene). The *RAD51L2* cDNA fragment was $^{32}$P-labeled using the T7 QuickPrime kit (Amersham Pharmacia) and hybridized in 6× SSC and 0.25% non-fat milk at 50°C. The library filters were washed with 0.5× SSC and 0.1% SDS at 50°C and exposed to X-ray film for 16 h. Two positive clones, cDNA1.1 and cDNA1.2, were identified and were further analyzed.

### 2.3. cDNA Sequencing

The sequence from the ends of the two cDNA clones was obtained by using T7 and T3 primers and BigDye Terminator Reagent mix (PE Applied Biosystems). Based on the sequence obtained, the two cDNAs represented the murine homologue of human *RAD51L2* cDNA. Complete cDNA sequence was obtained by undertaking a step-wise sequencing approach from the two ends of the two cDNAs, using primers specific to the murine *Rad51l2* cDNA sequence.

### 2.4. 5′ RACE to identify 5′ end of RAD51L2 cDNA

The 5′ end of the murine *Rad51l2* cDNA was obtained by using the 5′ RACE (Rapid Amplification of cDNA Ends) System (Life Technologies). Total RNA from an adult testis was used to synthesize the cDNA. The 5′ end of *Rad51l2* was amplified using a reverse primer, csl-8 (5′-GCACATC-TACTGCCAATTGC-3′) and subsequently re-amplified using nested reverse primer, csl-7 (5′-CACCA-CAAACTTCTGTCGTC-3′) and a forward primer specific to 5′adapter sequence. The PCR products were sub-cloned into the pGEM T-vector (Promega) and electroporated into the DH10B cells (Life Technologies). The transformed colonies were hybridized with a $^{32}$P- labeled *Rad51l2* cDNA probe. The positive clones were sequenced using T7 and SP6 primers.

### 2.5. Screening BAC library and sequencing the 5′ end of the gene

Filters containing mouse (129S6/SvEvTac) genomic library, RPCI-22, in BAC vector pBACe3.6 were obtained from Roswell Park Cancer Institute. The filters were hybridized with $^{32}$P-labeled insert of *Rad51l2* cDNA1.1 in Church buffer (Church, 1984) at 65°C for 16 h. The filters were washed with 0.2× SSC and 0.1% SDS solution at 65°C and exposed to X-ray film. Five positive clones were identified. One of the BAC clones, pBACRad51l2-514 was used for further characterization and subcloning of a *Rad51l2* genomic fragment. The BAC DNA was digested with *Eco*RI restriction enzyme and separated on a 0.8% agarose gel. The DNA was transferred to Hybond $N^+$ membrane (Amersham Pharmacia) and hybridized with the *Rad51l2* cDNA1.1. Three positive fragments, 20, 8, and 3 kb in size were detected. Further, hybridization of

the blot with multiple $^{32}P$ -end labeled 20-mer oligonucleotides showed that the 20 kb fragments contained most of the coding region of the cDNA. This 20 kb *Eco*RI fragment was subsequently subcloned into pBluescript SK + plasmid (Stratagene). Using murine *Rad51l2* specific primers, the sequence around the first exon was generated and analyzed using Genscan (Burge and Karlin, 1997) and TestCode (Fricket, 1982) programs.

### 2.6. Interspecific mouse backcross mapping

Interspecific backcross progeny were generated by mating (C57BL/6J × *M. spretus*) $F_1$ females and C57BL/6J males as described (Copeland and Jenkins, 1991). A total of 205 $N_2$ mice were used to map the *Rad51l2* locus (see text for details). DNA isolation, restriction enzyme digestion, agarose gel electrophoresis, Southern blot transfer and hybridization were performed essentially as described (Jenkins et al., 1982). All blots were prepared with Hybond – $N^+$ nylon membrane (Amersham). The probe, a 510 bp (nucleotides 1371–1880 of AF324883) fragment from the 3′ UTR of *Rad51l2* cDNA, was labeled with [$\alpha^{32}$P] dCTP using a random primed labeling kit (Stratagene); washing was done to a final stringency of 0.1 × SSCP, 0.1% SDS, 65°C. A major fragment of 3.4 kb was detected in *Xba*I digested C57BL/6J DNA and a major fragment of 3.2 kb was detected in *Xba*I digested *M. spretus* DNA. The presence or absence of the 3.2 kb *Xba*I *M. spretus*-specific fragment was followed in backcross mice.

A description of the probes and RFLPs for most loci linked to *Rad51l2* including *Scya11, Mpo* and *Nog* has been reported previously (Valenzuela et al., 1995; Gonzalo et al., 1996; Fujii et al., 1998). One locus, *Rad51l3* has not been reported previously for our interspecific backcross. The probe, a 450 bp fragment (nucleotides 1191–1640 of AF034955) from the 3′UTR of *Rad51l3* cDNA, detected *Hin*dIII fragments of 12.0 kb (C57BL/6J) and 2.7 kb (*M. spretus*). The 2.7 kb *Hin*dIII *M. spretus*-specific fragment was followed in backcross mice. Recombination distances were calculated using Map Manager, version 2.6.5. Gene order was determined by minimizing the number of recombination events required to explain the allele distribution patterns.

### 2.7. Northern analysis

Multiple tissue Northern blots containing polyA$^+$ RNA from various tissues of mice as well as embryos at different days of gestation were obtained from Clontech. The blots were hybridized with a $^{32}P$- labeled 443 bp fragment between the nucleotides 469–912 of the murine *Rad51l2* cDNA in 4 × SSCP, 1 × Denhardt's solution, 1% SDS, 100 μg/ml of sheared salmon sperm DNA at 65°C. for 16 h. The blots were washed with 0.2 × SSCP and 0.1% SDS solution at 65°C and exposed to X-ray film for 3 days. The blots were stripped in 0.5% SDS solution and then hybridized as described above with *β-actin* probe as a loading control.

## 3. Results

### 3.1. Human and murine Rad51l2

Sequence analysis of the murine *Rad51l2* cDNAs and its comparison with the sequence of its human homologue revealed that the first fourteen amino acids were not included in the cDNA clone 1.1 (Fig. 1). The cDNA library was rescreened using the murine cDNA as a probe but no new clones were identified. In order to obtain the sequence of the cDNA encoding the first fourteen amino acids of the murine protein, a 5′ Rapid amplification of cDNA ends (5′ RACE) technique was used. The sequence revealed the murine protein lacks the first nine amino acids present in the human protein (Figs. 1 and 2A). The tenth amino acid in the human sequence is methionine, which appears to be the initiating methionine of the murine protein. The apparent initiation codon of the murine cDNA does not contain the consensus translation initiation sequence (Kozak, 1986) as the ATG is preceded by a polyA sequence. In order to determine if this is the authentic 5′ end of the cDNA, we sequenced the genomic region around exon 1 of the murine *Rad51l2* gene. The sequence obtained from the 5′ RACE clone is identical to the sequence of the genomic region suggesting that the RACE sequence represents the true 5′ end of *Rad51l2* and is not an artifact of the procedure (Fig. 2). However, the genomic region containing the 5′ end of the cDNA has 639-bases of open reading frame including two in-frame ATG codons upstream of the putative initiating codon of *Rad51l2* (Fig. 2). However, this region does not appear to be a part of the *Rad51l2* transcript, as it was not identified by the 5′ RACE technique. In addition, it has not been found in any of the cDNA clones that were obtained from two additional cDNA libraries. These *Rad51l2* cDNA were amplified by PCR from the cDNA library using one primer specific to the *Rad51l2* transcript and another primer specific to cDNA cloning vector. The PCR products were sub-cloned and sequenced. Several PCR clones contained the region identified by 5′ RACE but none contained the upstream region containing the two in-frame ATG codons (data not shown). This upstream sequence has not been found in any of the EST clones present in the murine EST database (Fig. 1). However, we have identified an EST clone (GenBank Accession number BB591625) that contains the sequence identified by 5′ RACE. None of the amino acids from the upstream region are conserved in the human sequence.

We next examined this sequence using the Genscan program, which has been designed to identify putative protein coding sequences (Burge and Karlin, 1997). Surprisingly, in spite of the fact that the sequence contains a 639 bases long open reading frame (Fig. 2A), the first 351 bases were found to be non-coding and the ATG sequence identified from 5′ RACE technique is recognized as the translation start site. To determine if the first 351 bases of the open reading frame were not identified as coding sequences because of the composition of the sequence, we analyzed

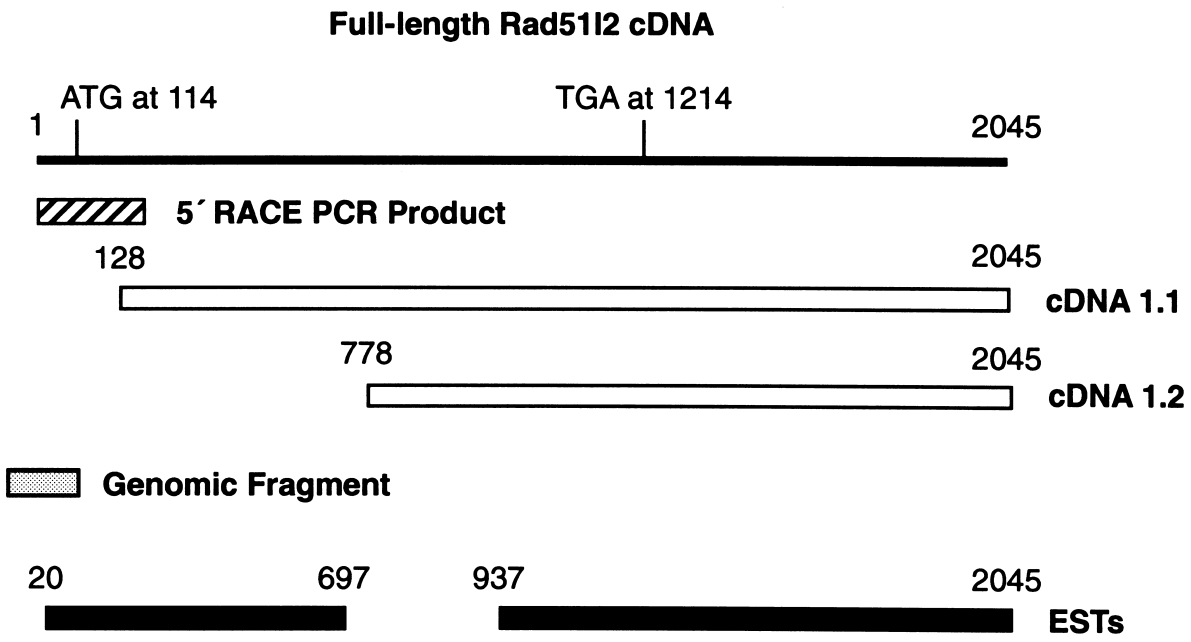


Fig. 1. Cloning of full-length murine *Rad51l2* cDNA. The sequence of the 2045 bases of the full-length cDNA was obtained from two cDNA clones, 1.1 and 1.2 (open bar) and the 5′RACE product (hatched bar). The sequence of the cDNA was confirmed with the sequence of the genomic locus (shaded bar) and fifteen EST sequences (solid bar) obtained from murine EST database. The *Rad51l2* EST containing sequences 20–697 corresponds to BE533505 and BB591625 while the region between 937–2045 is represented by 13 ESTs: AI464887, BE5322496, AI662180, AA267603, BB304345, BB241472, BB104381, BB234087, BB062709, AV253651, BB012221, BB277448, AV307754. Numbers at each end of the bars represent the sequence that corresponds to the full-length *Rad51l2*cDNA sequence (AF324883).

the sequence using TestCode program (Fricket, 1982). The TestCode program has been designed to help identify genes in the absence of the knowledge of codon preferences for the sequence being examined. It is based on the period three compositional constraints found in regions known to be coding and non-coding. Results of the TestCode analysis are shown as a plot that is divided into three regions based on the level of confidence that a given sequence is coding or non-coding. The TestCode result shows that the 5′-end sequence obtained from the RACE technique is a coding sequence with 95 percent level of confidence (Fig. 2B). In contrast, the upstream open reading frame identified from the genomic sequence is a non-coding sequence with either the same level of confidence or it falls within the 'window of vulnerability' where statistics can make no significant prediction.

Based on these analyses, we conclude that the murine *Rad51l2* encodes a 2045-base transcript, which translates into a 366-amino acid protein (GenBank Accession number AF324883). The murine protein lacks the first nine amino acids present in its human homologue. In addition, there is a single one amino acid gap in the murine protein after amino acid 347 when the two proteins are aligned (Fig. 3). Overall, the murine and human proteins are 86% identical. Based on conserved amino acid substitutions, the Rad51l2 protein is 93% similar between the two species. The murine Rad51l2 protein contains the two nucleotide binding motifs identified in the human protein (Fig. 3).

The murine *Rad51l2* gene consists of eight exons. The location of the exon-intron boundaries is shown in Fig. 3. The murine transcript is about 2.0 kb, which is larger than the 1.3 kb size of the human transcript (Dosanjh et al., 1998). Although the lengths of the coding regions are very similar, the murine transcript contains an 831-base 3′ untranslated region (3′UTR) which is much larger than the 122 bases of human 3′UTR. The significance of this relatively large 3′ UTR in the murine transcript is unknown.

### 3.2. Genetic mapping of *Rad51l2*

The mouse chromosomal location of *Rad51l2* was determined by interspecific backcross analysis using progeny derived from matings of [(C57BL/6J × *Mus spretus)*$F_1$ X C57BL/6J] mice. This interspecific backcross mapping panel has been typed for over 3000 loci that are well distributed among all the autosomes as well as the X chromosome (Copeland and Jenkins, 1991). C57BL/6J and *M. spretus* DNAs were digested with several enzymes and analyzed by Southern blot hybridization for informative restriction fragment length polymorphisms (RFLPs) using a mouse *Rad51l2* cDNA probe. The 3.2 kb *Xba*I *M. spretus* RFLP (see Section 2) was used to follow the segregation of the *Rad51l2* locus in backcross mice. The mapping results indicated that *Rad51l2* is located in the central region of mouse chromosome 11 linked to *Scya11, Rad51l3, Mpo* and *Nog*. Although 61 mice were analyzed for every marker and are shown in the segregation analysis (Fig. 4), up to 134 mice were typed for some pairs of markers. Each locus was

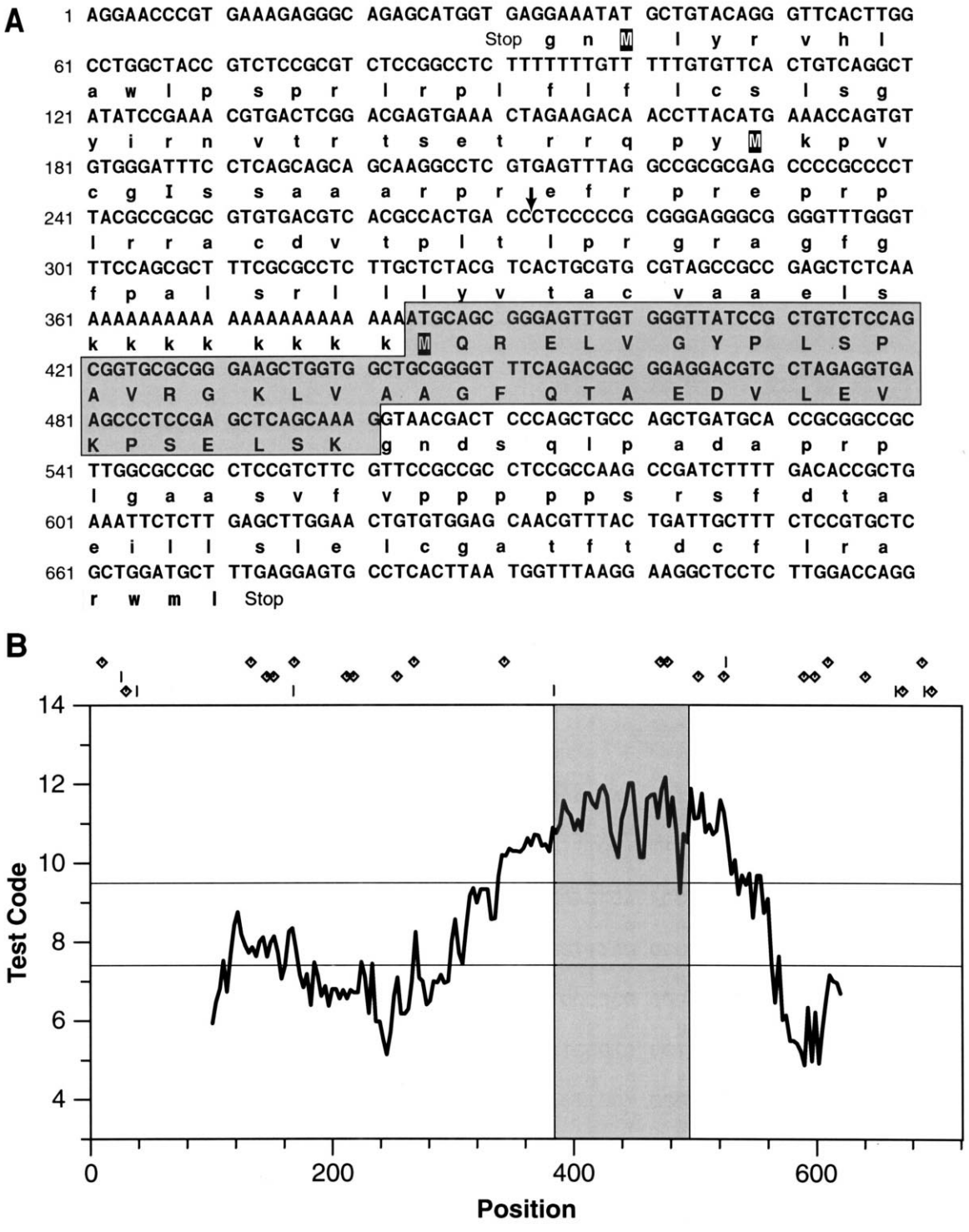


Fig. 2. Sequence of the genomic region near the 5′ end of the gene. (A) Nucleotide sequence of the 720 bases of genomic DNA and the amino-acid sequence of the 636-base open reading frame around exon 1 of the murine *Rad51l2* gene. Amino-acid sequence in enclosed in the shaded box represents the protein coding sequence present in the cDNA while the amino-acid sequence in lower-case is not included in the Rad51l2 protein. The arrow indicates the 5′ end of the RACE products. The three in-frame methionines present in the 639-base open reading frame are shown in enclosed box. (B) Results of TestCode analysis of the 720 bases of genomic sequence shown in A. The *x*-axis represents the sequence position and *y*-axis shows the corresponding TestCode score. Those scores that are above the two horizontal lines represent coding sequence with 95% level of confidence and those below are non-coding with same confidence level. No significant prediction can be made about the sequence that has a TestCode score between the two lines. The shaded region represents the sequence present in the coding region of the cDNA. In the plot, the markings above the curve show potential start sites (short vertical line) and stop codons (diamond) in the three reading frame.

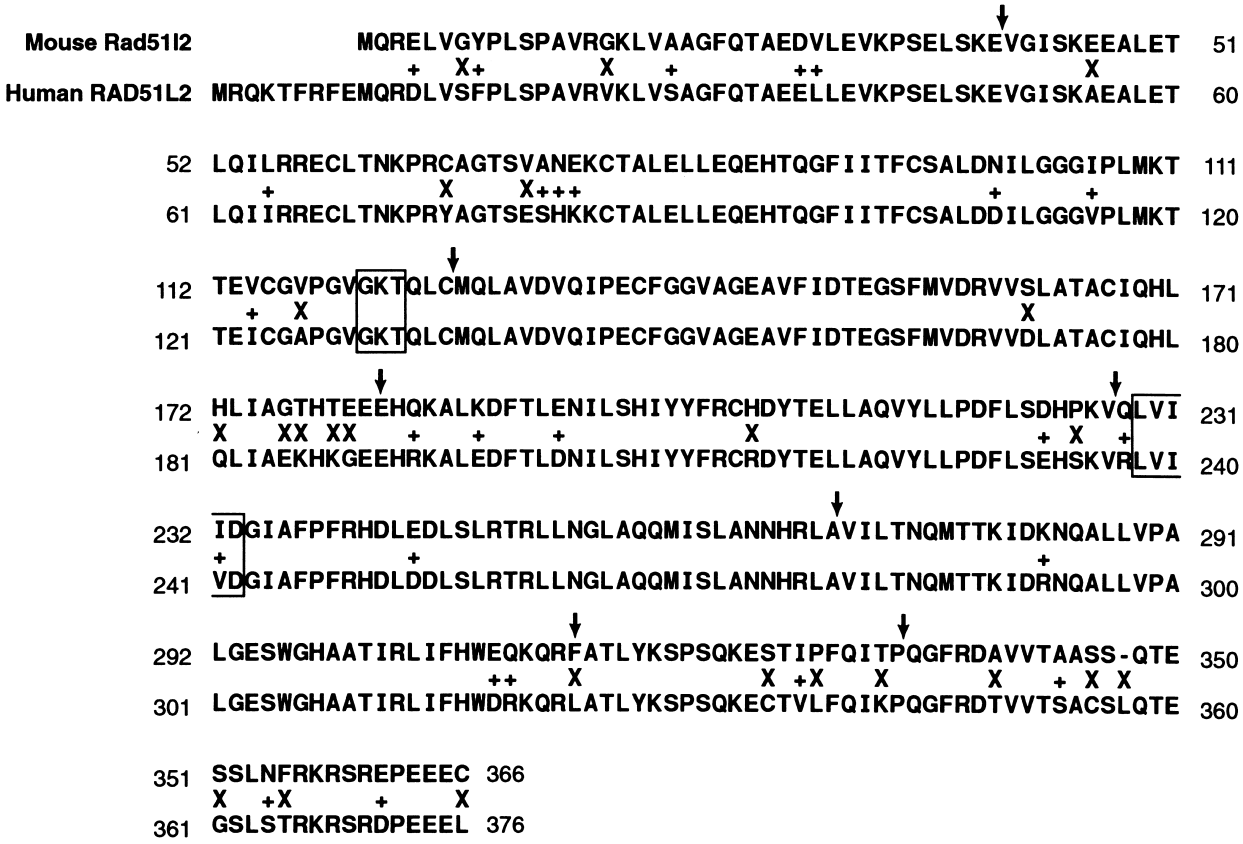


Fig. 3. Comparison of predicted amino acid sequences of the human and murine Rad51l2 proteins. The upper line represents the single-letter amino acid sequence of the murine protein and the lower represents the human protein sequence. A single gap (−) in the murine amino acid sequence between amino acids 347 and 348 has been introduced to achieve maximum alignment. ' + ' represents similar amino acids present at the given position in the two proteins and 'X' represents non-conserved amino acid change. The seven arrows indicate the boundaries of the eight exons present in the *Rad51l2* gene. The amino acids in the two boxes represent nucleotide binding site.

analyzed in pairwise combinations for recombination frequencies using the additional data. The ratios of the total number of mice exhibiting recombinant chromosomes to the total number of mice analyzed for each pair of loci and the most likely gene order are: centromere- *Scya11* – 1/118 – *Ral51l3* – 2/107 – *Rad51l2* – 0/101 – *Mpo* – 1/134 – *Nog*. The recombination frequencies [expressed as genetic distances in centiMorgans (cM) ± the standard error] are – *Scya11* – 0.9 ± 0.8 – *Rad51l3* – 1.9 ± 1.3 – [*Rad51l2, Mpo*] – 0.8 ± 0.7 – *Nog*. No recombinants were detected between *Mpo* and *Rad51l2* in 101 animals typed in common suggesting that the two loci are within 3.0 cM of each other (upper 95% confidence limit).

### 3.3. Expression analysis of Rad51l2

The expression of the *Rad51l2* transcript was examined in various tissues by Northern analysis. *Rad51l2* is expressed in the heart, brain, spleen, lung, liver, kidney and testis but was not detected in skeletal muscle (Fig. 5A). Relatively, more *Rad51l2* RNA was detected in testis when compared to other tissues. *Rad51l2* is expressed in embryos at various developmental stages (Fig. 5B). The expression was found to be higher between 11 and 15 days of gestation compared to day 7 or 17. The size of the transcript is 2.0 kb, which is the expected length based on the cDNA sequence. In every tissue where the 2.0 kb transcript is present, a very weak 3.0 kb transcript is also detected. At present, it is not known if the 3.0 kb transcript represents an alternatively spliced form of *Rad51l2* or is due to cross-hybridization to another member of the Rad51-like family.

## 4. Discussion

We have used the human *RAD51L2* cDNA as a probe to isolate its murine homologue. The murine *Rad51l2* gene encodes a 366 amino acid protein that is 86% identical to the human protein. Rad51l2 is 10 amino acids shorter than its human homologue, which includes the first nine amino acids and an additional amino acid near the carboxy-terminus. We have sequenced multiple cDNA clones and the 5′-end of the transcript. Results of the analysis of the genomic region using the Genscan and TestCode programs are consistent with the start site of the murine *Rad51l2* transcript. These evidences suggest that the murine Rad51l2

protein lacks the first nine amino acids present in the human RAD51L2. It is interesting to note that when we analyzed the human cDNA sequence using the Genscan program (data not shown), the first ATG sequence of the human cDNA sequence was not identified as the initiating codon. Instead, the second ATG (encoding the 10th amino acid of RAD51L2) of the human sequence was selected as the initiating codon. This suggests that the human protein may not contain the first nine amino acids that have been predicted based on the cDNA sequence.

We have mapped the *Rad51l2* gene to the region of mouse chromosome 11 that is syntenic with human chromosomes 17q (summarized in Fig. 4), consistent with the assignment of *RAD51L2* to 17q22-q23 (Human Genome database). We have compared our interspecific map of chromosome 11 with a composite mouse linkage map that reports the map location of many uncloned mouse mutations (provided from Mouse Genome Database, a computerized database maintained at The Jackson Laboratory, Bar Harbor, ME). *Rad51l2* maps in a region of the composite map that lacks mouse mutations with a phenotype that might be expected for an alteration in this locus (data now shown). In mice, *Rad51l2* is tightly linked (1.9 ± 1.3 cM) to another member of the *Rad51*-like family, *Rad51l3* (Fig. 4). However they are not present in tandem suggesting that they may not evolved as a result of gene duplication. This is also supported by the fact that the sequence of the two proteins is quite diverged (Thacker, 1999; Thompson and Schild, 1999). In humans, the two genes map on the same chromosome but are located in two different regions: *RAD51L2* maps to 17q22-23 while *RAD51L3* maps to 17q11 (Fig. 4).

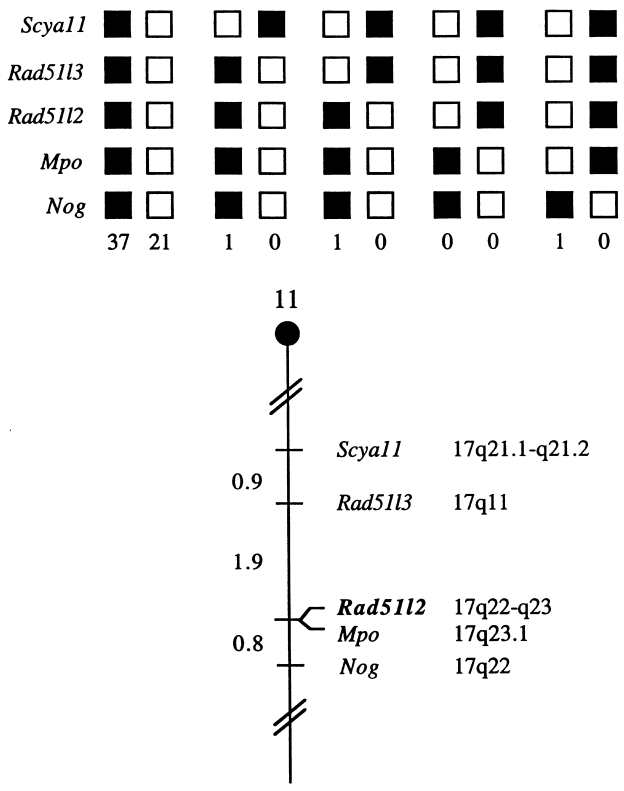


Fig. 4. *Rad51l2* maps in the central region of mouse chromosome 11. *Rad51l2* was placed on mouse chromosome 11 by interspecific backcross analysis. The segregation patterns of *Rad51l2* and flanking genes in 61 backcross animals that were typed for all loci are shown at the top of the figure. For individual pairs of loci, more than 61 animals were typed (see text). Each column represents the chromosome identified in the backcross progeny that was inherited from the (C57BL/6J × *M. spretus*) $F_1$ parent. The shaded boxes represent the presence of a C57BL/6J allele and white boxes represent the presence of a *M. spretus* allele. The number of offspring inheriting each type of chromosome is listed at the bottom of each column. A partial chromosome 11 linkage map showing the location of *Rad51l2* in relation to linked genes is shown at the bottom of the figure. Recombination distances between loci in centimorgans are shown to the left of the chromosome and the positions of loci in human chromosomes, where known, are shown to the right. References for the human map positions of loci cited in this study can be obtained from GDB (Genome Data Base), a computerized database of human linkage information is maintained by The William H. Welch Medical Library of The Johns Hopkins University (Baltimore, MD).

The expression of *Rad51l2* at various stages of embryonic development as determined by Northern analysis suggests that the gene may play an important role during embryogenesis. It is also expressed in various adult tissues except for skeletal muscle, where no expression was detected. The human gene has also been reported to have a similar wide spread expression pattern (Dosanjh et al., 1998). However, the human transcript is expressed in the skeletal muscle. In both mouse and human tissues, testis shows the highest expression of the transcript. The wide range of tissues expressing the *Rad51l2* gene suggests that it may play a vital function in mice as well as humans. A more detailed expression analysis using *in situ* hybridization technique will show the specific cell types that express this gene, which may provide clues to its biological role. The generation of mutation in *Rad51l2* will provide valuable information about its biological function. Mutations generated in other members of the *Rad51* family like *Rad51, Rad51l1* and *Rad51l3* have revealed their role in embryonic development and homologous DNA repair (Lim and Hasty, 1996; Shu et al., 1999; Pittman and Schimenti, 2000). Mutations in these genes result in early embryonic lethality suggesting that each of these genes play an important role in developmental processes and are not functionally redundant. The study of mice with a loss of function mutation in the *Rad51l2* gene will help us define the role of this gene as well as improve our understanding of the DNA repair process in mammalian cells. The functional significance of recent finding that *RAD51L2* is amplified and overexpressed in some primary tumors can be tested in transgenic mice (Barlund et al., 2000).

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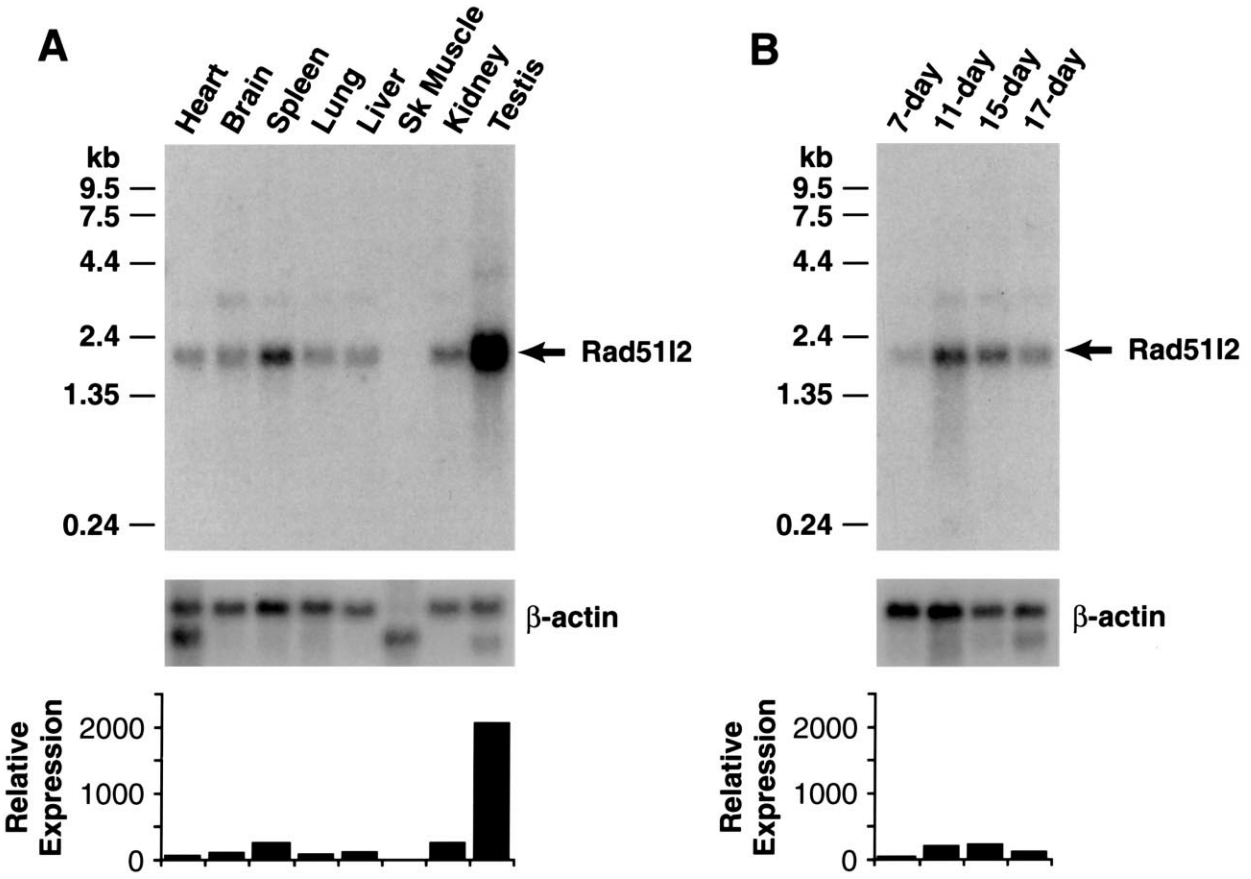


Fig. 5. Expression analysis of *Rad51l2*. (A) Results of Northern analysis using a blot containing polyA RNA from multiple tissues, hybridized with a 443 bp *Rad51l2* cDNA probe. A 2.0 kb transcript is detected in all tissues except skeletal muscle. The middle panel shows the result of the re-hybridization of the same blot with β-*actin* probe to show the relative amount of RNA present in each lane. The lower panel is a graph showing the relative expression level of *Rad51l2* transcript in various tissues after normalizing for loading differences using β-*actin* as control. (B) Northern blot containing polyA RNA from embryos at various stages of development shows expression of *Rad51l2* transcript during embryogenesis. To show the relative amount of RNA in each lane, the same blot was re-hybridized with the β-*actin* probe (middle panel). The relative levels of the *Rad51l2* transcript at various stages in shown in the graph (lower panel) after controlling for loading differences.

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