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SEQUENCE UPDATE

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Isolation and characterization of *OsDMC1*, the rice homologue of the yeast *DMC1* gene essential for meiosis

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Abstract Yeast *DMC1* is a meiosis-specific gene required for homologous chromosome pairing in meiosis. Using degenerate primers designed according to amino acid motifs conserved in yeast Dmc1 and *Arabidopsis* AtDmc1, we obtained full-length cDNA of a rice homologue of the *DMC1* gene (*OsDMC1*) by RT-PCR and rapid amplification of cDNA ends (RACEs). OsDmc1 exhibited 53% amino acid sequence identity to yeast Dmc1 and 81% to AtDmc1. *OsDMC1* was expressed at high-levels in reproductive organs, low-levels in roots, and undetectable levels in leaves and seedlings. Southern blot analyses revealed that *OsDMC1* is one of two *DMC1* homologues present in rice.

Keywords Meiosis · Rice · *DMC1*

Introduction

The life cycle of plants alternates between a diploid sporophytic generation and a haploid gametophytic generation. Meiosis is a key event of this transition. Meiosis differs from mitosis in that a single round of DNA replication is followed by two sequential cell divisions so that an initially diploid meiotic cell generates haploid gametes. In consequence, union of male and female gametes restores the normal cellular chromosome complement. The normal segregation of chromosomes during meiosis is dependent on the successful pairing of homologous chromosomes to form a bivalent during the zygotene stage of prophase I. The pairing of homologous chromosomes relies mainly on homologous recombination and synaptonemal complex polymerization (Kleckner 1996; Zickler and Kleckner 1998).

In yeast, molecular genetic analysis of meiosis has led to the isolation of some genes essential to meiosis, such as *DMC1*, *RAD51* and *HOP1* (Hollingsworth and Byers 1990; Bishop et al. 1992; Shinohara et al. 1992). *DMC1* and *RAD51* proteins (Dmc1 and Rad51) are two homologues of *E. coli RecA* protein (recA), which polymerizes on DNA to form a nucleofilment, promotes strand exchange and is required for homologous recombination and the recombinational repair of double strand breaks (Story and Steitz 1992, Story et al.1992). Dmc1 and Rad51 co-localize during the zygotene stage of meiotic prophase I, and play an important role in recombination-mediated homologous chromosome pairing and in the strand exchange. Rad51 is also involved in mitotic recombinational DNA repair, whereas *DMC1* is a meiosis-specific gene required for normal levels of meiotic reombination and normal synaptonemal complex (SC) formation (Bishop et al. 1992; Bishop 1994). *DMC1* mutants are defective in reciprocal recombination, fail to form normal SCs, and arrest late in meiotic prophase (Bishop et al. 1992).

In recent years, homologues of *DMC1* have been found in several higher eukaryotic organisms, such as humans (Habu et al. 1996), mice (Habu et al. 1996; Pittman et al. 1998; Yoshida et al. 1998) and *Arabidopsis thaliana* (Klimyuk and Jones 1997; Doutriaux et al. 1998). Human Dmc1 has homologous pairing and strand exchange activities similar to yeast Dmc1 (Li et al. 1997). Mouse Dmc1 is required for homologous chromosome synapsis during meiosis (Yoshida et al. 1998). In *Arabidopsis DMC1* mutants, homologous chromosomes could not pair to form bivalents and ten univalents were regularly found in pollen mother cells and megaspore mother cells undergoing meiosis (Couteau et al. 1999). This suggests that functions of *DMC1* are conserved in higher eukaryotes.

The rice homologue *OsDMC1* will appear in the EMBL, Gen Bank and DDBJ nucleotide sequence databases under accession number AB046620.

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Rice is an important crop, specially in Asia. The isolation of genes necessary for meiosis is a key step to understand the molecular mechanism of reproductive development of rice. Understanding these genes will be important to the study of male or female sterility and apomictic reproduction of rice. In this paper, we report the isolation and characterization of the rice homologue of yeast *DMC1*, named *OsDMC1*.

Materials and methods

Plant materials

Rice plants (*Oryza sativa* L. ssp. *japonica*) were grown under standard greenhouse conditions. Cultivar Nipponbare (ssp. *japonica*) was used in this experiment. Spikelets in which pollen mother cells were in meiosis were collected and used as a source of RNA for the identification of *OsDMC1*. Leaves were collected from 3-week old plants. Roots and seedlings were harvested from seeds germinated on sterile-water soaked papers.

Extraction of nucleic acids

Total RNA was isolated with a Trizol kit (GIBICO-BRL) according to the manufacturer's protocol, and was then treated with RNase-free DNaseI (TaKaRa) to remove residual genomic DNA. Genomic DNA was extracted from seedlings by cetyltrimethylammonium bromide (CTAB) according to the method of Murray and Thompson (1980)

Isolation of cDNA and genomic clones

Two pairs of degenerate primers were designed according to amino acid motifs conserved in *DMC1* and *AtDMC1* proteins (Dmc1 and AtDmc1). These primers are MP1: GGNATHAAYGCNGGNGAYGT; MP2: GGNAARGTNGCNTAYATHGA; MP3:ACNGCNACRTTRAAYTCYTCNGC and MP4: GCRTGNGCNARNTGNCCNCC. The combination of MP1 and MP4 was used in the first round of PCR; otherwise the combination of MP2 and MP3 was used for reamplification of the first-round PCR products.

To isolate cDNA clones, 4 µg of total RNA from spikelets was combined with 0.5 µg of oligo dT primer in a volume of 12 µl and denatured at 70°C for 10 min. cDNA was synthesized in a reaction volume of 20 µl SuperScript II RNase H$^-$ reverse transcriptase (GIBCO-BRL). The RT-PCR reaction was performed with 1 µl cDNAs as templates in 20 µl of reaction buffer (10 mM Tris-HCl, pH8.3, 50 mM KCl, 2 mM MgCl2, 0.05% Nonidet P–40) containing 40 pmol of each of two selected primers, 200 µM dNTPs (Sangon) and 2.5 units of Pfu DNA polymerase (5u/µl, Sangon). The PCR products were analyzed by electrophoresis. A 320-bp cDNA fragment, obtained after reamplification of first-round PCR products, was gel-purified and cloned into the pGEM-T vector (Promega).

For isolation of genomic clones, 100 ng genomic DNA was used as a template instead of the cDNA template; other conditions were same. The PCR products were analyzed with electrophoresis. A 698-bp DNA fragment, obtained after reamplification of first-round PCR products, was gel-purified and cloned into pGEM-T vector.

Identification of cDNA ends (RACE)

The full-length *OsDMC1* cDNA was isolated by rapid amplification of cDNA ends (RACEs) (Frohman et al. 1988). For 3' RACE, first-strand cDNA was synthesized with an oligo-dT adapter primer (CTGATCTAGAGGTACCGGATCTTTTTTTTTTTTTTTT). Specific cDNA was then amplified by two rounds of PCR with the gene-specific nested primers MP5:CCGGCCTGAACGAATTGT and MP6:TGGGATGGATGCCAATGC and the adapter primer CTGATCTAGAGGTACCGGATC. The amplified fragment was gel-purified and cloned into the pGEM-T vector.

The 5' region of the *OsDMC1* transcript was obtained with a GIBCO-BRL 5' RACE kit by using the gene-specific primers MP7:TCTCTCAGCAATCGGCACAA and MP8:AACAGCATTGGCATCCAT. The amplified fragment was gel-purified and ligated into pGEM-T vector.

DNA sequence analysis

DNA was sequenced by the dideoxy chain termination method with an automated sequencer model 377 (Applied Biosystems). The degree of amino acid sequence identity was determined with the BLAST program (Altschui et al. 1997).

Southern blot hybridization

Twenty micrograms of genomic DNA was separately digested with *Eco*RI, *Hind*III or *Pst*I. After the digested DNA was subjected to electrophoresis on a 0.8% agarose gel, the DNA fragments were blotted onto a Hybond N$^+$ nylon membrance (Amersham). Hybridization was carried out with the DIG-labeled 698-bp genomic DNA fragment of *OsDMC1* at 68°C according to the manufacturer's protocol (Boehringer Mannheim).

RT-PCR analysis of *OsDMC1* expression

The sense and antisense primers specific to *OsDMC1* cDNA were GTCCAAGCAGTACGACGAAGG and TTCCTCGACATGTGCAAAGT. As a control, primers specific to the rice tubulin gene *tubA* (accession number X91806), TCAGATGCCCAGTGACAGA and TTGGTGATCTCGGCAACAGA, were used.

First-strand cDNA was synthesized with SuperScript II RNase H$^-$ reverse transcriptase (200 u/µl, GIBCO-BRL) according to the manufacturer's protocol. PCR was performed in a mixture of 50 µl that contained 1 µl of the diluted first-strand cDNA, 10 pmol each of the gene-specific primers, 0.4 mM dNTPs, 1×GC PCR buffer and 2.5 u LATaq DNA polymerase (5 u/µl, TaKaRa) for 25 cycles. The PCR products were separated on a 1% agarose gel, blotted onto a Hybond N$^+$ nylon membranes (Amersham) and hybridized with the DIG-labeled *OsDMC1* cDNA probe at 68°C according to the manufacturer's manual (Boehringer Mannheim).

Results and discussion

Molecular cloning of the *OsDMC1* gene

With first-strand cDNAs synthesized with RNA from young spikelets as templates, primers MP1 and MP4 amplified weak smear products separated on agarose gels. After an aliquot of the PCR products was reamplified with MP2 and MP3, a single 320-bp cDNA fragment was obtained and cloned into the pGEM-T vector. Nucleotide sequence and BLAST analysis showed that the cDNA sequence was highly homologous to exon sequences of *DMC1* and *AtDMC1*. It had a continuous ORF and encoded a 109 amino acid sequence with 58% identity to Dmc1 and 91% identity to AtDmc1, indicating that this cDNA sequence was a partial cDNA of the *DMC1* homolog of rice. Based on these results, the rice *DMC1* gene was designed as *OsDMC1*.

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CGCCGGCCGCCTCCGCCTACAGGTGCGGGGGGTCTGTGTGAGCAGATGGCGCCG
                                                M  A  P
TCCAAGCAGTACGACGAAGGCGGGCAGCTCCAGCTCATGGATGCAGAGAGGATC
 S  K  Q  Y  D  E  G  G  Q  L  Q  L  M  D  A  E  R  I
GAGGAGGAGGAGGAGTGCTTCGAGTCCATCGACAAGTTAATCTCGCAAGGGATA
 E  E  E  E  E  C  F  E  S  I  D  K  L  I  S  Q  G  I
AACTCTGGAGATGTGAAGAAGCTGCAGGATGCTGGTATCTACACTTGCAATGGC
 N  S  G  D  V  K  K  L  Q  D  A  G  I  Y  T  C  N  G
CTCATGATGCATACAAAGAAGAGCCTGACAGGAATCAAGGGATTATCTGAAGCA
 L  M  M  H  T  K  K  S  L  T  G  I  K  G  L  S  E  A
AAGGTAGACAAGATCTGTGAAGCAGCTGAAAAGCTTCTGAGCCAGGGCTTCATG
 K  V  D  K  I  C  E  A  A  E  K  L  L  S  Q  G  F  M
ACAGGAAGTGATCTCCTTATCAAGCGGAAGTCTGTTGTTCGGATCACCACTGGG
 T  G  S  D  L  L  I  K  R  K  S  V  V  R  I  T  T  G
AGCCAGGCACTTGATGAGCTGCTTGGCGGAGGGATTGAAACACTCTGCATCACA
 S  Q  A  L  D  E  L  L  G  G  G  I  E  T  L  C  I  T
GAGGCATTTGGAGAGTTCCGATCAGGGAAGACCCAGTTGGCTCACACTCTATGT
 E  A  F  G  E  F  R  S  G  K  T  Q  L  A  H  T  L  C
GTCTCCACTCAGCTTCCAATTCACATGCATGGGGGGAATGGGAAGGTCGCTTAT
 V  S  T  Q  L  P  I  H  M  H  G  G  N  G  K  V  A  Y
ATCGATACTGAGGGAACATTCCGGCCTGAACGAATTGTGCCGATTGCTGAGAGA
 I  D  T  E  G  T  F  R  P  E  R  I  V  P  I  A  E  R
TTTGGGATGGATGCCAATGCTGTTCTTGATAATATCATATATGCTCGTGCATAC
 F  G  M  D  A  N  A  V  L  D  N  I  I  Y  A  R  A  Y
ACCTATGAGCACCAGTACAACCTTCTCCTTGGCCTTGCCGCTAAGATGGCTGAA
 T  Y  E  H  Q  Y  N  L  L  L  G  L  A  A  K  M  A  E
GAGCCTTTCAGGCCTCTGATTGTGGATTCTGTGATCGCACTATTCCGTGTCGAT
 E  P  F  R  P  L  I  V  D  S  V  I  A  L  F  R  V  D
TTCAGTGGCAGGGGTGAACTTGCAGAGCGCCAGCAAAAATTGGCACAGATGCTC
 F  S  G  R  G  E  L  A  E  R  Q  Q  K  L  A  Q  M  L
TCCCGCCTTACTAAAATAGCTGAGGAGTTCAATGTTGCAGTGTACATCACCAAC
 S  R  L  T  K  I  A  E  E  F  N  V  A  V  Y  I  T  N
CAAGTGATTGCCGACCCAGGTGGTGGAATGTTCATAACTGACCTGAAAAAACCA
 Q  V  I  A  D  P  G  G  G  M  F  I  T  D  L  K  K  P
GCGGGAGGCCACGTGCTGGCGCATGCAGCTACCATCCGGTTGATGCTGAGGAAA
 A  G  G  H  V  L  A  H  A  A  T  I  R  L  M  L  R  K
GGCAAAGGAGAGCAGCGTGTCTGCAAGATCTTTGATGCCCCTAACCTGCCTGAG
 G  K  G  E  Q  R  V  C  K  I  F  D  A  P  N  L  P  E
GGAGAAGCTGTTTTCCAGGTAACATCAGGTGGAATAATGGATGCGAAAGACTGA
 G  E  A  V  F  Q  V  T  S  G  G  I  M  D  A  K  D  *
ATGTTCATTGAGGGTGCTTCCTGTCTTCAAATTATTCAGGTTTCTCCTAGTAAA
TTCCTGCATAGGCCATATAGGTTTGGTACATTGACTAAACTACTACTGCTACTT
TGCCACTGTCGAGGAAATATGCAACCTCATTTATCCAGACGATTATACCTTAAA
ATGGGTATTTTTCTATGCTTATGAGATCAACAGTTGTACTGAAACAAATACAGT
ATTGGCTTAAGTGAAAATATATAAGAGGGTTACATGATGGTAAAAAAAAAAA
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Fig. 1 *OsDMC1* cDNA and deduced amino acid sequence. The translation initiation codon is indicated in *bold*. A potential polyadenylation signal is *underlined*

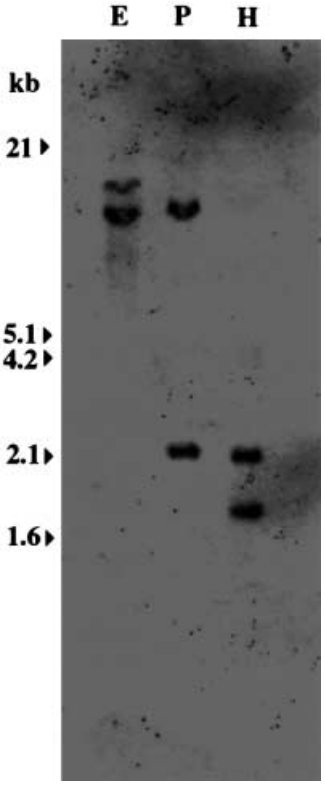

Fig. 2 Southern blot analysis of *OsDMC1* gene. Twenty micrograms of genomic DNA was separately digested with *Eco*RI (*E*), *Pst*I (*P*) or *Hind*III (*H*), and separated on an 0.8% agarose gel. Hybridization was performed with the DIG-labeled 698-bp genomic DNA fragment of *OsDMC1*. DNA size standards in kb are shown to the *left*

With genomic DNA as a template, a single 698-bp fragment was amplified under the PCR conditions described. Comparison of the 698-bp DNA sequence with the 320-bp cDNA sequence revealed that the DNA sequence had four exons interrupted by three introns. The sequence edited from the four exons was identical to the 320-bp cDNA sequence. The results indicated that the 698-bp fragment was a genomic DNA fragment of the *OsDMC1* gene.

These primers specific to the 320-bp cDNA sequence were designed to isolate the 3' and 5' ends of *OsDMC1* cDNA by RACEs. Combining the 320-bp cDNA sequence with its 5' and 3' sequences resulted in a full-length cDNA of 1348 bp with a 5' UTR of 45 bp, a 3' UTR of 268 bp, and an ORF of 1035 bp, which predicted a 344 amino acid polypeptide (OsDmc1) (Fig. 1). A proposed poly(A) signal, ACTAAA, is localized at nucleotide positions 1168–1173. The poly (A) tail starts at position 1337. *OsDMC1* cDNA is the first cDNA sequence of *DMC1* homologs identified from monocots reported to date.

OsDmc1 had a calculated molecular mass of 37.6 kDa and an isoelectric point (pI) of 5.53. It contained motif GEFRSGKT (residues 133–140) which matches the well-known motif A consensus sequence G/AXXXXGKT/S for NTP-binding conserved in many NTP-binding proteins (Story et al. 1992). Optimal alignment revealed that it had 53% overall identity to yeast Dmc1 (Bishop et al. 1992) and 81% to *Arabidopsis* AtDmc1 (Klimyuk and Jones 1997)

Two *DMC1* homologues are present in rice

In order to determine whether there are other *DMC1* homologues in the rice genome, genomic DNA was digested to completion with either *Eco*RI, *Pst*I or *Hind*III, which do not cut into the 698-bp genomic DNA fragment of *OsDMC1*, and then subjected to Southern blot analysis. Hybridization with the 698-bp fragment used as a probe showed two bands in all cases (Fig. 2), even though the blots were washed to high stringency. This suggests that there are two *DMC1* homologs in the rice genome. *OsDMC1* is one of the two-copy gene family. This is obviously different from *DMC1* of yeast and *AtDMC1* of *Arabidopsis*, which are all single-copy genes (Bishop et al. 1992; Klimyuk and Jones 1997).

Expression of *OsDMC1*

To examine the expression patterns of *OsDMC1*, a cDNA probe generated by PCR containing an ORF of *OsDMC1* and labeled with DIG was used to probe the

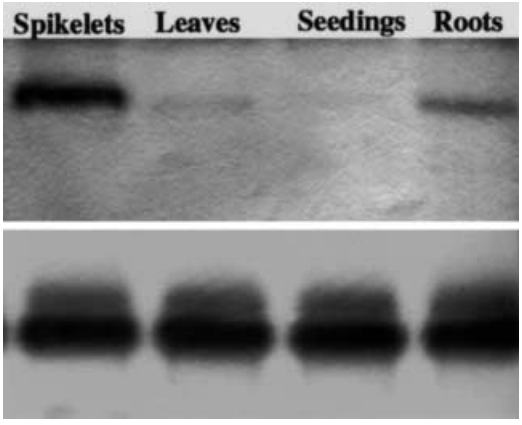

Fig. 3 RT-PCR analysis of *OsDMC1* expression. First-strand cDNA was synthesized with total RNA from various organs and used as templates for PCR with a gene-specific primer set (see Materials and methods). PCR products were separated by a 1% agarose gel, blotted onto a Hybond N^+ nylon membrane and hybridized with the DIG-labeled cDNA probe containing open reading frame of *OsDMC1*. *Top*: organ specificity of *OsDMC1* expression. *Bottom*: control with tubulin gene *tubA*

gel blots of total RNA from spikelets, leaves, seedlings and roots. No hybridization signals were detected even when 50 µg of total RNA was loaded (data not shown), suggesting that *OsDMC1* is expressed at a low level. This difficulty has been encounted when analyzing expression of plant meiotic genes *AtDMC1* (Klimyuk and Jones 1997), *SYN1* (Bai et al. 1999) and *AtPelota* (Caryl et al. 2000) with northern hybridization. We therefore used RT-PCR to analyze expression of this gene. RT-PCRs were conducted with equal amounts of first-strand cDNAs prepared from equal amounts of total RNA isolated from these tissues. Figure 3 shows that *OsDMC1* was expressed at high levels in reproductive organs (spikelets), low-levels in roots, and undetectable levels in leaves and seedlings. These results were similar to those obtained with *Arabidopsis AtDMC1*, which showed high-level expression in reproductive organs and low-level expression in leaf tissues when analyzed by RT-PCR (Klimyuk and Jones 1997). In situ hybridization and *AtDMC1* promoter-GUS fusion analyses revealed that *AtDMC1* expression was restricted to meiotic cells in anthers and carpel (Klimyuk and Jones 1997). *AtDMC1* mutation severely disturbed meiotic chromosome behavior that resulted in low male and female fertility. *Arabidopsis dmc1* mutants can produce 1.5% viable progeny (Couteau et al. 1999), which is not the case for mouse or yeast mutants in which meiosis is completely arrested in prophase (Bishop et al. 1992; Pittman et al.1998; Yoshida et al. 1998). Couteau et al. (1999) suppose that its low chromosome number offers a pathway for *Arabidopsis* to partly escape the consequences of a *dmc1* mutation. Rice has mid-range chromosome number. We are dissecting the function of *OsDMC1* in meiosis and analyzing its effect on reproductive development and vegetable growth of rice with antisense RNA.

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