

# Filament formation and robust strand exchange activities of the rice DMC1A and DMC1B proteins

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## ABSTRACT

The DMC1 protein, a meiosis-specific DNA recombinase, catalyzes strand exchange between homologous chromosomes. In rice, two *Dmc1* genes, *Dmc1A* and *Dmc1B*, have been reported. Although the *Oryza sativa* DMC1A protein has been partially characterized, however the biochemical properties of the DMC1B protein have not been defined. In the present study, we expressed the *Oryza sativa* DMC1A and DMC1B proteins in bacteria and purified them. The purified DMC1A and DMC1B proteins formed helical filaments along single-stranded DNA (ssDNA) and double-stranded DNA (dsDNA), and promoted robust strand exchange between ssDNA and dsDNA over five thousand base pairs in the presence of RPA, as a co-factor. The DMC1A and DMC1B proteins also promoted strand exchange in the absence of RPA with long DNA substrates containing several thousand base pairs. In contrast, the human DMC1 protein strictly required RPA to promote strand exchange with these long DNA substrates. The strand-exchange activity of the *Oryza sativa* DMC1A protein was much higher than that of the DMC1B protein. Consistently, the DNA-binding activity of the DMC1A protein was higher than that of the DMC1B protein. These biochemical differences between the DMC1A and DMC1B proteins may provide important insight into their functional differences during meiosis in rice.

## INTRODUCTION

Meiosis combines two successive rounds of nuclear division, meiosis I and meiosis II, with a single round of DNA replication to produce haploid gametes from diploid cells in eukaryotes (1). Meiotic homologous recombination occurs between homologous chromosomes during cell division at meiosis I. This recombination between homologous chromosomes ensures their correct segregation at meiosis I through the formation of chiasmata, which physically connect homologous chromosomes (1–3).

Meiotic homologous recombination is initiated by the formation of a double-strand break (DSB), which is introduced by the SPO11 protein (4–6). Single-stranded DNA (ssDNA) tails are then produced at the DSB site, and they invade the homologous double-stranded DNA (dsDNA). This strand-invasion step, called homologous pairing, forms new Watson–Crick base pairs (heteroduplex) between the invading strand and its complementary strand of parental dsDNA. After this homologous-pairing step, the heteroduplex region is expanded by the subsequent strand-exchange step, to ensure correct pairing between homologous chromosomes by extensive homology matching between paired chromosomes. The *Escherichia coli* RecA protein was the first enzyme found to catalyze the homologous-pairing and strand-exchange steps (7–10). Two RecA homologues, the RAD51 and DMC1 proteins, have been identified in eukaryotes (11–14), and they have also been shown to catalyze homologous pairing and strand exchange (15–21).

The RAD51 and DMC1 proteins bind to ssDNA produced at DSB sites, and form extensive helical

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nucleoprotein filaments along the DNA (20–25), like the RecA protein (26,27). These nucleoprotein filaments capture intact dsDNA, and form the ternary complex containing ssDNA and dsDNA within the filament. The homologous-pairing and strand-exchange reactions are accomplished by the ternary complex. Therefore, the ability to form a filament along DNA is an important property for a RecA-type recombinase, such as the RAD51 and DMC1 proteins.

The RAD51 protein is expressed in both meiotic and mitotic cells (11,13), but the DMC1 protein is only present in meiotic cells (12,14), suggesting that the DMC1 protein functions as a specific factor for meiotic homologous recombination. The DMC1 protein was first discovered in yeast (12), and subsequently has been found in many mammals (14) and plants (28–35). The knockout of the *dmc1* gene in the mouse causes asynapsis and sterility (36,37), as in *Dmc1*-deficient yeast (12), and, in *Arabidopsis*, mutations in the *Dmc1* gene result in meiotic defects (38). These facts indicate that the DMC1 protein is actually an essential factor for meiotic recombination.

The yeast and mammalian genomes contain only one *Dmc1* gene. Among plants, the *Arabidopsis* genome also contains a single *Dmc1* gene. On the other hand, in rice, two *Dmc1* genes, *Oryza sativa Dmc1A* and *Dmc1B*, which are located on chromosomes 12 and 11, respectively, have been reported (32–35). An RNA interference study indicated that the *Dmc1A* and *Dmc1B* genes are required for the pairing between homologous chromosomes during meiosis in rice (39). The individual functions of these *Oryza sativa Dmc1A* and *Dmc1B* genes have not been clarified yet, but differences in their expression profiles during meiosis have been reported (34). Therefore, the rice DMC1A and DMC1B proteins may have some distinct functions during meiosis.

Previously, the *Oryza sativa* DMC1A protein, purified under denaturing conditions, was found to possess homologous-pairing activity with short oligonucleotides (40,41); however, its recombinase activity, such as filament formation and extensive strand exchange, has not been studied yet. In addition, no biochemical experiments have been performed with the *Oryza sativa* DMC1B protein. Therefore, in the present study, we purified the *Oryza sativa* DMC1A and DMC1B proteins without denaturation to evaluate their recombinase activities. We found that the purified DMC1A and DMC1B proteins formed helical filaments along DNA, and exhibited robust strand-exchange activities *in vitro*.

## MATERIALS AND METHODS

### Protein preparations

The *Oryza sativa Dmc1A* and *Dmc1B* genes were expressed even in rice somatic cells, which were maintained on culture medium containing an auxin, 2,4-dichlorophenoxyacetic acid (2,4-D) (33,34). Accordingly, full-length cDNA sequences encoding the *Oryza sativa* DMC1A and DMC1B proteins (NCBI accession nos. AB079873 and AB079874, respectively) were amplified from total RNA preparations from the cultured cells of rice (*O. sativa*,

cv. Nipponbare), by using the standard protocols for reverse transcription PCR (RT-PCR) and rapid amplification of cDNA ends (5'-RACE and 3'-RACE). The DNA fragments containing the *Oryza sativa Dmc1A* and *Dmc1B* coding sequences were then inserted into the pZER0-2 plasmid (Invitrogen, Carlsbad, CA, USA), and were further subcloned into the NdeI-BamHI sites of the pET-15b vector (Novagen, Darmstadt, Germany). These *Oryza sativa* DMC1A and DMC1B expression vectors were introduced into the *Escherichia coli* strain BL21-CodonPlus(DE3)-RIL (Stratagene, La Jolla, CA, USA) or the *E. coli recAA* strain BLR(DE3) (Novagen, Darmstadt, Germany) supplemented with the CodonPlus-RIL plasmid. The cells harboring the DMC1A and DMC1B expression vectors were cultured at 37°C in 5l of LB medium supplemented with 0.5% glucose, ampicillin and chloramphenicol. The protein expression was induced by adding IPTG to a final concentration of 1 mM, when the OD<sub>600</sub> of the culture was about 0.5, and the incubation temperature was decreased to 25°C. We tested the induction of DMC1A or DMC1B protein expression at 18, 25 and 30°C, and confirmed that there are few differences in the protein production under these temperature conditions. We then compared the solubility of the DMC1A proteins produced under the 18 and 25°C conditions, and found that the protein generated under the 25°C conditions bound more effectively to the Ni-beads. Therefore, we employed the 25°C conditions for the protein expression. The cells were harvested after an overnight incubation (12–18 h), and the cell pellet was frozen at –80°C. The DMC1A and DMC1B proteins were produced as hexahistidine-tagged proteins. The cell pellet was thawed, and was suspended in buffer A [50 mM Tris-HCl (pH 8.0 at 25°C), 500 mM NaCl, 1 mM DTT, 10% glycerol] supplemented with 10 mM imidazole and 1 mM PMSF. The cells were disrupted by sonication, and the insoluble fraction was removed by centrifugation at 4°C. The supernatant was mixed with 2 ml of Ni-Sepharose 6 Fast Flow resin (GE Healthcare Bio-sciences, Uppsala, Sweden) for 60 min at 4°C, and was packed into an empty column. The hexahistidine-tagged DMC1A and DMC1B proteins bound to the Ni-Sepharose resin were eluted with an 80 ml linear gradient of 10–500 mM imidazole in buffer A. Fractions containing the DMC1A and DMC1B protein were collected, and the hexahistidine tag was removed with thrombin protease (1 unit per mg of the DMC1A or DMC1B protein; GE Healthcare Bio-sciences, Uppsala, Sweden) during overnight dialysis against buffer B [20 mM Tris-HCl (pH 8.0 at 25°C), 2 mM 2-mercaptoethanol, 0.25 mM EDTA, and 10% glycerol] supplemented with 400 mM KCl (for the DMC1A protein) or 100 mM KCl (for the DMC1B protein). Removal of the hexahistidine tag was confirmed by SDS-PAGE. The DMC1A sample, but not the DMC1B sample, was centrifuged at 10 000 r.p.m. for 5 min to remove aggregates, and the supernatant was diluted with the same volume of buffer C [20 mM Tris-HCl (pH 8.0 at 25°C), 1 mM DTT, 0.25 mM EDTA and 10% glycerol]. The sample was then applied to a ToyoScreen AF-Heparin HC-650M 5-ml column (TOSOH, Tokyo, Japan). The DMC1A and DMC1B proteins were eluted with a 40 ml linear gradient of

0.2–1.1 M KCl (0.1–1.1 M KCl for the DMC1B protein) in buffer C, and the fractions containing the proteins were collected. The KCl concentration of the DMC1A and DMC1B protein solutions was adjusted to 500 mM with buffer C containing 2 M KCl, and the proteins were concentrated with an Amicon Ultra-15 centrifugal filter device (30 000 NMWL; Millipore, Billerica, MA, USA). The purified DMC1A and DMC1B proteins were dialyzed against buffer D [20 mM HEPES-KOH (pH 7.5 at 25°C), 500 mM KCl, 1 mM DTT and 10% glycerol]. Aliquots of the purified DMC1A and DMC1B proteins were frozen in liquid N<sub>2</sub>, and were stored at –80°C. We confirmed that freezing did not affect the DNA-binding activities of the proteins. The human DMC1 protein was purified by the method described previously (42), except for the use of Ni-Sepharose resin and a ToyoScreen AF Heparin column (TOSOH, Tokyo, Japan), instead of the Ni-NTA agarose resin (QIAGEN, Hilden, Germany) and the Heparin-Sepharose column (GE Healthcare Bio-sciences, Uppsala, Sweden), respectively. Human RPA was expressed in *E. coli* cells, and was purified according to the published protocol (43). The protein concentration was determined using the Bradford method, with bovine serum albumin (Nacalai Tesque, Kyoto, Japan) as the standard.

#### MALDI-TOF-MS analysis

One microliter of 5% trifluoroacetic acid (TFA) was added to 10 µl of a 1 mg/ml *Oryza sativa* DMC1A or DMC1B protein sample, and the mixture was bound to a ZipTip (C18) pipette tip (Millipore, Billerica, MA, USA). After the protein was bound, the tip was first rinsed with 0.1% TFA and 5% methanol, and then rinsed with 0.1% TFA. The DMC1A or DMC1B protein was eluted with 0.1% TFA and 80% acetonitrile, and was deposited onto the sample plate, which was precoated with a dried layer of sinapic acid (Sigma-Aldrich, St Louis, MO, USA). A MALDI-TOF mass spectrometry analysis was performed with an AXIMA-CFR mass spectrometer (Kratos Analytical, Manchester, UK). Bovine carbonic anhydrase II was used as a standard protein for external calibration. Each single spectrum was obtained as an accumulation of 250 laser shots.

#### DNA substrates

ϕX174 virion DNA (circular ssDNA) and ϕX174 RF I DNA (supercoiled dsDNA) were purchased from New England Biolabs (Ipswich, MA, USA). ϕX174 linear dsDNA was prepared by digesting ϕX174 RF I DNA at the PstI site. All DNA concentrations are expressed in moles of nucleotides.

#### The strand-exchange assay

The *Oryza sativa* DMC1A protein, the DMC1B protein, and the human DMC1 protein were each incubated with 25 µM ϕX174 circular ssDNA at 37°C for 10 min. After this incubation, 1 µl of 20 µM RPA was added to the reaction mixture, which was incubated at 37°C for 10 min. The reactions were then initiated by the addition of 1 µl of 200 µM ϕX174 linear dsDNA in 10 µl of 28 mM

HEPES-KOH buffer (pH 7.5), containing 200 mM KCl, 1 mM DTT, 1 mM ATP, 1 mM MgCl<sub>2</sub>, 3% glycerol, 0.1 mg/ml bovine serum albumin, 20 mM creatine phosphate and 75 µg/ml creatine kinase, and were continued for 1 h. It should be noted that a trace amount of nicked circular dsDNA, which was not visible by ethidium bromide staining but was detected by SYBR Gold staining, was present as a contaminant in the ϕX174 linear dsDNA used in this study. The reactions were stopped by the addition of 0.08% SDS and 0.4 mg/ml proteinase K (Roche Applied Science, Basel, Switzerland), and the samples were further incubated at 37°C for 20 min. The deproteinized reaction products were separated by 1% agarose gel electrophoresis in 1× TAE buffer (ice-cold, 40 mM Tris-acetate and 1 mM EDTA) at 3.0 V/cm for 4 h. The products were visualized by SYBR Gold (Invitrogen, Carlsbad, CA, USA) staining. Band intensities were quantified by an LAS-1000 imaging analyzer (Fujifilm, Tokyo, Japan) equipped with the Image Gauge software (44).

#### Assays for DNA binding

The ϕX174 circular ssDNA (10 µM) or the linear ϕX174 dsDNA (10 µM) was mixed with the *Oryza sativa* DMC1A protein, the DMC1B protein or the human DMC1 protein in 10 µl of a standard reaction solution, containing 20 mM HEPES-KOH (pH 7.5), 1 mM DTT, 0.1 mg/ml bovine serum albumin, 1 mM MgCl<sub>2</sub>, 200 mM KCl, 4% glycerol and 1 mM ATP. The reaction mixtures were incubated at 37°C for 10 min, and were then analyzed by 0.8% agarose gel electrophoresis in 1× TAE buffer at 3.0 V/cm for 3 h. The bands were visualized by SYBR Gold (for ssDNA) or ethidium bromide (for dsDNA) staining. In the competitive DNA binding assay, the ϕX174 circular ssDNA (20 µM) and the supercoiled ϕX174 dsDNA (20 µM) were mixed with the *Oryza sativa* DMC1A protein, the DMC1B protein, or the human DMC1 protein in 10 µl of a standard reaction solution, containing 28 mM HEPES-KOH (pH 7.5), 200 mM KCl, 1 mM DTT, 1 mM ATP, 1 mM MgCl<sub>2</sub>, 4% glycerol and 0.1 mg/ml bovine serum albumin. The reaction mixtures were incubated at 37°C for 10 min, and were then analyzed by 1% agarose gel electrophoresis in 1× TAE buffer at 3.0 V/cm for 4 h. The bands were visualized by SYBR Gold staining.

#### ATPase activity

The *Oryza sativa* DMC1A protein, the DMC1B protein, and the human DMC1 protein, purified from the *E. coli* *recAΔ* strain BLR(DE3) (Novagen, Darmstadt, Germany), were each incubated with 1 mM ATP (Roche, ATP sodium salt) in 28 mM HEPES-KOH buffer (pH 7.5), containing 200 mM KCl, 1 mM MgCl<sub>2</sub>, 1 mM DTT, 4% glycerol and 0.1 mg/ml bovine serum albumin, in the presence or absence of ssDNA (40 µM) or dsDNA (40 µM). The reactions were performed at 37°C. After a 10 min pre-incubation in the absence of ATP, the reactions were initiated by adding 1 mM ATP. At the indicated times, a 20 µl aliquot of the reaction mixture was mixed with 30 µl of 100 mM EDTA to quench the reaction. The amount of inorganic phosphate released

was determined by a colorimetric assay, as described previously (45).

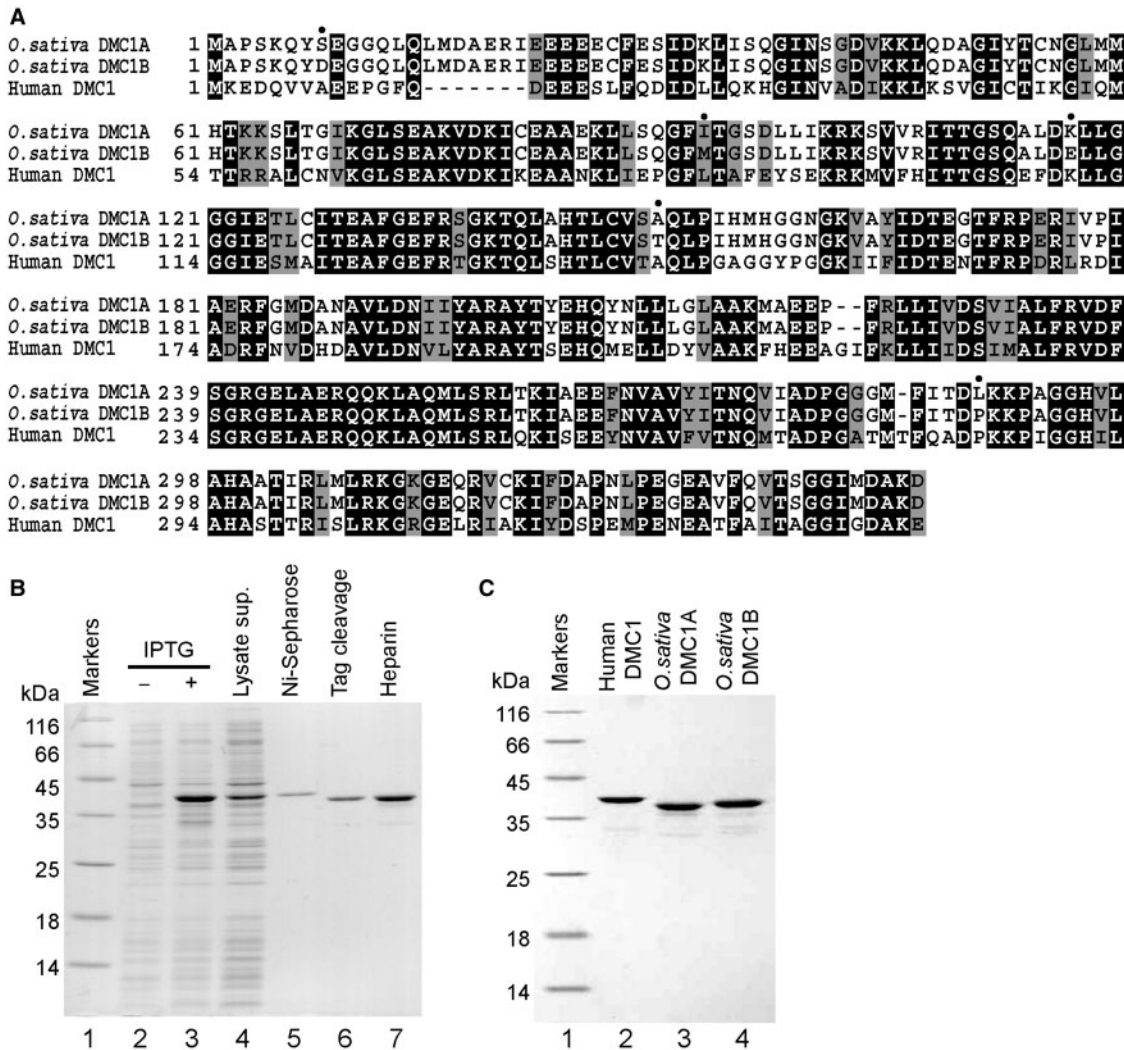
**Electron microscopy**

The *Oryza sativa* DMC1A and DMC1B proteins (1 μM) were each mixed with φX174 replicative form II DNA (1 μM) or φX174 circular ssDNA (1 μM) in 20 mM HEPES-KOH buffer (pH 7.5), containing 200 mM KCl, 1 mM MgCl<sub>2</sub>, 1 mM DTT, 4% glycerol and 1 mM ATP or 1 mM ATPγS, and were incubated for 15 min at 37°C. Samples (3 μl) were adsorbed on a carbon coated Cu grid (400 meshes per inch), which was subjected to glow discharge for 20s, and were stained with 2% uranium acetate. The samples were examined with a JEM2000FX electron microscope (JEOL, Tokyo, Japan).

**RESULTS**

**Purification of the *Oryza sativa* DMC1A and DMC1B proteins without denaturation**

The rice genome encodes two *Dmc1* genes, *Dmc1A* and *Dmc1B* (32–35). In the present study, we purified the *Oryza sativa* DMC1A and DMC1B proteins. The *Oryza sativa* *Dmc1A* and *Dmc1B* genes were cloned by RT-PCR, and the predicted amino acid sequences are shown in Figure 1A. The DMC1A protein was overexpressed in *Escherichia coli* cells as a fusion protein, with an N-terminal hexahistidine tag (His<sub>6</sub> tag) containing a cleavage site for thrombin protease (Figure 1B, lane 3). Although a substantial amount of the DMC1A protein was present in the soluble fraction, a large amount of the protein still remained in the insoluble fraction (Figure 1B, lane 4).



**Figure 1.** Purification of the *Oryza sativa* DMC1A and DMC1B proteins. (A) Amino acid sequences of the *Oryza sativa* DMC1A and DMC1B proteins, and the human DMC1 protein. The sequences were aligned with the ClustalX software (65), and the figure was produced with the BOXSHADE program. Black and gray boxes indicate identical and similar amino acid residues, respectively. Black dots above the sequences denote the non-conserved amino acid residues between the DMC1A and DMC1B proteins. (B) Purification of the DMC1A protein. Proteins from each purification step were analyzed by SDS-PAGE with Coomassie Brilliant Blue staining. Lane 1, the molecular mass markers; lane 2, the whole cell lysate without IPTG; lane 3, the whole cell lysate with IPTG; lane 4, the soluble fraction of the cell lysates; lane 5, the Ni-Sepharose fraction; lane 6, the Ni-Sepharose fraction after thrombin protease treatment; lane 7, the ToyoScreen AF-Heparin HC-650M fraction. (C) The purified *Oryza sativa* DMC1A, *Oryza sativa* DMC1B and human DMC1 proteins. Lane 1, the molecular mass markers; lane 2, the human DMC1 protein (1 μg); lane 3, the *Oryza sativa* DMC1A protein (1 μg); lane 4, the *Oryza sativa* DMC1B protein (1 μg).

We purified the DMC1A protein from the soluble fraction by a method including Ni-Sepharose column chromatography, removal of the hexahistidine tag from the DMC1A portion with thrombin protease, and ToyoScreen AF-Heparin column chromatography (Figure 1B, lanes 5–7). The DMC1B protein was also purified by a procedure similar to that used for the DMC1A protein (Figure 1C).

We then measured the molecular masses of these *Oryza sativa* DMC1A and DMC1B proteins by mass spectroscopic analyses. The molecular masses predicted from the amino acid sequences of the recombinant DMC1A and DMC1B proteins are 37824 daltons and 37885 daltons, respectively. We performed the mass spectroscopic measurements six times. The average molecular mass of the DMC1A protein was 37817 daltons, with a standard deviation (SD) of 14.9. On the other hand, the average molecular mass of the DMC1B protein was 37903 daltons, with an SD value of 22.1. These results indicated that the purification method established in this study produced the correct DMC1A and DMC1B proteins.

As shown in Figure 2A, the purified DMC1A and DMC1B proteins hydrolyzed ATP in the presence of ssDNA. To eliminate the possibility of RecA contamination in the purified DMC1A and DMC1B fractions, we tested the ssDNA-dependent ATPase activities of the DMC1A and DMC1B proteins purified from *E. coli* cells lacking the *recA* gene. Like the human DMC1 protein, the DMC1A and DMC1B proteins hydrolyzed ATP less efficiently in the presence of dsDNA (Figure 2B). The  $k_{\text{cat}}$  values of the ssDNA-dependent ATP hydrolysis by the DMC1A and DMC1B proteins were about  $0.7 \text{ min}^{-1}$  and  $0.8 \text{ min}^{-1}$ , respectively. The reported  $k_{\text{cat}}$  values of the human DMC1 protein were  $1.5 \text{ min}^{-1}$  (18) and  $0.6 \text{ min}^{-1}$  (21), while that of the *Saccharomyces cerevisiae* Dmc1 protein was  $0.7 \text{ min}^{-1}$  (19). Therefore, the DMC1A and DMC1B proteins possess similar levels of ATPase activity, as compared to the human DMC1 and *S. cerevisiae* Dmc1 proteins.

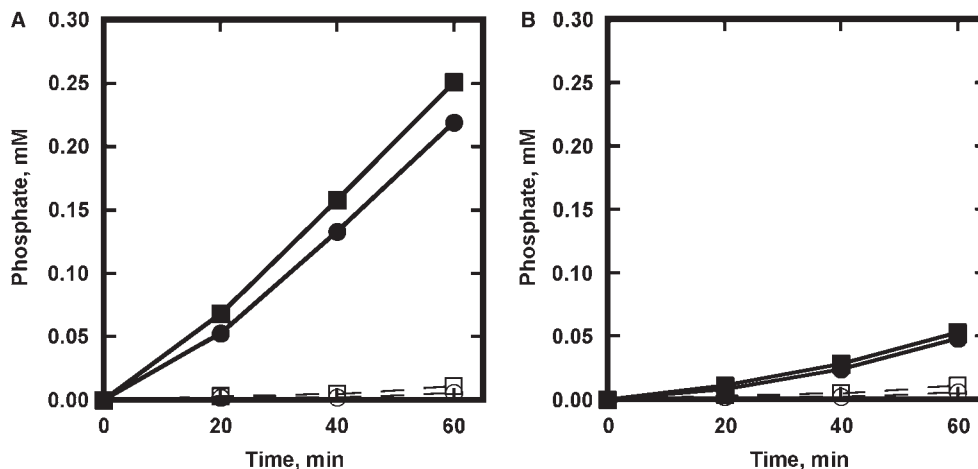
### Filament formation by the *Oryza sativa* DMC1A and DMC1B proteins

The DMC1 protein alone reportedly forms a ring structure (20,25,42,46), and binds to DNA as stacked rings (20,21,25,46,47). The DMC1 protein also forms a helical filament structure on DNA in the presence of ATP (20,21). The DMC1 filament formed along ssDNA in an ATP-dependent manner is considered to be the active form for homologous pairing and strand exchange (20). We tested whether the *Oryza sativa* DMC1A and DMC1B proteins could form the helical filament along ssDNA. Our electron microscopic analyses revealed that the DMC1A and DMC1B proteins formed helical filaments with ssDNA in the presence of ATP (Figure 3A and B). The formation of nucleoprotein filaments by the DMC1A and DMC1B proteins with ssDNA was also observed in the presence of ATP $\gamma$ S (Figure 3C and D), but not in the absence of nucleotide cofactors. DMC1A and DMC1B also formed filaments with dsDNA (Figure 3E). Therefore, these results indicated that the DMC1A and DMC1B proteins form nucleoprotein filaments along DNA, like the human DMC1 protein.

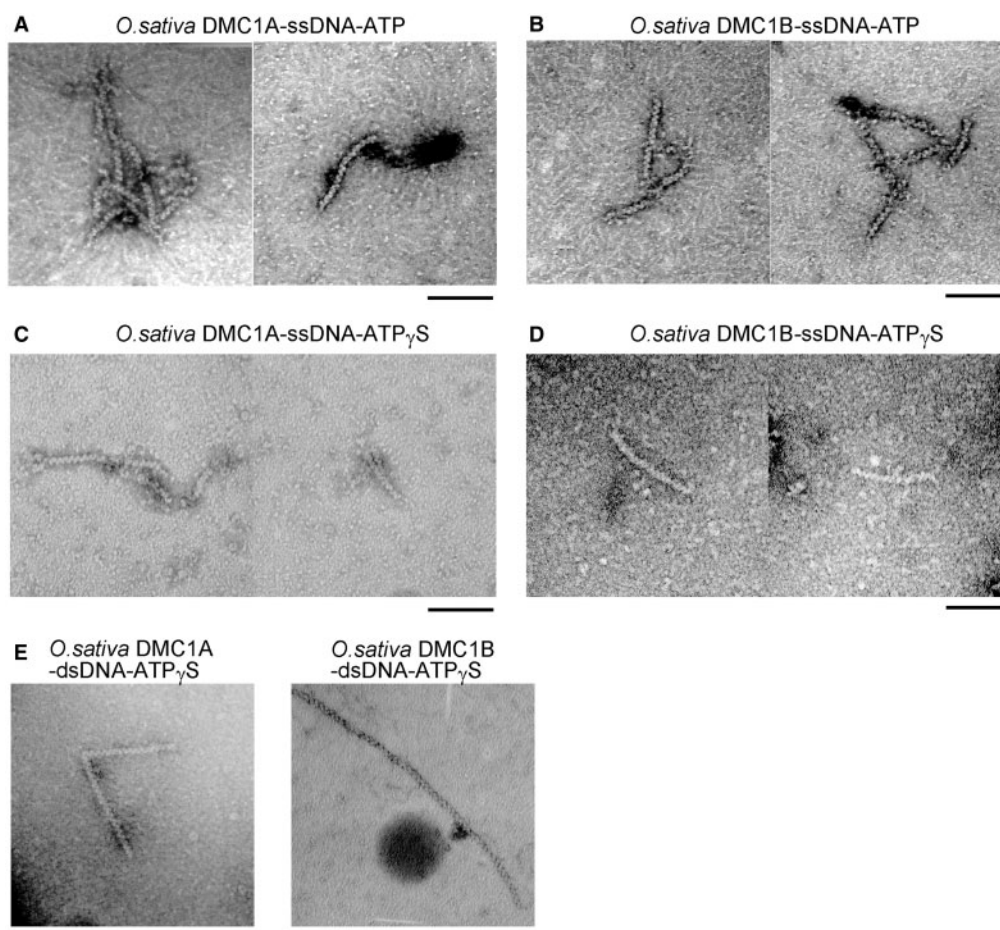
### Strand exchange activity of the *Oryza sativa* DMC1A and DMC1B proteins

We further studied the strand-exchange activities of the DMC1A and DMC1B proteins. The DMC1A protein reportedly promoted strand exchange with short oligonucleotides (41). In the present study, we studied the reaction with DNA substrates containing several thousand base pairs, in which extensive unidirectional migration of the DNA branch formed between ssDNA and dsDNA is promoted (9,10).

To test the extent of the strand-exchange activity of the DMC1A and DMC1B proteins,  $\phi$ X174 phage circular ssDNA (5386 bases) and linearized  $\phi$ X174 dsDNA (5386 base pairs) were used as DNA substrates. With this combination of DNA substrates, two reaction



**Figure 2.** The ATPase activities of the *Oryza sativa* DMC1A and DMC1B proteins. (A) The ssDNA-dependent ATPase activities of the DMC1A and DMC1B proteins. Time course experiments are shown. Circles and squares indicate experiments with the *Oryza sativa* DMC1A and DMC1B proteins, respectively. Closed symbols with solid lines and open symbols with broken lines indicate experiments in the presence and absence of ssDNA, respectively. (B) The dsDNA-dependent ATPase activities of the DMC1A and DMC1B proteins. Time course experiments are shown. Closed symbols with solid lines and open symbols with broken lines indicate experiments in the presence and absence of dsDNA, respectively.



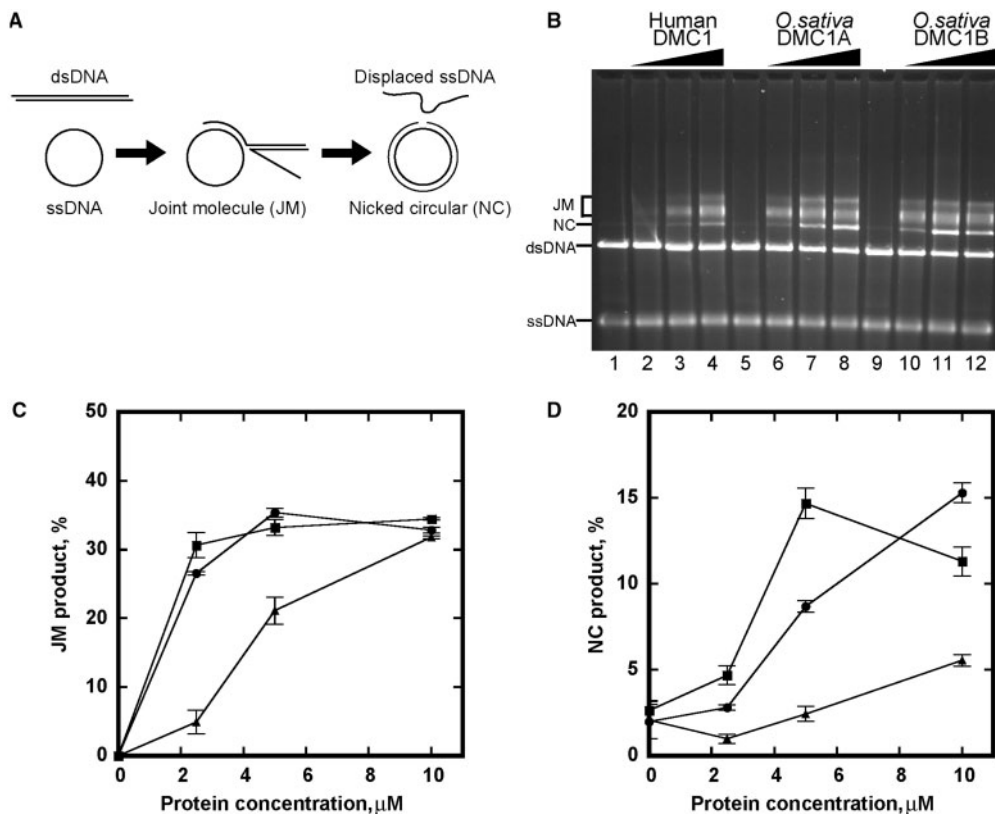
**Figure 3.** Electron microscopic images of the *Oryza sativa* DMC1A and DMC1B filaments. The DMC1A (A and C) and DMC1B (B and D) filaments in the presence of  $\phi$ X174 circular ssDNA and ATP (A and B) or ATP $\gamma$ S (C and D). (E) The DMC1A and DMC1B filaments in the presence of  $\phi$ X174 form II DNA and ATP $\gamma$ S. The black bar denotes 100 nm.

products were detected: a joint molecule between ssDNA and dsDNA (JM) and a nicked circular DNA (NC) (Figure 4A). The strand exchange must be promoted over a span of five thousand base pairs to form the NC product, a complete strand-exchange product, which is generated by the extensive branch migration activity of the DMC1 proteins (Figure 4A). The strand-exchange reactions were conducted in the presence of RPA (2  $\mu$ M), which is an essential factor for the strand exchange mediated by the human DMC1 protein (20,21).

As shown in Figure 4B–D, the DMC1A and DMC1B proteins exhibited robust strand-exchange activity. The strand-exchange activity of the DMC1A and DMC1B proteins was not detected in the absence of ATP (Supplementary Figure 1). The strand-exchange reactions were conducted in the presence of 200 mM KCl, which is required to efficiently promote the strand-exchange reaction by the human DMC1 protein (20). We tested the KCl requirement for the *Oryza sativa* DMC1A protein, and found that a 200 mM KCl concentration is also suitable for the strand-exchange reaction by the protein (Supplementary Figure 2). A significant amount of the NC product was formed in the reactions containing the

DMC1A and DMC1B proteins (Figure 4B and D), as compared to the reactions with the human DMC1 protein, indicating that the DMC1A and DMC1B proteins could promote strand exchange over five thousand base pairs. The human DMC1 protein purified from insect cells reportedly formed the strand-exchange products with about 25% of the input DNAs, in the presence of excess amounts of the protein (20). Our human DMC1 protein purified from bacterial cells formed the JM products with about 30% of the input DNAs, with excess amounts of the protein (10  $\mu$ M), indicating that the human DMC1 protein used for the control experiments of this study was as active as the protein purified from insect cells (20).

No synergistic action in the strand-exchange reaction was observed when the DMC1A and DMC1B proteins co-existed in various stoichiometries, although an additive effect was seen (Figure 5A). Intriguingly, the DMC1A and DMC1B proteins promoted strand exchange in the absence of RPA, whereas the human DMC1 protein strictly required RPA to promote strand exchange (Figure 5B). Therefore, the DMC1A and DMC1B proteins promote strand exchange more efficiently than the human DMC1 protein.



**Figure 4.** The strand-exchange activities of the *Oryza sativa* DMC1A and DMC1B proteins. (A) A schematic diagram of the strand-exchange assay. (B) The strand-exchange assay in the presence of RPA. The *Oryza sativa* DMC1A, *Oryza sativa* DMC1B and human DMC1 proteins were each incubated with  $\phi$ X174 circular ssDNA at 37°C for 10 min. After the addition of RPA,  $\phi$ X174 linear dsDNA was added to initiate the reaction. The reactions were continued for 60 min. The DNA products were then deproteinized, and were separated by agarose gel electrophoresis. The joint molecules and the nicked circular products are indicated by JM and NC, respectively. Lanes 1–4, human DMC1; lanes 5–8, DMC1A; lanes 9–12, DMC1B. The concentrations of the DMC1A, DMC1B and human DMC1 proteins were 0  $\mu$ M (lanes 1, 5 and 9), 2.5  $\mu$ M (lanes 2, 6 and 10), 5  $\mu$ M (lanes 3, 7 and 11), and 10  $\mu$ M (lanes 4, 8 and 12). (C and D) The band intensities of the JM (C) and NC (D) products in panel (B) were quantified as the peak volumes of densitometric scans. The ratios (%) of the products relative to the sum total of the peak volumes of all DNAs are plotted against the protein concentration. Average values from three independent experiments are shown with the standard error values. Circles, squares and triangles indicate experiments with the DMC1A, DMC1B and human DMC1 proteins, respectively.

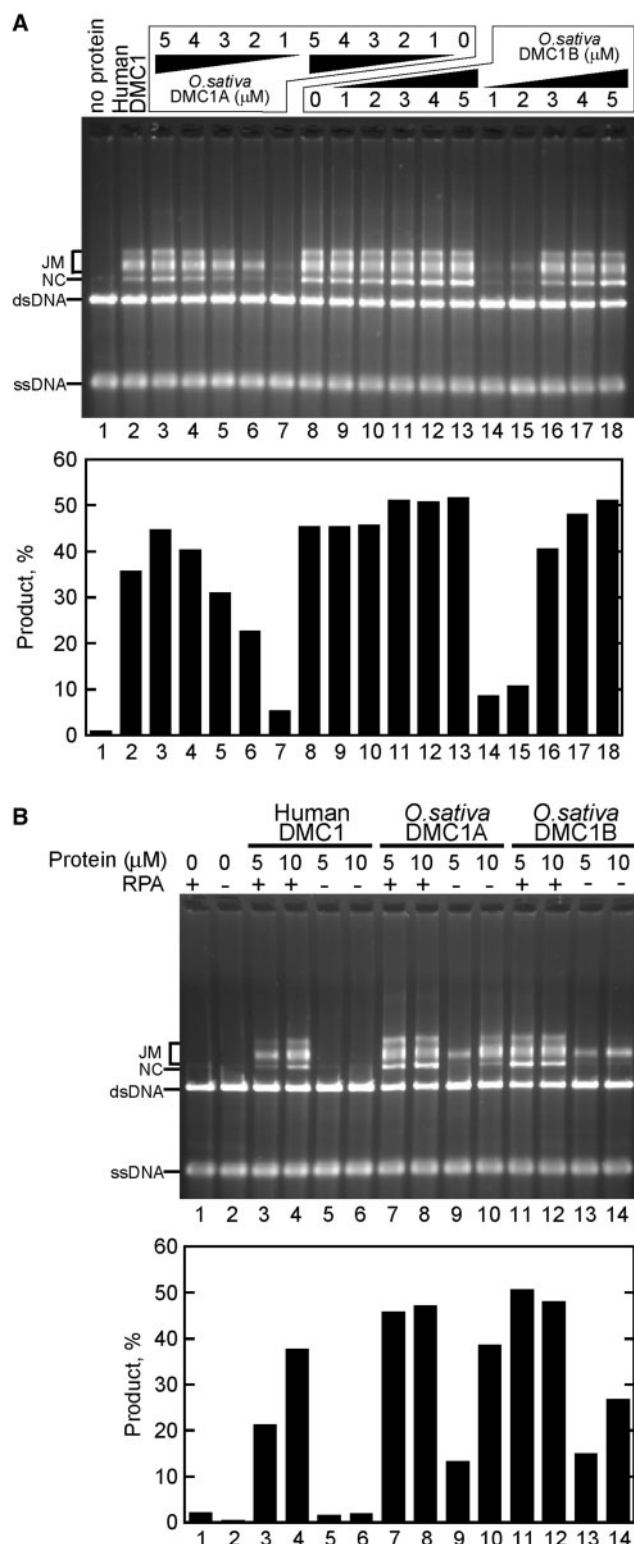
#### DNA-binding activity of the *Oryza sativa* DMC1A and DMC1B proteins

We next tested the DNA-binding activities of the DMC1A and DMC1B proteins. A gel mobility shift assay revealed that both the DMC1A and DMC1B proteins bound to ssDNA in the presence of ATP with higher affinity than the human DMC1 protein (Figure 6A). These DNA-binding properties of the DMC1A and DMC1B proteins were not affected by omitting ATP (Supplementary Figure 3). Under these reaction conditions, 4  $\mu$ M of the DMC1A and DMC1B proteins saturated 10  $\mu$ M of the ssDNA (Figure 6A, lanes 9 and 14). Therefore, the DMC1A and DMC1B proteins bind to ssDNA with a stoichiometry of about 3 nucleotides per monomer, like the other RecA-family proteins. DMC1A and DMC1B concentrations of about 1.0  $\mu$ M were sufficient for binding 50% of the input ssDNA to the protein (Figure 6B). On the other hand, the dsDNA-binding activities of the DMC1A and DMC1B proteins were not significant, as compared to that of the human DMC1 protein (Figure 6C and D). The DMC1A-DNA complexes migrated more slowly than the DMC1B-DNA complexes (Figure 6A and C).

These results suggested that the DMC1A protein binds to DNA more cooperatively than the DMC1B protein. Consistently, the strand-exchange activity of the DMC1A protein was also higher than that of the DMC1B protein, under the conditions without RPA (Figure 5B). A competitive DNA-binding assay revealed that the DMC1A and DMC1B proteins preferentially bound to ssDNA under the conditions used in the strand-exchange experiments (Figure 7), although the bands corresponding to the DMC1B-ssDNA complexes overlapped with the free dsDNA bands (lanes 10–12). In contrast, the human DMC1 protein did not exhibit a binding preference to either ssDNA or dsDNA under these reaction conditions (Figure 7, lanes 2–4). The high affinity binding and the marked preference for ssDNA by the DMC1A and DMC1B proteins may alleviate the requirement for RPA during the strand-exchange reaction.

#### DISCUSSION

In the homologous recombination processes, the strand-exchange step may play an essential role to ensure the



**Figure 5.** Additive action and effect of RPA in the strand-exchange activities of the *Oryza sativa* DMC1A and DMC1B proteins. (A) The strand-exchange assay in the presence of various amounts of the DMC1A and DMC1B proteins. The indicated amounts of the DMC1A and DMC1B proteins (total 5  $\mu$ M) were incubated with  $\phi$ X174 circular ssDNA at 37°C for 10 min. After the addition of RPA,  $\phi$ X174 linear dsDNA was added to initiate the reaction. The reactions were continued for 60 min. Lane 1, no DMC1; lane 2, 10  $\mu$ M human DMC1; lanes 3–8, DMC1A; lanes 9–12, DMC1A and DMC1B mixture, lanes 13–18, DMC1B. (B) The

correct pairing between homologous chromosomes, by searching for extensive spans of homology between paired DNA molecules, just after homologous pairing. Therefore, extensive strand-exchange activity may be especially important for meiotic homologous recombination, in which homologous chromosomes, but not sister chromatids, are utilized as substrates for recombination. The DMC1 and RAD51 proteins are known to promote strand exchange in eukaryotes, and the DMC1 protein may play a specific role in the extensive strand exchange during meiotic homologous recombination. The human DMC1 protein was previously reported to promote extensive strand exchange (20,21). However, extensive strand-exchange activity over several thousand base pairs has not been reported with the other DMC1 orthologues, thus far.

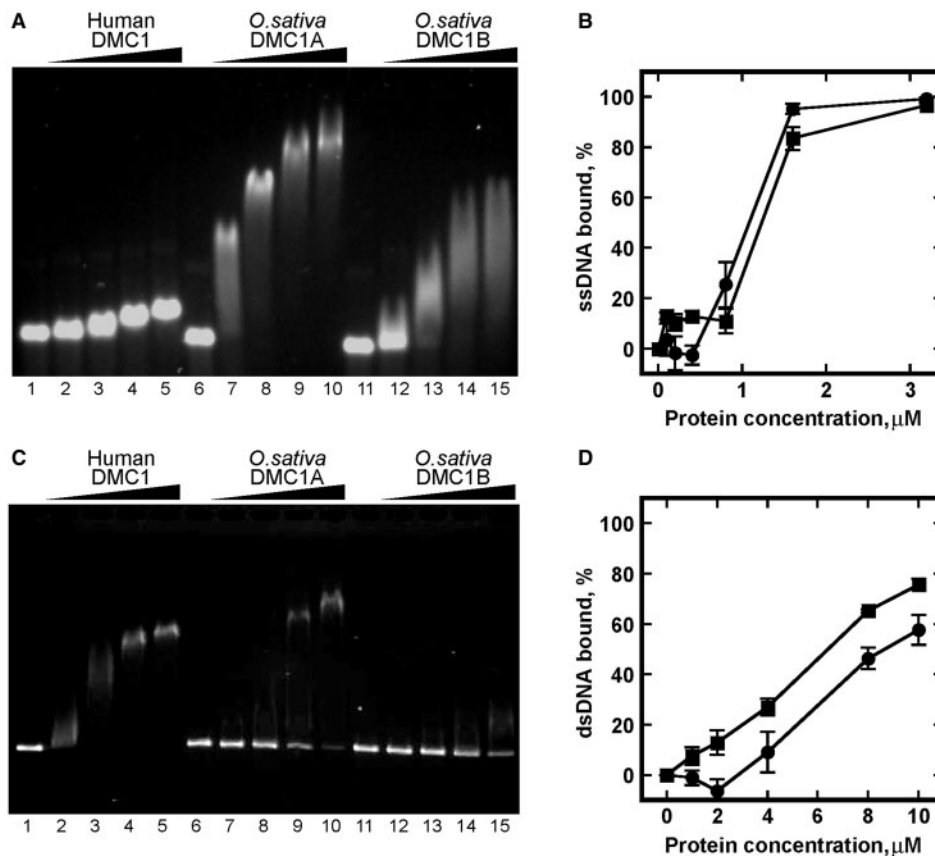
In the present study, we found that the *Oryza sativa* DMC1A and DMC1B proteins exhibited robust strand exchange, and promoted it over five thousand base pairs. Electron microscopic analyses revealed that the DMC1A and DMC1B proteins formed the helical nucleoprotein filament, which is considered as the active form for strand exchange (20,21). Interestingly, the DMC1A and DMC1B proteins did not require RPA to promote strand exchange with long ssDNA and dsDNA substrates, although the human DMC1 protein strictly requires RPA (20,21). These results suggest that the DMC1A and DMC1B filaments possess stronger strand-exchange activity than the human DMC1 protein.

The DMC1 protein is highly conserved among yeasts, vertebrates and plants. Only one *Dmc1* gene was found in genomes of yeasts and vertebrates; however, in rice, two *Dmc1* genes, *Dmc1A* and *Dmc1B*, have been reported (32–35). The *Dmc1A* and *Dmc1B* genes are considered to have duplicated about 7 million years ago (49,50). These two *Dmc1A* and *Dmc1B* genes display different expression profiles during meiosis (34), suggesting that the DMC1A and DMC1B proteins have distinct functions. In the present study, we found that the DMC1A and DMC1B proteins both exhibited robust strand-exchange activity; however, the strand-exchange activity of the DMC1A protein was obviously higher than that of the DMC1B protein. Consistently, the DMC1A protein displayed higher cooperative DNA-binding activity than the DMC1B protein.

The DMC1A and DMC1B proteins used in the present study have only five amino acid differences. These amino acid residues are Ser8/Asp8, Ile93/Met93, Lys117/Glu117, Ala150/Thr150 and Leu288/Pro288 (DMC1A/DMC1B). According to the structures of the RecA/Rad51/Dmc1 family proteins, these residues are expected to be located on the solvent surface. The Leu288/Pro288 residue is located in the L2-DNA binding region, suggesting that this residue may be directly involved in the DNA-binding

strand-exchange assay in the absence of RPA. The DMC1A, DMC1B and human DMC1 proteins were each incubated with  $\phi$ X174 circular ssDNA at 37°C for 10 min. Then,  $\phi$ X174 linear dsDNA was added to initiate the reaction. The reactions were continued for 60 min. Lanes 3–6, human DMC1; lanes 7–10, DMC1A; lanes 11–14, DMC1B. Lanes 1, 3, 4, 7, 8, 11 and 12 are control experiments with RPA. Lanes 2, 5, 6, 9, 10, 13 and 14 are experiments without RPA. The concentrations of the DMC1A, DMC1B and human DMC1 proteins were 0  $\mu$ M (lanes 1 and 2), 5  $\mu$ M (lanes 3, 5, 7, 9, 11 and 13), and 10  $\mu$ M (lanes 4, 6, 8, 10, 12 and 14).





**Figure 6.** The DNA-binding activities of the *Oryza sativa* DMC1A and DMC1B proteins. (A) The ssDNA-binding assay. The  $\phi$ X174 circular ssDNA (10  $\mu$ M) was incubated with the DMC1A, DMC1B or human DMC1 protein at 37°C for 10 min. The samples were analyzed by agarose gel electrophoresis. The bands were visualized by SYBR Gold staining. Lanes 1–5, human DMC1; lanes 6–10, DMC1A; lanes 11–15, DMC1B. The concentrations of the DMC1A, DMC1B and human DMC1 proteins were 0  $\mu$ M (lanes 1, 6 and 11), 1  $\mu$ M (lanes 2, 7 and 12), 2  $\mu$ M (lanes 3, 8 and 13), 4  $\mu$ M (lanes 4, 9 and 14) and 8  $\mu$ M (lanes 5, 10 and 15). (B) Graphic representation of the ssDNA-binding activities of the DMC1A and DMC1B proteins. Protein titration experiments with 0.1–3.2  $\mu$ M DMC1A and DMC1B were performed. Average values from three independent experiments are shown with the standard error values. Circles and squares indicate experiments with the DMC1A and DMC1B proteins, respectively. (C) The dsDNA-binding assay. The  $\phi$ X174 linear dsDNA (10  $\mu$ M) was incubated with the DMC1A, DMC1B or human DMC1 protein at 37°C for 10 min. The bands were visualized by ethidium bromide staining. Lanes 1–5, human DMC1; lanes 6–10, DMC1A; lanes 11–15, DMC1B. The concentrations of the DMC1A, DMC1B and human DMC1 proteins were 0  $\mu$ M (lanes 1, 6 and 11), 1  $\mu$ M (lanes 2, 7 and 12), 2  $\mu$ M (lanes 3, 8 and 13), 4  $\mu$ M (lanes 4, 9 and 14) and 8  $\mu$ M (lanes 5, 10 and 15). (D) Graphic representation of the dsDNA-binding activities of the DMC1A and DMC1B proteins. Protein titration experiments with 1–10  $\mu$ M DMC1A and DMC1B were performed, and average values from three independent experiments are shown with the standard error values. Circles and squares indicate experiments with the DMC1A and DMC1B proteins, respectively.

activity of the DMC1A and DMC1B proteins. The most drastic amino acid difference is between the DMC1A-Lys117 (basic amino acid) and DMC1B-Glu117 (acidic amino acid) residues. The Lys117/Glu117 and Ala150/Thr150 residues are located close to the Walker A-motif for ATP binding. Recently, the crystal structures of RecA-DNA complexes revealed that the ssDNA and the ATP bind to RecA-RecA interfaces within the active filament form (51). Therefore, the Lys117/Glu117 and Ala150/Thr150 residues may indirectly affect the ssDNA binding by the DMC1A and DMC1B proteins. These biochemical differences between the DMC1A and DMC1B proteins found in the present study may be the key to understanding the functional differences of these proteins during meiosis in rice.

The strand-exchange activity of the DMC1 proteins was reportedly stimulated by their cognate co-factors (52–57). These factors include proteins that may function on the chromatin structure, such as RAD54 and its related

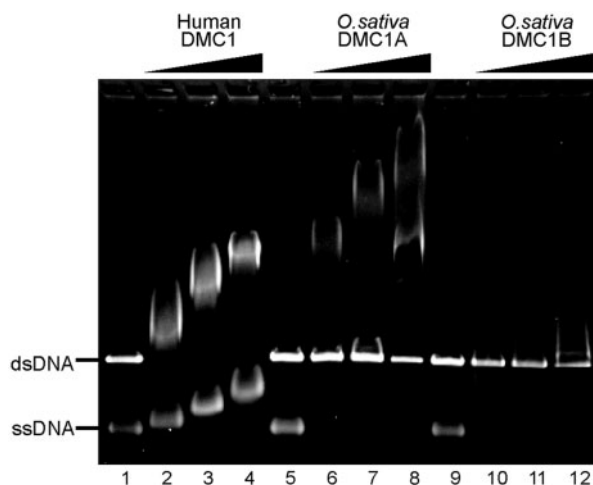
proteins (58–64). It is also possible that the DMC1A and DMC1B proteins interact with different co-factors *in vivo*, and these five amino acid differences may be responsible for such specific interactions of the DMC1A and DMC1B proteins with their cognate co-factors. Proteomics or/and comprehensive two-hybrid analyses will be useful to identify the co-factors that specifically interact with the DMC1A and DMC1B proteins.

#### SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

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**Figure 7.** The competitive DNA-binding assay. The  $\phi$ X174 circular ssDNA (20  $\mu$ M) and the  $\phi$ X174 linear dsDNA (20  $\mu$ M) were incubated with the DMC1A, DMC1B or human DMC1 protein at 37°C for 10 min. The bands were visualized by SYBR Gold staining. Lanes 1–4, human DMC1; lanes 5–8, DMC1A; lanes 9–12, DMC1B. The concentrations of the DMC1A, DMC1B and human DMC1 proteins were 0  $\mu$ M (lanes 1, 5 and 9), 2.5  $\mu$ M (lanes 2, 6 and 10), 5  $\mu$ M (lanes 3, 7 and 11) and 10  $\mu$ M (lanes 4, 8 and 12).

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## REFERENCES

- Petronczki, M., Siomos, M.F. and Nasmyth, K. (2003) Un ménage à quatre: the molecular biology of chromosome segregation in meiosis. *Cell*, **112**, 423–440.
- Neale, M.J. and Keeney, S. (2006) Clarifying the mechanics of DNA strand exchange in meiotic recombination. *Nature*, **442**, 153–158.
- Kleckner, N. (2006) Chiasma formation: chromatin/axis interplay and the role(s) of the synaptonemal complex. *Chromosoma*, **115**, 175–194.
- Keeney, S., Giroux, C.N. and Kleckner, N. (1997) Meiosis-specific DNA double-strand breaks are catalyzed by Spo11, a member of a widely conserved protein family. *Cell*, **88**, 375–384.
- Romanienko, P.J. and Camerini-Otero, R.D. (2000) The mouse Spo11 gene is required for meiotic chromosome synapsis. *Mol. Cell*, **6**, 975–987.
- Baudat, F., Manova, K., Yuen, J.P., Jasin, M. and Keeney, S. (2000) Chromosome synapsis defects and sexually dimorphic meiotic progression in mice lacking Spo11. *Mol. Cell*, **6**, 989–998.
- Shibata, T., DasGupta, C., Cunningham, R.P. and Radding, C.M. (1979) Purified Escherichia coli recA protein catalyzes homologous pairing of superhelical DNA and single-stranded fragments. *Proc. Natl Acad. Sci. USA*, **76**, 1638–1642.
- McEntee, K., Weinstock, G.M. and Lehman, I.R. (1979) Initiation of general recombination catalyzed in vitro by the recA protein of Escherichia coli. *Proc. Natl Acad. Sci. USA*, **76**, 2615–2619.
- Cox, M.M. and Lehman, I.R. (1981) Directionality and polarity in recA protein-promoted branch migration. *Proc. Natl Acad. Sci. USA*, **78**, 6018–6022.
- Kahn, R., Cunningham, R.P., DasGupta, C. and Radding, C.M. (1981) Polarity of heteroduplex formation promoted by Escherichia coli recA protein. *Proc. Natl Acad. Sci. USA*, **78**, 4786–4790.
- Shinohara, A., Ogawa, H. and Ogawa, T. (1992) Rad51 protein involved in repair and recombination in *S. cerevisiae* is a RecA-like protein. *Cell*, **69**, 457–470.
- Bishop, D.K., Park, D., Xu, L. and Kleckner, N. (1992) DMC1: a meiosis-specific yeast homolog of E. coli recA required for recombination, synaptonemal complex formation, and cell cycle progression. *Cell*, **69**, 439–456.
- Shinohara, A., Ogawa, H., Matsuda, Y., Ushio, N., Ikeo, K. and Ogawa, T. (1993) Cloning of human, mouse and fission yeast recombination genes homologous to RAD51 and recA. *Nat. Genet.*, **4**, 239–243.
- Habu, T., Taki, T., West, A., Nishimune, Y. and Morita, T. (1996) 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. *Nucleic Acids Res.*, **24**, 470–477.
- Sung, P. (1994) Catalysis of ATP-dependent homologous DNA pairing and strand exchange by yeast RAD51 protein. *Science*, **265**, 1241–1243.
- Baumann, P., Benson, F.E. and West, S.C. (1996) Human Rad51 protein promotes ATP-dependent homologous pairing and strand transfer reactions in vitro. *Cell*, **87**, 757–766.
- Gupta, R.C., Bazemore, L.R., Golub, E.I. and Radding, C.M. (1997) Activities of human recombination protein Rad51. *Proc. Natl Acad. Sci. USA*, **94**, 463–468.
- Li, Z., Golub, E.I., Gupta, R. and Radding, C.M. (1997) Recombination activities of HsDmcl protein, the meiotic human homolog of RecA protein. *Proc. Natl Acad. Sci. USA*, **94**, 11221–11226.
- Hong, E.L., Shinohara, A. and Bishop, D.K. (2001) Saccharomyces cerevisiae Dmcl protein promotes renaturation of single-strand DNA (ssDNA) and assimilation of ssDNA into homologous super-coiled duplex DNA. *J. Biol. Chem.*, **276**, 41906–41912.
- Sehorn, M.G., Sigurdsson, S., Bussen, W., Unger, V.M. and Sung, P. (2004) Human meiotic recombinase Dmcl promotes ATP-dependent homologous DNA strand exchange. *Nature*, **429**, 433–437.
- Bugreev, D.V., Golub, E.I., Stasiak, A.Z., Stasiak, A. and Mazin, A.V. (2005) Activation of human meiosis-specific recombinase Dmcl by  $Ca^{2+}$ . *J. Biol. Chem.*, **280**, 26886–26895.
- Ogawa, T., Yu, X., Shinohara, A. and Egelman, E.H. (1993) Similarity of the yeast RAD51 filament to the bacterial RecA filament. *Science*, **259**, 1896–1899.
- Benson, F.E., Stasiak, A. and West, S.C. (1994) Purification and characterization of the human Rad51 protein, an analogue of E. coli RecA. *EMBO J.*, **13**, 5764–5771.
- Sung, P. and Robberson, D.L. (1995) DNA strand exchange mediated by a RAD51-ssDNA nucleoprotein filament with polarity opposite to that of RecA. *Cell*, **82**, 453–461.
- Chang, Y.C., Lo, Y.H., Lee, M.H., Leng, C.H., Hu, S.M., Chang, C.S. and Wang, T.F. (2005) Molecular visualization of the yeast Dmcl protein ring and Dmcl-ssDNA nucleoprotein complex. *Biochemistry*, **44**, 6052–6058.
- Shibata, T., Cunningham, R.P., DasGupta, C. and Radding, C.M. (1979) Homologous pairing in genetic recombination: complexes of recA protein and DNA. *Proc. Natl Acad. Sci. USA*, **76**, 5100–5104.
- Flory, J., Tsang, S.S. and Muniyappa, K. (1984) Isolation and visualization of active presynaptic filaments of recA protein and single-stranded DNA. *Proc. Natl Acad. Sci. USA*, **81**, 7026–7030.
- Kobayashi, T., Hotta, Y. and Tabata, S. (1993) Isolation and characterization of a yeast gene that is homologous with a meiosis-specific cDNA from a plant. *Mol. Genet.*, **237**, 225–232.
- Kobayashi, T., Kobayashi, E., Sato, S., Hotta, Y., Miyajima, N., Tanaka, A. and Tabata, S. (1994) Characterization of cDNAs induced in meiotic prophase in lily microsporocytes. *DNA Res.*, **1**, 15–26.
- Sato, S., Hotta, Y. and Tabata, S. (1995) Structural analysis of a recA-like gene in the genome of Arabidopsis thaliana. *DNA Res.*, **2**, 89–93.
- Doutriaux, M.P., Couteau, F., Bergounioux, C. and White, C. (1998) Isolation and characterisation of the RAD51 and DMC1 homologs from Arabidopsis thaliana. *Mol. Gen. Genet.*, **257**, 283–291.

32. Ding,Z., Wang,T., Chong,K. and Bai,S. (2001) Isolation and characterization of OsDMC1, the rice homologue of the yeast DMC1 gene essential for meiosis. *Sex. Plant Reprod.*, **13**, 285–288.
33. Shimazu,J., Matsukura,C., Senda,M., Ishikawa,R., Akada,S., Harada,T., Tabata,S. and Niizeki,M. (2001) Characterization of a DMC1 homologue, *RiLIM15*, in meiotic panicles, mitotic cultured cells and mature leaves of rice (*Oryza sativa* L.). *Theor. Appl. Genet.*, **102**, 1159–1163.
34. Kathiresan,A., Khush,G.S. and Bennett,J. (2002) Two rice DMC1 genes are differentially expressed during meiosis and during haploid and diploid mitosis. *Sex. Plant Reprod.*, **14**, 257–267.
35. Metkar,S.S., Sainis,J.K. and Mahajan,S.K. (2004) Cloning and characterization of the DMC1 genes in *Oryza sativa*. *Curr. Sci.*, **87**, 353–357.
36. Pittman,D.L., Cobb,J., Schimenti,K.J., Wilson,L.A., Cooper,D.M., Brignull,E., Handel,M.A. and Schimenti,J.C. (1998) Meiotic prophase arrest with failure of chromosome synapsis in mice deficient for Dmcl1, a germline-specific RecA homolog. *Mol. Cell*, **1**, 697–705.
37. Yoshida,K., Kondoh,G., Matsuda,Y., Habu,T., Nishimune,Y. and Morita,T. (1998) The mouse RecA-like gene Dmcl1 is required for homologous chromosome synapsis during meiosis. *Mol. Cell*, **1**, 707–718.
38. Couteau,F., Belzile,F., Horlow,C., Grandjean,O., Vezon,D. and Doutriaux,M.P. (1999) Random chromosome segregation without meiotic arrest in both male and female meocytes of a dmcl1 mutant of Arabidopsis. *Plant Cell*, **11**, 1623–1634.
39. Deng,Z. and Wang,T. (2007) OsDMC1 is required for homologous pairing in *Oryza sativa*. *Plant Mol. Biol.*, **65**, 31–42.
40. Kant,C.R., Rao,B.J. and Sainis,J.K. (2005) DNA binding and pairing activity of OsDmcl1, a recombinase from rice. *Plant Mol. Biol.*, **57**, 1–11.
41. Rajanikant,C., Kumbhakar,M., Pal,H., Rao,B.J. and Sainis,J.K. (2006) DNA strand exchange activity of rice recombinase OsDmcl1 monitored by fluorescence resonance energy transfer and the role of ATP hydrolysis. *FEBS J.*, **273**, 1497–1506.
42. Kinebuchi,T., Kagawa,W., Enomoto,R., Tanaka,K., Miyagawa,K., Shibata,T., Kurumizaka,H. and Yokoyama,S. (2004) Structural basis for octameric ring formation and DNA interaction of the human homologous-pairing protein Dmcl1. *Mol. Cell*, **14**, 363–374.
43. Henricksen,L.A., Umbricht,C.B. and Wold,M.S. (1994) Recombinant replication protein A: expression, complex formation, and functional characterization. *J. Biol. Chem.*, **269**, 11121–11132.
44. Ishida,T., Takizawa,Y., Sakane,I. and Kurumizaka,H. (2008) The Lys313 residue of the human Rad51 protein negatively regulates the strand-exchange activity. *Genes Cells*, **13**, 91–103.
45. Matsuo,Y., Sakane,I., Takizawa,Y., Takahashi,M. and Kurumizaka,H. (2006) Roles of the human Rad51 L1 and L2 loops in DNA binding. *FEBS J.*, **273**, 3148–3159.
46. Passy,S.I., Yu,X., Li,Z., Radding,C.M., Masson,J.Y., West,S.C. and Egelman,E.H. (1999) Human Dmcl1 protein binds DNA as an octameric ring. *Proc. Natl Acad. Sci. USA*, **96**, 10684–10688.
47. Masson,J.Y., Davies,A.A., Hajibagheri,N., Van Dyck,E., Benson,F.E., Stasiak,A.Z., Stasiak,A. and West,S.C. (1999) The meiosis-specific recombinase hDmcl1 forms ring structures and interacts with hRad51. *EMBO J.*, **18**, 6552–6560.
48. Lee,M.H., Chang,Y.C., Hong,E.L., Grubb,J., Chang,C.S., Bishop,D.K. and Wang,T.F. (2005) Calcium ion promotes yeast Dmcl1 activity via formation of long and fine helical filaments with single-stranded DNA. *J. Biol. Chem.*, **280**, 40980–40984.
49. Wu,J., Kurata,N., Tanoue,H., Shimokawa,T., Umehara,Y., Yano,M. and Sasaki,T. (1998) Physical mapping of duplicated genomic regions of two chromosome ends in rice. *Genetics*, **150**, 1595–1603.
50. The Rice Chromosomes 11 and 12 Sequencing Consortia (2005) The sequence of rice chromosomes 11 and 12, rich in disease resistance genes and recent gene duplications. *BMC Biol.*, **3**, 20.
51. Chen,Z., Yang,H. and Pavletich,N.P. (2008) Mechanism of homologous recombination from the RecA-ssDNA/dsDNA structures. *Nature*, **453**, 489–494.
52. Enomoto,R., Kinebuchi,T., Sato,M., Yagi,H., Kurumizaka,H. and Yokoyama,S. (2006) Stimulation of DNA strand exchange by the human TBPIP/Hop2-Mnd1 complex. *J. Biol. Chem.*, **281**, 5575–5581.
53. Pezza,R.J., Petukhova,G.V., Ghirlando,R. and Camerini-Otero,R.D. (2006) Molecular activities of meiosis-specific proteins Hop2, Mnd1, and the Hop2-Mnd1 complex. *J. Biol. Chem.*, **281**, 18426–18434.
54. Haruta,N., Kurokawa,Y., Murayama,Y., Akamatsu,Y., Unzai,S., Tsutsui,Y. and Iwasaki,H. (2006) The Swi5-Sfr1 complex stimulates Rhp51/Rad51- and Dmcl1-mediated DNA strand exchange in vitro. *Nat. Struct. Mol. Biol.*, **13**, 823–830.
55. Sarai,N., Kagawa,W., Kinebuchi,T., Kagawa,A., Tanaka,K., Miyagawa,K., Ikawa,S., Shibata,T., Kurumizaka,H. and Yokoyama,S. (2006) Stimulation of Dmcl1-mediated DNA strand exchange by the human Rad54B protein. *Nucleic Acids Res.*, **34**, 4429–4437.
56. Ploquin,M., Petukhova,G.V., Morneau,D., Déry,U., Bransi,A., Stasiak,A., Camerini-Otero,R.D. and Masson,J.Y. (2007) Stimulation of fission yeast and mouse Hop2-Mnd1 of the Dmcl1 and Rad51 recombinases. *Nucleic Acids Res.*, **35**, 2719–2733.
57. Pezza,R.J., Voloshin,O.N., Vanevski,F. and Camerini-Otero,R.D. (2007) Hop2/Mnd1 acts on two critical steps in Dmcl1-promoted homologous pairing. *Genes Dev.*, **21**, 1758–1766.
58. Alexiadis,V. and Kadonaga,J.T. (2002) Strand pairing by Rad54 and Rad51 is enhanced by chromatin. *Genes Dev.*, **16**, 2767–2771.
59. Jaskelioff,M., Van Komen,S., Krebs,J.E., Sung,P. and Peterson,C.L. (2003) Rad54p is a chromatin remodeling enzyme required for heteroduplex DNA joint formation with chromatin. *J. Biol. Chem.*, **278**, 9212–9218.
60. Alexeev,A., Mazin,A. and Kowalczykowski,S.C. (2003) Rad54 protein possesses chromatin-remodeling activity stimulated by the Rad51-ssDNA nucleoprotein filament. *Nat. Struct. Biol.*, **10**, 182–186.
61. Alexiadis,V., Lusser,A. and Kadonaga,J.T. (2004) A conserved N-terminal motif in Rad54 is important for chromatin remodeling and homologous strand pairing. *J. Biol. Chem.*, **279**, 27824–27829.
62. Wolner,B. and Peterson,C.L. (2005) ATP-dependent and ATP-independent roles for the Rad54 chromatin remodeling enzyme during recombinational repair of a DNA double strand break. *J. Biol. Chem.*, **280**, 10855–10860.
63. Zhang,Z., Fan,H.Y., Goldman,J.A. and Kingston,R.E. (2007) Homology-driven chromatin remodeling by human RAD54. *Nat. Struct. Mol. Biol.*, **14**, 397–405.
64. Kwon,Y., Chi,P., Roh,D.H., Klein,H. and Sung,P. (2007) Synergistic action of the *Saccharomyces cerevisiae* homologous recombination factors Rad54 and Rad51 in chromatin remodeling. *DNA Repair (Amst)*, **6**, 1496–1506.
65. Thompson,J.D., Gibson,T.J., Plewniak,F., Jeanmougin,F. and Higgins,D.G. (1997) The CLUSTAL\_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.*, **25**, 4876–4882.