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The mouse and human homologs of *DMC1*, the yeast meiosis-specific homologous recombination gene, have a common unique form of exon-skipped transcript in meiosis

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ABSTRACT

Genetic recombination in meiosis plays an important role in generating diversity of genetic information. In yeast an *Escherichia coli RecA*-like gene, *DMC1*, is expressed in meiotic prophase and its product co-localizes with Rad51 protein on zygotene chromosomes. We have cloned the mouse and human homologs of the yeast *DMC1* gene. The predicted human and mouse DMC1 proteins showed 54.1% sequence identity with yeast Dmc1 protein. The domain II region, highly conserved in the *E.coli* RecA-like protein family, was also found in the mammalian DMC1 proteins, including the two ATP binding motifs and DNA binding sites within the region. *In situ* hybridization analysis revealed expression of the mouse *Dmc1* gene in testicular germ cells in meiosis; RT–PCR showed expression in embryonal ovaries. These findings suggest that DMC1 plays an important role in meiotic homologous recombination. From both the man and mouse we have isolated an alternative spliced form of *Dmc1* cDNA (*Dmc1-d*), which is deleted for a region between the two motifs involved in nucleotide binding. Since the alternatively spliced *Dmc1-d* transcript was detected in both male and female germ cells, the encoded protein DMC1-D may have a novel role in mammalian genetic recombination in meiosis.

INTRODUCTION

Much of the study of genetic recombination at the molecular level has been carried out with prokaryotes and lower eukaryotes. In *Escherichia coli* RecA protein is known to polymerize on DNA and form helical filaments in order to catalyze the central steps of DNA recombination, homologous pairing and strand exchange of DNA in the presence of adenosine triphosphate (ATP) (1–5). In *Saccharomyces cerevisiae* two classes of genes are required for meiotic recombination. One class includes the *RAD52* (*RAD50–57*) epistasis group of genes (6–8) involved in both mitotic DNA repair and meiotic recombination through interaction with double-strand breaks (DSBs) in DNA. Among them, Rad51 protein of yeast is structurally and functionally similar to RecA protein of *E.coli*. (9–12). The genes belonging to the other class are *HOP1* (13), *SPO11* (14), *RED1* (15), *REC102* (16), *ZIP1* (17) and *Dmc1* (18), which are essential only for meiotic recombination.

DMC1 (disrupted meiotic cDNA) is a gene which plays a role specifically in meiotic recombination in yeast (18). Yeast *dmc1* mutants fail in reciprocal recombination and accumulate DSB recombination intermediates. They are incapable of forming normal synaptonemal complex, thus causing an arrest of late meiotic prophase at a meiosis-specific 'cell cycle checkpoint' that monitors various defects of chromosome metabolism. The Dmc1 protein is structurally and evolutionally related to the gene products of yeast *RAD51* and *E.coli RecA*.

In yeast, Dmc1 and Rad51 proteins have been shown to co-localize within spread meiotic nuclei (19), suggesting their co-operative function in meiotic recombination. However, Dmc1 and Rad51 exhibit different expression patterns during meiosis, even though they are structurally similar to each other. Furthermore, Rad51 complex formation is required for Dmc1 association, presumably to promote recombination events, whereas Rad51 forms complexes in meiotic nuclei independently of Dmc1. Thus Dmc1 and Rad51 apparently have different functions in meiosis, but they may function to initiate recombination, e.g. in early recombination nodules (RNs), some of which might become the late RNs and chiasmata (20).

In higher eukaryotes *RAD51* homologs have been isolated from the chicken (21), human (22) and mouse (23,24). They encode

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proteins sharing >80% similarity to the yeast Rad51 protein and ~50% similarity to *E.coli* RecA protein. The mouse RAD51 homolog partially suppresses sensitivity of the yeast *rad51-1* mutation to DNA damaging agents (23). The purified human RAD51 proteins bind and form filaments with single-stranded or duplex DNA using DNA-dependent ATPase activity (25). Since RAD51 proteins are expressed in spermatocytes in the mouse testis and in lily anther cells in meiosis they are thought to be involved in meiotic homologous recombination.

A eukaryotic *DMC1* homolog has been cloned from the plant *Lilium longflorium* as a meiosis-specific cDNA (*LIM15*) (26). The predicted amino acid sequence showed similarity with yeast Dmc1, Rad51, Rad55 (27) and Rad57 proteins (28). LIM15 protein co-localizes with lily RAD51 protein on zygotene chromosomes of the lily in meiosis (29). In mammals the homolog of the yeast *DMC1* gene has not been identified and its structure and role in genetic homologous recombination have not been revealed. Here we describe the isolation of human and mouse homologs of the yeast *DMC1* gene, which have a common novel alternative spliced form. They are expressed in spermatocytes in the testis during meiotic prophase. Though the meiotic recombination system of lower eukaryotes like yeast should essentially be the same as that of higher eukaryotes, including mammals, our finding of novel alternatively spliced mRNAs may suggest the presence of a unique common modification of the system in mammals.

MATERIALS AND METHODS

cDNA cloning of mouse and human *DMC1* genes

Two oligonucleotides (5′-CAAGGATCCGAC/TACIGAA/GGGIACITTC/TA/CGICCIGA-3′ and 5′-CAATCTAGAIG/CA/(CT)IAA/GC/TTCICCICG/TICC-3′, where I is inosine) were designed to code for two peptides in the conserved regions of *S.cerevisiae* Dmc1 and mouse RAD51 proteins. The oligonucleotides were used as primers in a reverse transcriptase-polymerase chain reaction (RT–PCR). For this total RNA from the mouse 129/Sv testis was used as a template. The 230 bp fragment generated was subcloned and used as a probe to screen a λZAP cDNA library prepared from 129/Sv mouse testis RNAs. A positive clone, pMDM1, was isolated and both DNA strands were sequenced. A mouse *Dmc1* cDNA was used as a probe to screen a human testis cDNA library in pCDM8 (Clontech). A human clone referred to as pHDM1 was isolated. For 5′-RACE (Clontech) two synthetic oligonucleotides (5′-TGAGCTCTCC-TCTTCACTTTCGCAAGCT-3′ and 5′-TGAAGCATGAGCC-AGAATGTGTCCCC-3′) were used and cDNA was obtained by following the manufacturer's instructions.

Genomic DNA cloning

For determining exon/intron organization four oligonucleotides were synthesized.

L1 primer: 5′-TTCGTACTGGAAAAACTCAGCTGTATC-3′
L2 primer: 5′-CTTGGCTGCGACATAATCAAGTAGCTCC-3′
M1 primer: 5′-TGACAGCTCAACTTCCAGGAACAGGCG-3′
M2 primer: 5′-TAGTATAGGCACGTGCATAGAGCACGT-3′

The genomic DNA from mouse 129/Sv liver was used as a template for amplification. PCR was carried out in 100 µl rTth-XL (Perkin-Elmer Cetus) supplemented with 40 pmol 5′ and 3′ primers, 0.4 mM each deoxynucleotide triphosphate, 1.1 mM $MgCl_2$ and 1 U rTth DNA polymerase XL (Perkin-Elmer Cetus). The amplification products were gel purified, subcloned into pCRII (Invitrogene) and sequenced.

RT–PCR

Single-stranded cDNA was synthesized from 1 µg poly(A)$^+$ RNA from male mice testes at 5 weeks of age and 1 µg total RNA from embryonal ovaries at 14 days postcoitum using an oligo(dT)$_{18}$ primer with Superscript II (Gibco-BRL). PCR was performed using the L1, L2, M1 and M2 primers as shown above. The amplification products were subcloned into pCRII and sequenced.

RNA blot analysis

Total RNAs were isolated from adult mouse tissues with guanidinium thiocyanate by the single step method, poly(A)$^+$ RNAs were absorbed on oligo(dT)–cellulose and eluted with a high salt buffer. Total RNAs (35 µg) or poly(A)$^+$ RNAs (5 µg) were separated on a 1.2% agarose–2.2 M formaldehyde gel and blotted onto a nitrocellulose membrane filter (0.45 µm; Micron Separations Inc.). The filter was prehybridized for 1 h and then hybridized at 42°C for 16 h in 6× SSC, 50% formamide, 5× Denhardt's solution, 120 mg/ml sheared salmon sperm DNA and 0.1% SDS with an [α-^{32}P]dCTP-labeled cDNA probe. The probes used were mouse *Dmc1* cDNA, including nucleotide positions 1–2204 of pMDM1 (probe A), 538–783 of *Dmc1* cDNA, which is spliced out in *Dmc1-d* cDNA (probe B), a 446 bp fragment from the 5′ part (nt 1–446) of *Dmc1* cDNA (probe C), a 469 bp fragment from the 5′ part (nt 1–469) of *Rad51* cDNA (probe D) and the human *GAPDH* (glyceraldehyde 3-phosphate dehydrogenase) gene as a control (Clontech). Each 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.

In situ hybridization

In situ hybridization was performed by a modified method of Cox *et al*. described previously, with the inclusion of high stringency wash steps (30), which were necessary to reduce non-specific binding of the *Dmc1* and *Rad51* probes. The fixed testes were embedded in paraffin wax and sections of 6 µm thickness were prepared.

Antibodies

Polyclonal antisera were raised in rabbits using three peptides: peptide A, MKEDQVVQEESGFQ, corresponding to the N-terminal region of mouse DMC1; peptide B, VTAQLPGTG-GYSGG, corresponding to amino acid residues 141–157 of mouse DMC1; peptide C, VEEESFGPQPISRLE, corresponding to the N-terminal region of mouse RAD51. They were conjugated with keyhole limpet hemocyanin with glutaldehyde (Fig. 1A). Immunoglobulin was purified from the antiserum with an antigen-conjugated Sepharose column. The immunoglobulin was eluted with a low pH buffer (0.2 M glycine–HCl, pH 2.8) and immediately neutralized.

Expression of mouse DMC1 protein

PCR-generated fragments of the mouse *Dmc1*, *Dmc1-d* and *Rad51* cDNAs, each containing all the coding region, were confirmed to be free of mutations by DNA sequencing and were

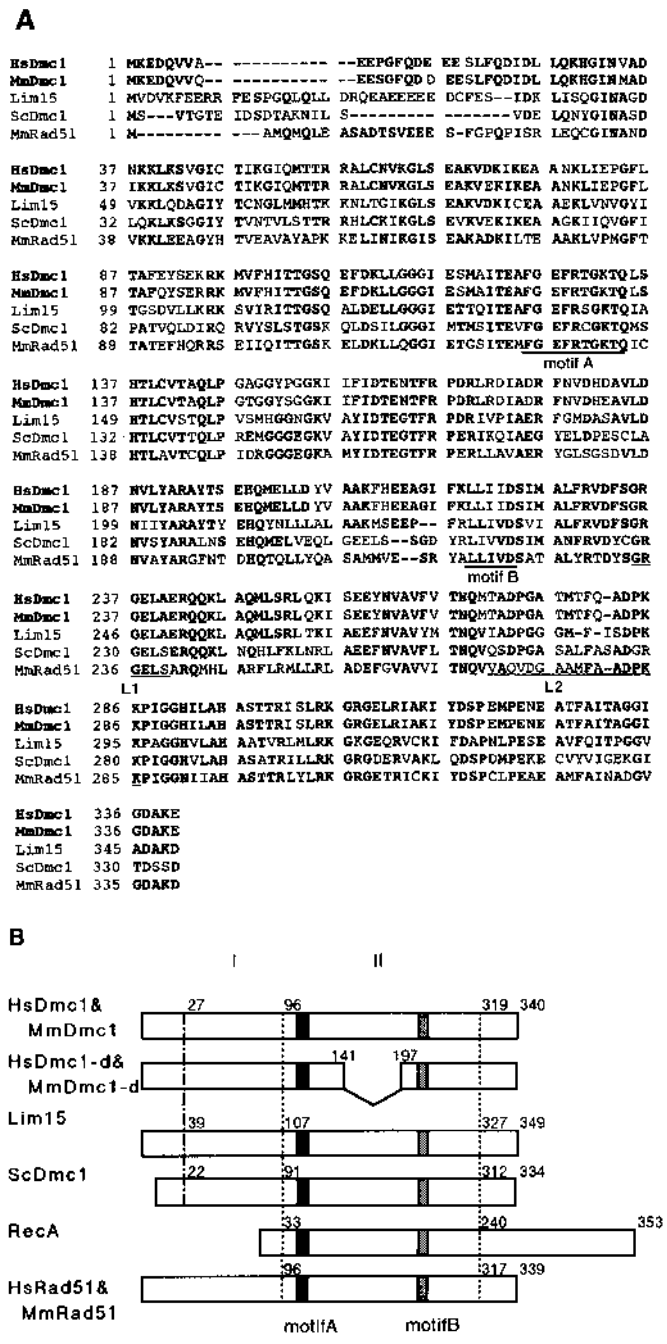

Figure 1. (A) Sequence alignment of mouse, human, lily and yeast DMC1 and mouse RAD51 proteins. Bold characters mark identity between all sequences. Two consensus sequences for nucleotide binding sites (motif A and motif B) and L1 and L2, which are referred to as the disordered loop segments, responsible for double- and single-strand DNA binding, are underlined. (**B**) Domain structures of the DMC1 protein in the *RecA*-like gene sub-family (32). Hs, *Homo sapiens*; Mm, *Mus musculus*; Sc, *Saccharomyces cerevisiae.*

subcloned into a modified T7 overexpression vector. Each of the constructs encodes mouse DMC1, DMC1-D or RAD51 with a histidine tag at the N-terminus. The proteins were expressed in BL21 (DE3) pLysS bacteria (Stratagene) by induction with IPTG. Proteins were collected after cell lysis by sonication in a solution containing 20 mM phosphate, pH 7.8, 0.5 mM dithiothreitol, 10% glycerol.

Western blot analysis

The cells from mouse tissues were washed in ice-cold phosphate-buffered saline (PBS). After centrifugation the cell pellet was resuspended in sample buffer and denatured by boiling for 10 min. The protein samples (30 µg/lane) were electrophoresed on 12.5% SDS–polyacrylamide gels and transferred to PVDF filters (Millipore). The filters were blocked with PBS containing 5% dried milk and then probed with diluted antibodies (×500). The filters were treated with horseradish peroxidase-conjugated anti-rabbit IgG and the signals detected using DAB solution (0.5 mg/ml 3,3′-diaminobenzidine, 0.02% $CoCl_2$, 0.03% H_2O_2 in PBS).

RESULTS

Molecular cloning of mouse and human *Dmc1* genes

To identify the mouse *Dmc1* homolog we designed two oligonucleotides to code for two peptides highly conserved between yeast Dmc1 and mouse RAD51 proteins (amino acid residues 155–162 and 228–233 of yeast Dmc1) as described in Materials and Methods. The oligonucleotides were used to clone part of mouse *Dmc1* cDNA by reverse transcriptase-directed polymerase chain reaction (RT–PCR). Using poly(A)$^+$ RNA from mouse testes and RT–PCR we succeeded in amplifying a fragment of ~230 bp. Sequence analysis revealed a fragment of ~230 bp having a continuous open reading frame (ORF). Translation of the ORF predicted strong homology to the yeast Dmc1 protein. Using the 230 bp fragment as a probe cDNA clones were isolated from a mouse testis cDNA library. Restriction analysis of the positive cDNA clones showed that one of the five corresponded to the yeast *DMC1* gene. The clone (pMDM1) contained an insert of 2203 bp. Sequence analysis revealed a long ORF of 1020 nt (nt 156–1175), which predicted a product of 340 amino acids. A presumed polyadenylation signal, AATAAA, is present at nucleotide positions 2065–2070. The poly(A) tail starts at position 2191.

The human *DMC1* homolog, *hDMC1*, has been also isolated from a human testis cDNA library using the above mouse *Dmc1* cDNA as a probe. For this we initially obtained five cDNA clones. Sequencing of the longest cDNA clone revealed that the clone was missing a 5′ region. To obtain the sequence of the missing 5′-end of *hDMC1* mRNA we employed the 5′-RACE method using two oligonucleotides from the 5′-end of the cDNA clones as specific antisense primers. The PCR amplification yielded fragments of 800–1000 bp in size. These fragments were subcloned into pCRII and subsequently sequenced. Thus these sequences further extended the previously isolated cDNA clone to the 5′-end, which included an in-frame methionine start codon at position 180–896 of the longest 5′-RACE sequence. Combining the 5′ sequence with the cDNA clone resulted in a cDNA of 2292 bp in total length with an ORF corresponding to a 340 amino acid polypeptide with similarity to mouse DMC1 (Fig. 1A). A potential polyadenylation signal was at position 2179 and a poly(A) tail was present at the end of the insert.

Sequence comparison with the yeast Dmc1 and LIM15 proteins

The predicted amino acid sequence of the mouse DMC1 protein showed significant homology with its yeast and lily counterparts. Optimized alignment revealed 54.1 and 61.2% overall sequence identity of the protein with yeast DMC1 and lily LIM15 respectively (Fig. 1A). In the domain I region (Fig. 1B) the structure of mouse DMC1 protein (amino acid residues 27–95) is more similar to yeast Dmc1 protein (55.7% identity, amino acid residues 22–91) and the lily LIM15 protein (48.6%, amino acid residues 39–107) than mouse RAD51 (42.9%, amino acid residues 17–96). As this region is important for interaction between Rad51 and Rad51 or Rad51 and Rad52 in yeast (1,5,31), domain I may be essential for self-association of DMC1 proteins or interaction with other proteins. The strongest homology was found in the domain II region of mouse DMC1 protein (amino acid residues 96–319). In this region amino acid identities of mouse DMC1 protein to lily LIM15, yeast DMCI and mouse RAD51 proteins were 68.2, 59.0 and 57.2% respectively. The region included specific portions of purine nucleotide binding sites, designated motifs A and B, and parts interacting with single- and double-stranded DNAs, thus being conserved among different species. In the C-terminal region the mouse DMC1 protein (amino acid residues 319–340) revealed 61.9, 57.1 and 38.1% identity with mouse RAD51, lily LIM15 and yeast Dmc1 protein respectively. Although no specific biochemical function has been ascribed to this region, the conservation between mouse DMC1 and RAD51 proteins implies that this region may be important in recombination, for example, a region involved in interaction with other components of the recombination machinery (32,33).

Alternative splicing generates heterogeneity in the *DMC1* domain II region

Sequence analysis showed that four out of the five mouse *Dmc1* cDNAs isolated constituted a novel form of *Dmc1* mRNA lacking 164 bases within the domain II region, but otherwise identical. The deleted form of cDNA, referred to as *Dmc1-d*, had a nucleotide sequence of 1920 bp containing an ORF (nt 156–1010) for a 285 amino acid polypeptide with a molecular weight of 31.663 kDa. The predicted product had a deletion at amino acid residues 142–196 within the DMC1 domain II region. The junctional amino acid at the deletion point was glycine, instead of a serine at residue 196 as in the DMC1 polypeptide. In addition, *Dmc1-d* cDNA had truncations of 5 bp at the 5′-end and of a 3′ non-coding region downstream of position 2086 bp of *Dmc1* cDNA.

We also isolated human *DMC1-D* cDNA encoding a 285 amino acid polypeptide. As was the case for mouse *Dmc1-d*, human *DMC1-D* cDNA had a deletion at residues 142–196 of human DMC1 protein. Due to the deletion, the junctional amino acid residue 196 of DMC1-D was converted from serine to glycine. In the 5′ region a further sequence difference was also found between human *DMC1-D* cDNA (positions 1–28) and *DMC1* cDNA (positions 1–19), suggesting the presence of another alternative splice site for the 5′ non-coding exon.

To analyze the exon–intron structure over the alternatively spliced region of the mouse *Dmc1* gene we amplified mouse genomic DNA (129/Sv) by PCR using primers corresponding to both the missing and the flanking sequences of the deleted region of the mouse *Dmc1-d* cDNA (Fig. 2A). The amplified products were isolated as four independent overlapping clones spanning an area of ~5 kb.

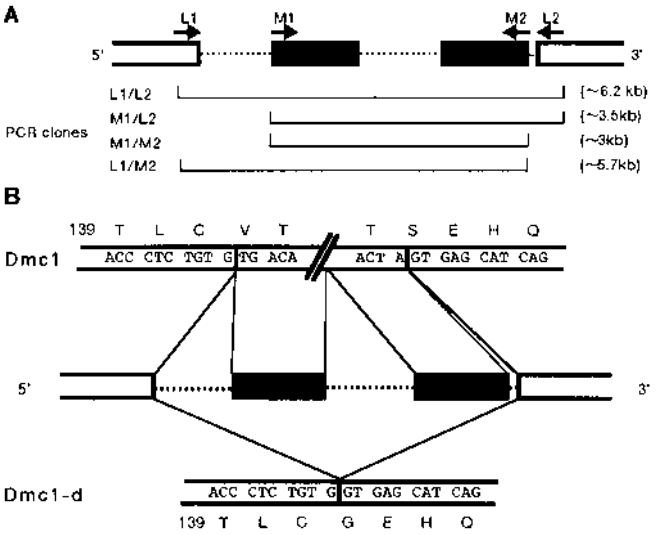

Figure 2. Partial structure of the mouse *Dmc1* gene around the deleted region of *Dmc1-d* cDNA. (**A**) Schematic representation of the genome organization. The positions corresponding to PCR primers are designated L1, L2, M1 and M2 and the resulting PCR products are also shown. (**B**) Schematic representation of alternative splicing of the *Dmc1* gene.

Subsequent subcloning and sequencing revealed the presence of two exons and three introns within the deleted region (Fig. 2B). These results clearly demonstrate that *Dmc1-d* cDNA corresponds to mRNA alternatively spliced by skipping the two exons, resulting in the 57 amino acid deletion and one amino acid substitution, Ser (AGT)→Gly (GGT), at the alternative splice junction site of the DMC1 protein.

Expression of Dmc1 and Dmc1-d mRNAs

Northern blot analysis with a mouse *Dmc1* cDNA probe (probe A) showed the presence of RNA species of 1.9 and 2.2 kb (Fig. 3A). These RNAs were detected only in the testis, so far as we could ascertain. This was in contrast to the case of mouse *Rad51* mRNA, which was expressed in the thymus, spleen, testis and ovary, where cells were proliferating. To examine whether the two different hybridization bands correspond to the two alternatively spliced mRNA species we carried out a Northern blot analysis using as probe the fragment nt 538–783 of *Dmc1* cDNA (probe B), which is spliced out in *Dmc1-d* mRNA. However, the probe hybridized to both mRNA species (Fig. 3B). Furthermore, Northern blot analysis of poly(A)$^+$ and poly(A)$^-$ RNAs indicated that both the 1.9 and 2.2 kb RNAs were polyadenylated. Thus the two bands include poly(A)$^+$ mRNAs containing the deleted sequences in *Dmc1-d* cDNA. Because the size of our cDNA (2203 bp) corresponded to the longer band (2.2 kb), the shorter band (1.9 kb) might possibly correspond to an alternative polyadenylated form of the cDNA. The relationship between the two mRNA species and the alternatively spliced cDNAs remains to be determined.

To confirm the presence of the two spliced forms of mRNAs in germ cells we carried out RT–PCR (Fig. 4). The two oligonucleotides flanking the deleted region were used to amplify cDNAs from the mouse adult testes and from mouse embryonal ovaries at 14 days postcoitum, when the female germ cells were in meiotic prophase. We observed amplified products of 230 and 76

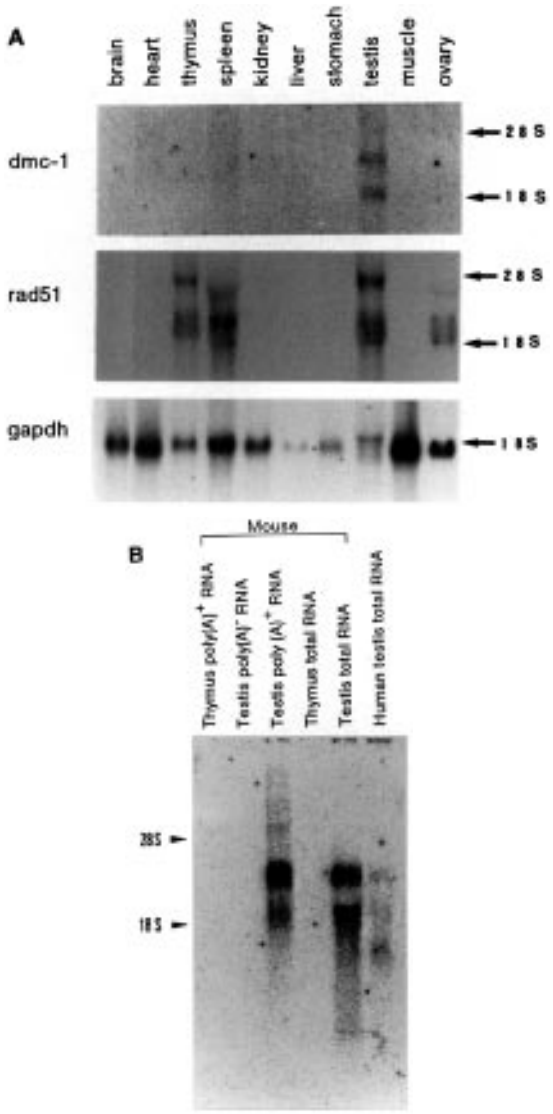

Figure 3. RNA blot analysis. (**A**) Expression patterns of mouse *Dmc1* and mouse *Rad51* in various mouse tissues. A blot with samples, each containing an equal amount (40 µg) of total RNA from the indicated mouse tissues was hybridized with a mouse *Dmc1* cDNA probe (probe A) and then reprobed with a mouse *Rad51* cDNA probe (probe D). The same blot was hybridized with human *GAPDH* cDNA. (**B**) Hybridization of poly(A)$^+$ RNA and poly(A)$^-$ RNA with a *Dmc1* probe. A blot with samples of 1 µg poly(A)$^+$ RNA, 40 µg poly(A)$^-$ RNA, 40 µg total RNA from mouse testis and total RNA from mouse spleen was hybridized with mouse *Dmc1* cDNA (probe B).

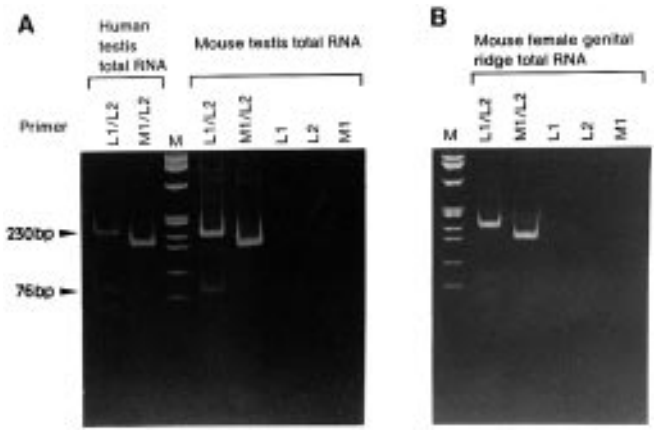

Figure 4. Detection of *Dmc1* and *Dmc1-d* transcripts by RT–PCR analysis. First strand cDNAs were obtained from poly(A)$^+$ RNA of mouse testis at 5 weeks of age, total RNA from ovaries from 14 days postcoitum embryos and total RNA from human testis and were amplified by PCR. (**A**) Total human testis cDNA was subjected to amplification with L1 and L2 primers (lane 1) and M1 and L2 primers (lane 2). Total mouse testis cDNA was subjected to PCR with L1 and L2 primers (lane 4) and M1 and L2 primers (lane 5). The mouse testis cDNA was amplified with a single oligomer, either with L1 (lane 6), L2 (lane 7) or M1 (lane 8) as a negative control. The DNA size markers are in lane 3. (**B**) The cDNA from mouse embryonal ovaries was amplified with L1 and L2 primers (lane 2) and M1 and L2 primers (lane 3). As negative controls the cDNA was mixed solely with either L1 (lane 4), L2 (lane 5) or M1 (lane 6) primer and amplified. The size markers are in lane 1.

bp from both the testis and embryonal ovary, corresponding to the expected sizes of *Dmc1* and *Dmc1-d* cDNA respectively. Cloning and sequencing analysis of the fragments showed that they were indeed derived from *Dmc1* and *Dmc1-d* cDNA respectively. The same results were also obtained by RT–PCR of human testis RNA.

DMC1 and *DMC1-d* mRNA expression during spermatogenesis

To confirm that testicular cells were capable of expressing *Dmc1* or *Dmc1-d* transcripts we carried out *in situ* hybridization (Fig. 5). As antisense probes we used probe C (Fig. 5a) to detect a 5′ part of *Dmc1* cDNA (nt 1–446 of *Dmc1* cDNA) and probe B (Fig. 5c) to detect specifically a *Dmc1* mRNA region that was absent in *Dmc1-d* cDNA (nt 538–783 of *Dmc1* cDNA). We also used probe D (Fig. 5e) specific for *Rad51*, containing the 5′ part of *Rad51* cDNA. The antisense probes, as well as the respective control sense RNA probes (Fig. 5b, d and f), were synthesized with RNA polymerase. We verified that the *Rad51*-specific probe D did not cross-hybridize with *Dmc1* transcripts by Northern blot analysis (Fig. 3A).

To examine the expression of *Dmc1*, *Dmc1-d* and *Rad51* genes during spermatogenesis mouse testes of 5 weeks of age were freeze-sectioned and hybridized with probes B, C and D. The probes were labeled with digoxigenin. The results are shown in Figure 5a–f. High accumulation of *Dmc1* mRNAs was observed in spermatocytes in meiosis using probe C (Fig. 5c and d). Weaker signals were observed in the same cells when we used probe B (Fig. 5a and b). Thus *Dmc1* transcripts, presumably together with *Dmc1-d*, were present in spermatocytes during spermatogenesis. *Rad51* transcripts were detected in spermatogonia and spermatocytes (Fig. 5e and f).

DMC1 and DMC1-D protein expression during spermatogenesis

To examine the distribution of DMC1 and DMC1-D proteins in the adult testis we raised rabbit polyclonal antibodies against a peptide corresponding to an N-terminal region (peptide A, MKEDQVVQEESGFQ) of mouse DMC1 protein (amino acid residues 1–14) and against another peptide corresponding to a region at residues 141–154 (peptide B, VTAQLPGTGGYSGG) of mouse DMC1 protein present within the deleted region of the DMC1-D protein. We also prepared antibody against an N-terminal region (peptide C, VEEESFGPQPISRLE) of mouse

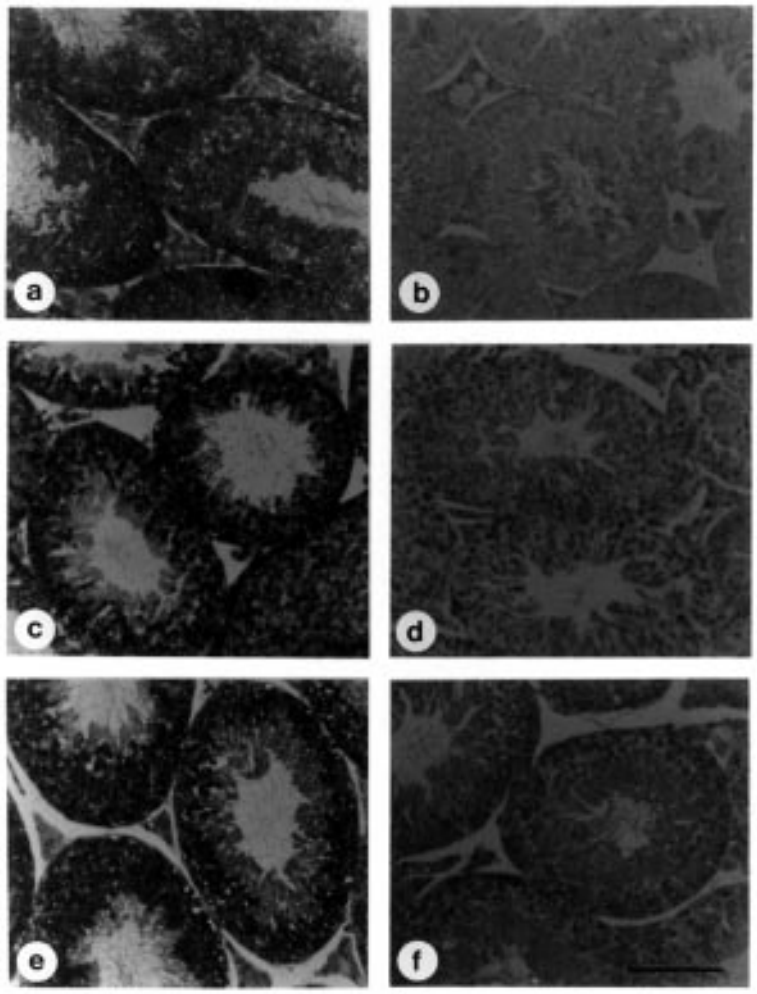

Figure 5. Expression of *Dmc1* and *Dmc1-d* in the adult mouse testis. *In situ* hybridization was performed on specimens as described in Materials and Methods. Sections of the adult mouse testis were hybridized with antisense probe C containing sequences coding for the N-terminus of DMC1 protein (**a**), with antisense probe B containing a part of the sequence corresponding to the deleted region of DMC1-d protein (**c**) and with probe D including the N-terminal coding sequence of RAD51 protein. The sense RNAs were used for hybridizations as controls for probe C (**b**), for probe B (**d**) and for probe D (**f**). The scale bar is 100 μm.

RAD51 protein. To verify antibody specificities the recombinant mouse DMC1, DMC1-D and RAD51 proteins with histidine tags were expressed in *E.coli* (Fig. 6A). Western blot experiments showed that neither anti-peptide A nor anti-peptide B antiserum reacted with His-tagged RAD51 protein and that anti-peptide C serum did not recognize either the mouse DMC1 or DMC1-D proteins with His tags. We carried out Western blot analysis against mouse tissues (testis and liver). As shown in Figure 6B, extracts from the adult testes contained two positive bands with the mobilities expected for DMC1 (37 kDa) and DMC1-D (31 kDa) proteins when immunoblots were stained with an antibody specific for the N-terminal part of DMC1 protein (anti-peptide A). Since anti-peptide C reacts significantly more weakly with DMC-1D protein than DMC-1 protein, as shown in Figure 3A, the amount of DMC1-D protein in testis could be much more than the trace amount as detected by immunoblot in Figure 3B. The low molecular weight band below 31 kDa should be non-specifically reacting components in the extracts. The same blot stained with antibody specific to the deleted part of DMC1-D protein (anti-peptide B) showed the presence of only one positive band of DMC1 protein (37 kDa). DMC1 and DMC1-D proteins, as well as RAD51 protein, were detected in the testis but not in the liver. These results suggest that both DMC1 and DMC1-D proteins are expressed in the testis, presumably during spermatogenesis.

DISCUSSION

DMC1 protein structure

The ORF of *Dmc1* cDNA predicts a 340 amino acid protein with a calculated mass of 37 kDa. The primary structure of mouse DMC1 showed more significant overall similarity with DMC1 proteins from different species than with RAD51 protein. Nevertheless, the conservation (53.6%) of the overall structure between mouse and human DMC1 and RAD51 proteins was significant. This suggests that DMC1 and RAD51 proteins have similar biochemical characteristics to exert the same type of function. The domain II region of mouse DMC1 protein showed a high sequence identity of 57.2% with mouse RAD51. This

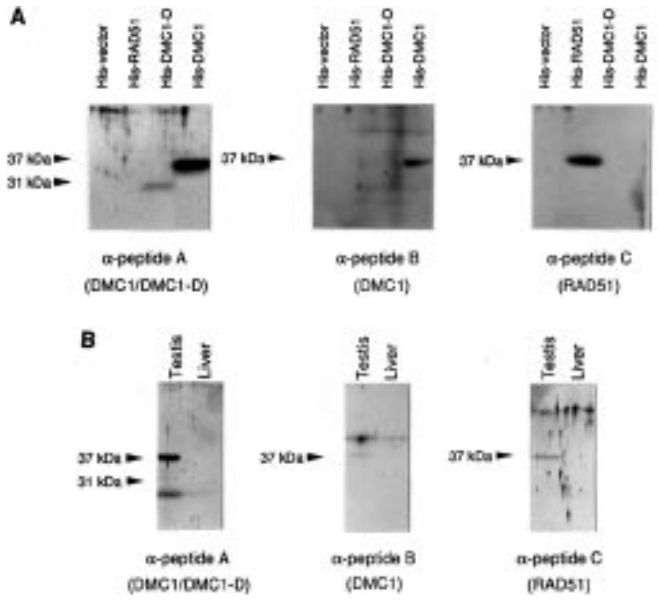

Figure 6. Western blot analysis of DMC1, DMC1-D and RAD51 proteins. (**A**) *Escherichia coli* BL21 (DE3) pLysS cells were transformed with plasmids containing the coding sequences of DMC1, DMC1-D and RAD51 proteins with 6× histidine tags at the N-termini and their gene expression was induced with IPTG. The bacterially expressed His-tagged proteins were subjected to SDS–PAGE and Western blots were carried out. His-DMC1, His-DMC1-D, His-RAD51 and a negative control (His-vector) were examined. The filters were probed with respective polyclonal antisera: anti-peptide A, anti-peptide B and anti-peptide C. The filter containing the bacterial cell lysates, including one with the control plasmid without the insert DNA, was probed with a mixture of antisera of anti-peptide A, anti-peptide B and anti-peptide C. (**B**) Expression of mouse DMC1, DMC1-D and RAD51 proteins in mouse organs. Whole cell lysates of the mouse testes (lanes 1, 3 and 5) and liver (lanes 2, 4 and 6) were immunoblotted with anti-peptide A (lanes 1 and 2), with anti-peptide B (lanes 3 and 4) or anti-peptide C (lanes 5 and 6). Each lane contains an equal amount of protein (30 μg).

region contains the consensus sequences for nucleotide binding known as motif A (GPESSGKT) and motif B (VIVVD) (32) and is also important for single- and double-stranded DNA binding and the strand exchange reaction in the case of bacterial RecA protein (12,31).

Expression of Dmc1 and RAD51 in meiosis

High frequencies of genetic recombination occur during meiotic prophase. The first step of homologous recombination in meiosis begins with searching for homologous DNA, which results in chromosome pairing during leptotene (34–36). This step presumably includes DNA–DNA interaction. DSBs are widely induced during meiosis in *S.cerevisiae*, at recombinational hot spots. Such DSBs might lead to heteroduplex formation as a recombination intermediate on the way to crossovers, which persist throughout pachytene (37). This reaction may be mediated by RecA-like protein functions. In the lily RAD51 protein is expressed through interphase to pachytene and LIM15 protein mainly at leptotene and zygotene (29). These proteins co-localize on leptotene and zygotene chromosomes. Our *in situ* hybridization analysis revealed that mouse *Dmc1* mRNA was expressed in spermatocytes in meiosis and mouse *Rad51* mRNA in the spermatogonia and spermatocytes (38). Thus DMC1 and RAD51 proteins expressed in meiotic prophase are supposed to catalyze DSB repair of DNA, resulting in pairing of homologous DNA in meiosis. The DSB repair-like homology search would be followed by intimate synapsis and synaptonemal complex formation during the zygotene and pachytene stages.

Deletion of DMC1 protein

In mammals testis-specific RNA splicing is observed in c-*abl* (39), *Wnt-1* (40), *Oct-2* (41), *Sry* (42), *CREM* and *CREB* transcripts (43). In yeast meiosis-specific RNA splicing is observed in the *MER2* (44) and *SPO13* transcripts (45). We found *Dmc1* splicing variants from the mouse and human testis cDNA libraries. Furthermore, by RT–PCR we confirmed that *Dmc1* alternative splicing took place not only in the testis during spermatogenesis, but also in the embryonic ovary during oogenesis. These suggest that the alternative splicing of *Dmc1* in mammals could be involved in homologous recombination and/or progression of the meiotic cell cycle.

Dmc1-d cDNA does not contain the region at nucleotide positions 577–741 of mouse *Dmc1* cDNA within the domain II region (142–196 amino acid region) that is conserved in the *RecA*-like gene family (3). Since this deleted region locates close to motifs A and B necessary for ATP binding, DMC1-D protein may have a low binding affinity for nucleotides and low ATPase activity. Since a recent report described ATPase activity as not being required for the strand exchange reaction with bacterial RecA (46,47), DMC1-D protein may still have strand exchanging activity. Otherwise, the protein thus modified might have a novel role in meiotic recombination. The loss of 167 amino acids likely leads to drastic changes in the three-dimensional structure of the protein itself, thus modifying single- and double-stranded DNA binding activity, resulting in modulation of recombination events as a positive or negative regulator during meiosis, perhaps within a 'recombinosome' complex (33).

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