Comparison of activity of OsDmc1A recombinase of rice (*Oryza sativa*) in presence of Ca\(^{2+}\) and Mg\(^{2+}\) ions

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Recombinases are known to play an important role in the homology search and strand exchange during meiosis as well as homologous recombination (HR)-mediated DNA repair specifically require Mg\(^{2+}\) ion for their activity. The Ca\(^{2+}\) has been shown to stimulate the strand exchange activity of hDmc1 and ScDmc1 by forming the extended filaments on DNA. *Oryza sativa* disrupted meiotic cDNA1A (OsDmc1A), a homologue of yeast and human Dmc1 from rice shows the hallmark functions of recombinase. Here, we report the effects of Ca\(^{2+}\) and Mg\(^{2+}\) on OsDmc1A activity from rice (*Oryza sativa*). OsDmc1A showed a concentration-dependent binding with both single-stranded DNA (ssDNA) and double-stranded DNA (dsDNA) substrates in presence of Mg\(^{2+}\) or Ca\(^{2+}\). The ssDNA and dsDNA binding activities, as well as renaturation activity of OsDmc1A were similar in the presence of Ca\(^{2+}\) or Mg\(^{2+}\). Increasing the Ca\(^{2+}\) or Mg\(^{2+}\) increased the DNA binding, renaturation and strand exchange of OsDmc1A. But, OsDmc1A showed only a slight stimulation of strand exchange activity in presence of Ca\(^{2+}\), when compared the activity in presence of Mg\(^{2+}\). Electron microscopy showed that OsDmc1A formed ring-like structures in presence of Mg\(^{2+}\) or Ca\(^{2+}\). However, OsDmc1A formed filament like structures with both ss and dsDNA in presence of Mg\(^{2+}\) or Ca\(^{2+}\). Taken together, Ca\(^{2+}\) did not affect OsDmc1A recombinase activity significantly.

**Keywords:** OsDmc1 recombinase, Homologous recombination, FRET assay, Electron microscopy, Meiosis, *Oryza sativa*, Rice

The process of homologous recombination (HR) is important for maintaining the genomic integrity and genetic diversity. In eukaryotes, this process is well-recognized in meiosis where homologous chromosomes pair and exchange the genetic information\(^1\). It also occurs during DNA repair in normal cells\(^2\). Rad51 and its meiotic homologue Dmc1 play a central role in HR\(^3\). HR is initiated at double-strand breaks produced during DNA damage or by the action of specific endonucleases during meiosis. Single-stranded DNA (ssDNA tails) are produced at the double-strand break sites. Rad51 and Dmc1 form nucleoprotein complexes and invade the undamaged double-stranded DNA. The strand invasion and homologous pairing results in formation of a heteroduplex between invading strand and its complementary strand on dsDNA\(^1,2\). The strand exchange then follows to ensure correct pairing between the homologous chromosomes by extensive homology search. These activities demonstrate that Rad51 and Dmc1 are the eukaryotic homologues of *E. coli* RecA, which performs homologous pairing and strand exchange reactions. The nucleoprotein filament formed by these proteins lies at the core of the process of HR\(^2\). It has been shown that Rad51 and Dmc1 bind ssDNA non-specifically to form helical filaments, although exact number of subunits per turn is a matter of debate\(^5,6\). During recombination in eukaryotic cells, the nucleoprotein filament formed by these recombinases can recombine with either sister chromatid or with one of the two homologous chromatids.

The Ca\(^{2+}\) has been found to stimulate the strand exchange activity of human recombinases *viz.* hRad51 and hDmc1\(^6,7\) and the mechanism of stimulation is different for both of these recombinases. Ca\(^{2+}\) ion stimulate the hRad51 activity by modulating ATPase activity\(^6\), whereas of hDmc1 stimulation could be accounted by conformational changes induced by free Ca\(^{2+}\) binding to a site distinct from Mg-ATP site on the protein, resulting in more stable hDmc1-ssDNA complexes\(^7\). Ca\(^{2+}\) is reported to stimulate the strand...
exchange activity in yeast and exhibits low recombinase activity in vitro. ScDmc1 forms octameric rings and bind to ssDNA as both ring and filamentous forms. Ca²⁺ is also found to stimulate the activity by increasing the tendency of filament formation, indicating that filament form is more active.

In spite of importance of HR in plants, the biochemistry of this process is not well understood. Earlier, we have reported the preliminary biochemical characterization of OsDmc1A protein from rice. OsDmc1A is reported to show all the classical hallmark biochemical properties of recombinase, which include ssDNA and dsDNA binding in presence of Mg²⁺, ssDNA-dependent ATPase and D loop formation; these activities are homology and ATP dependent. The strand exchange reaction of OsDmc1A between duplex DNA and its homologous single-stranded DNA have been analyzed by FRET assays. Recently, it has been observed that OsDmc1A and B from rice (Japonica) have robust strand exchange activity, over 5000 base pairs in presence of replication protein A (RPA). OsDmc1A has shown much higher strand exchange activity than that of OsDmc1B. The rice Dmc1A and B proteins exhibit formation of helical filaments along ssDNA.

Since Ca²⁺ has been shown to stimulate the activity of the recombinase proteins from yeast and human, in this study, we have examined the effect of Ca²⁺ on OsDmc1A in order to find out the functional differences between the plant meiotic recombinases with that of yeast and human recombinases. We have found that substitution of Ca²⁺ for Mg²⁺ cause a slight stimulation of the strand exchange activity of OsDmc1A, while renaturation activity remains unaltered.

**Materials and Methods**

**Materials**

ATP, MgCl₂ and CaCl₂ were purchased from Sigma Chemical Co, USA. All the reagents and solutions were used under sterile conditions. ΦX-174 Virion DNA was purchased from New England Biolabs (England). Two complementary oligonucleotides (55 mers) for renaturation and strand exchange assay were obtained from Metabion, Germany with the following sequence: PhiC: 5’-CGATA CGCTC AAAGT CAATAA TAATC AGCCG GACAT TCGAG AAGGT AAACG’-3’, PhiW: 5’-CGTCT TTCAT ACCCT TCTGA ATGTC AGCTG GATTA TTTTG ACTTT GAGCG TGATC-3’, M13C: 5’-CTACA ACGCC TGTAG CATTC CACAG A CAGC CCTCA TAGTT AGCGT AACGA GATCG-3’, Phi-W and Phi-WC were labeled with fluorescein and rhodamine B at 5’ and 3’ ends, respectively. Renaturation and strand exchange reactions were monitored using Fluorescence spectrophotometer F-4010 (Hitachi, Japan). OsDMC1 cDNA from rice was cloned and protein was purified as described previously.

**DNA binding assays**

ssDNA and dsDNA binding assays were done as described previously. A 20 µl of reaction mixture containing indicated amounts of OsDmc1A, 20 mM HEPES (pH 7.9), 2.0 mM ATP, 10.0 mM CaCl₂ or MgCl₂, 3.0% glycerol, 1.0 mM dithiothreitol and 100 µg/ml bovine serum albumin was incubated at 37°C for 5 min and reaction was started by the addition of either 1.0 µg of ΦX-174 virion ssDNA per reaction (ssDNA binding assay) or 0.5 µg of PCR amplified dsDNA per reaction (dsDNA binding assay). The reaction was stopped by adding 2.0 µl of 2.0% glutaraldehyde. After incubation with glutaraldehyde for 20 min at 37°C, 4.0 µl of 30% glycerol was added. Complexes were analyzed on 0.8% agarose gel in 1X Tris-acetate-EDTA running buffer at 10 V/cm. DNA in the complexes was visualized by staining the gel with ethidium bromide.

**Forster resonance energy transfer (FRET) assay**

FRET is a sensitive and simple technique to probe the DNA strand dynamics during renaturation and strand exchange in real time without subjecting the sample for deproteinization. During FRET phenomenon, a non-radiative transfer of energy takes place between the donor dye and an acceptor dye when these dyes are in their Forster distance. We used two complementary oligonucleotides (55 bases) labeled with fluorescein and rhodamine B at 5’ and 3’ ends, respectively. If these two dyes are juxtaposed as a result of renaturation, or move apart as a result of strand exchange, FRET or loss of FRET conditions will result, respectively.

**Renaturation assay by FRET**

Two complementary single-stranded oligonucleotides labeled with fluoresceine at 5’ end rhodamine B at 3’ end individually were used in this assay. When two complementary DNA strands are in single-stranded state, fluoresceine and rhodamine dyes are apart and no FRET occurs, as a result fluoresceine emission is maximum. As protein-mediated or spontaneous renaturation takes place, two complementary strands anneal to make a duplex
molecule. As a result, fluoresceine and rhodamine dyes are juxtaposed and FRET takes place, which in turn decreases the fluoresceine emission intensity. This assay is straight forward and simple to read the reaction in real time without the removal of protein from the reaction mixtures\textsuperscript{10}.

Briefly, the reaction mixture (100 µl) containing 20 mM HEPES (pH 7.9), 2 mM ATP, indicated amount of MgCl\(_2\) or CaCl\(_2\), 3.0% glycerol, 1.0 mM DTT and 3.0 µM of OsDmc1A was pre-incubated at 37°C for 5 min with Phi-C oligonucleotide (27.5 µM of nucleotides) labeled with rhodamine B at 3' end. Complementary oligonucleotide Phi-W (27.5 µM of nucleotides) labeled with fluorescein at 5' end was added and decrease in fluorescein emission intensity as a result of FRET, was measured at 522 nm after excitation at 490 nm using F-4010 Hitachi fluorescence spectrophotometer. Fluorescence of each reaction was normalized to one by dividing the each time point fluorescence value by the initial fluorescence value and decrease in fluorescence was plotted against time.

**Strand exchange assay by FRET**

Figure 4A shows the cartoon representing the design of the FRET assay used for the strand exchange reaction mediated by OsDmc1A protein. Fluoresceine and rhodamine labeled complementary single-stranded oligonucleotides were used for making the double-stranded oligonucleotide by thermal denaturation process. Unlabeled single-stranded oligonucleotide was pre-synapsed with OsDmc1A protein and strand exchange reaction was started by adding the duplexed oligonucleotide. Due to the strand exchange reaction, unlabeled oligonucleotide replaces the rhodamine labeled strand which in turn reduces the FRET effect. This leads to the increase in the fluoresceine emission intensity representing the ongoing strand exchange reaction\textsuperscript{10}.

Briefly, the reaction mixture (100 µl) containing 20 mM HEPES (pH 7.9), 2 mM ATP, indicated amounts of MgCl\(_2\) or CaCl\(_2\), 3.0% glycerol, 1.0 mM DTT and 3.0 µM of OsDmc1A was pre-incubated with unlabeled Phi-C oligonucleotide (27.5 µM of nucleotides) for 5 min at 37°C. Duplex 55-mer made from fluorescein labeled Phi-W and rhodamine B labeled Phi-C was added (27.5 µM of nucleotides) as homologous duplex. Increase in fluorescence emission intensity of fluorescein at 522 nm after excitation at 490 nm was measured at 30 s intervals for 10 min due to the loss of FRET as a result of strand exchange. M13C was used as non-homologous DNA for monitoring the spontaneous, as well as non-specific strand exchange activity. Initial fluorescence of the each reaction was bought to the zero and increase in fluorescence was plotted against time.

**Electron microscopy of OsDmc1A-DNA complexes**

Electron microscopy of OsDmc1A protein was done according to the procedure described by Rajanikant \textit{et al}\textsuperscript{12} with slight modifications. The reaction mixture (10 µl) containing 20 mM HEPES (pH 7.9), 2 mM ATP, 10.0 mM MgCl\(_2\) or CaCl\(_2\), 3.0 % (w/v) glycerol, and 500 ng of PhiX-174 ss- or ds DNA was incubated at 37°C for 5.0 min. OsDmc1A protein (6.0 µM) was added and incubation was continued for further 20 min at 37°C and samples were fixed with 0.2% glutaraldehyde for 20 min at 37°C. Samples were diluted to 1:8 ratio with 1.0X reaction buffer and 5 µl of each sample was spotted on 100 mesh carbon coated copper grids freshly activated under UV lamp for 1.0 h. After 5 min, these grids were stained with 1.0% aqueous uranyl acetate solution for 2.0 min, followed by air-drying at room temperature. Digital images were acquired at 120 KV using TechnaiG\textsuperscript{2} (Phillips) electron microscope.

**Sequence analysis**

Dmc1 protein sequences for OsDmc1A (Japonica rice AAK55555, Indica rice AAM76793), \textit{Arabidopsis} Dmc1 (AtDmc1 AAC49617), \textit{Zea mays} Dmc1 (ZmDmc1 CAG35023), \textit{Saccharomyces cerevisiae} Dmc1 AAA34571), \textit{Coprinus} Dmc1 (Copr1m Dmc1 BAA89533), \textit{Homo sepians} Dmc1 (ScDmc1 ACG30372) and \textit{MmDmc1} (Mus Musculus Dmc1 BAA19082) were downloaded from NCBI website (www.ncbi.nlm.nih.gov). Sequences were aligned with MUSCLE (v3.7) multiple alignment software. Dendrogram was constructed using PhyML program (v3.0aLRT), implementing the maximum likelihood method on www.phylogeny.fr website.

**Results**

We compared the DNA binding, renaturation and strand exchange activities of OsDmc1A protein in the presence of either Mg\textsuperscript{2+} or Ca\textsuperscript{2+}. Figure 1 shows the ssDNA binding activity of OsDmc1A in presence of Ca\textsuperscript{2+} and Mg\textsuperscript{2+} ions. OsDmc1 showed concentration-dependent binding to ssDNA substrates in the presence of 10 mM of Mg\textsuperscript{2+} or Ca\textsuperscript{2+} (Fig. 1A and B); in both cases, OsDmc1A protein binding started at low concentration (1.0 µM) and increased thereafter. It did not show any difference in the binding efficiency when compared between Ca\textsuperscript{2+} and Mg\textsuperscript{2+} with ssDNA.
ssDNA binding of OsDmc1A (3.0 µM) at different concentrations of Mg\textsuperscript{2+} and Ca\textsuperscript{2+} showed optimal binding at 5.0-7.5 mM and 7.5-10.0 mM of Mg\textsuperscript{2+} and Ca\textsuperscript{2+}, respectively (Fig. 1C and D). We further observed a minimal binding of the protein to ssDNA even in absence of Mg\textsuperscript{2+} and Ca\textsuperscript{2+}. However, the protein bound to ssDNA equally well in presence of Mg\textsuperscript{2+} and Ca\textsuperscript{2+}, especially at the concentrations exceeding 5.0 mM. Thus, it was proposed that Ca\textsuperscript{2+} had no effect on ssDNA binding properties of OsDmc1A, when compared in presence of Mg\textsuperscript{2+}.

Similarly, in case of dsDNA (Fig. 2), minimal binding of OsDmc1A protein with dsDNA substrates was observed under the conditions tested. Additionally, it was found that the protein-dsDNA complexes barely entered the gel. However, we did not observe any differences in the protein binding with dsDNA in the presence of Mg\textsuperscript{2+} or Ca\textsuperscript{2+}. Though the protein binding to dsDNA was low, we noticed the protein concentration-dependency of the formation of nucleo-protein complexes (Fig. 2A and B). However, protein binds with dsDNA even in absence of both the cations and binding increased with increase in the concentration of ions. The optimal binding of OsDmc1A was observed at 5-10 mM of either Mg\textsuperscript{2+} or Ca\textsuperscript{2+} (Fig. 2C and D). Taken together, binding affinity of OsDmc1 to dsDNA was comparable in presence of either Mg\textsuperscript{2+} or Ca\textsuperscript{2+}.

Figure 3A shows the schematic representation of the design of FRET assay used for measuring the renaturation activity of OsDmc1A. Figure 3B shows time-dependent renaturation reaction mediated by OsDmc1A protein in presence of 10 mM of Mg\textsuperscript{2+} or Ca\textsuperscript{2+} monitored by FRET. As time of reaction progressed, a decrease in emission intensity was observed. We also studied the same reactions in absence of the protein and in presence of either 10 mM of Mg\textsuperscript{2+} or Ca\textsuperscript{2+}, which represent the spontaneous renaturation under the tested conditions. The extent of spontaneous renaturation was less, compared to the renaturation in presence of OsDmc1A protein. However, no difference was observed between the extent of decrease in intensity in presence of Mg\textsuperscript{2+} and Ca\textsuperscript{2+}, indicating that rate of renaturation was similar in both cases. We further compared the renaturation activity in presence of lower concentrations (0-10 mM) of cations (Fig. 3C) and
plotted the extent of decrease in fluorescence intensity, which represented the extent of renaturation reaction as a function of ion concentration. OsDmc1A mediated the renaturation activity to the minimal extent in the reactions, where no cations were added. At 5.0 mM of Mg$^{2+}$ or Ca$^{2+}$, renaturation activity reached maximum and not much difference was observed when ionic concentration was increased further to 10.0 mM. At each concentration (0-10 mM) of Mg$^{2+}$ or Ca$^{2+}$, the extent of renaturation activity was similar, indicating that Ca$^{2+}$ did not affect the renaturation reaction, when compared the same in presence of Mg$^{2+}$.

Figure 4B shows the time-dependent strand exchange reaction in presence of 10 mM of Mg$^{2+}$ or Ca$^{2+}$. There was minimal simulation of strand exchange by Ca$^{2+}$ initially, but activities in presence of Ca$^{2+}$ or Mg$^{2+}$ reached similar extent after 10 min, indicating that there was no significant difference in strand exchange activity in presence of Ca$^{2+}$ or Mg$^{2+}$.

In case of non-homologous DNA (M13C) in presence of Ca$^{2+}$ or Mg$^{2+}$, the extent of strand exchange activity was minimal. OsDmc1A required minimum of 2.5 mM of Ca$^{2+}$ or Mg$^{2+}$ for its strand exchange activity and activity increased in presence of 2.5 mM of Ca$^{2+}$ or Mg$^{2+}$ and remained the same at 5.0 mM of Ca$^{2+}$ or Mg$^{2+}$ (Fig. 4C). At 10 mM of cation concentration, we observed a marginal increase in the strand exchange activity of OsDmc1A. However, there was no difference in the strand exchange activity with particular concentration of Ca$^{2+}$ or Mg$^{2+}$.

Figure 5 shows the electron microscope images of OsDmc1A in presence of Ca$^{2+}$ or Mg$^{2+}$. OsDmc1A formed rings in absence of ssDNA and dsDNA (Fig. 5A and B). The preponderance of compact rings was higher in the presence of Ca$^{2+}$. We observed 10-times more ring-like structures in the presence of Ca$^{2+}$, when compared in presence of Mg$^{2+}$. OsDmc1A recombinase formed helical filaments upon binding to ss- or dsDNA. (Fig. 5C-F).

Figure 6 shows the evolutionary distances in the sequences of Dmc1 protein in different species. The plant, fungal and mammal Dmc1 proteins formed three distinct groups, suggesting a discrete evolutionary blueprint for conserved Dmc1 protein in the eukaryotes. The monocot and dicots also showed divergence in Dmc1 sequence. The divergence of Dmc1 protein from yeast and mammals suggests that though the function is conserved, they show small differences in the activity at molecular level.
Fig. 4—Strand exchange activity of OsDmc1A by FRET [(A) Schematic representation of the design of FRET assay used for measuring the strand exchange activity of OsDmc1A. Replacement of the juxtaposed dyes (fluoresceine and rhodamine B) due to the strand exchange result in loss of FRET, which, in turn, increases the emission intensity of fluoresceine; (B) strand exchange activity of 3.0 µM of OsDmc1A in presence of 10 mM of Mg$^{2+}$ or Ca$^{2+}$. Strand exchange reaction activity in presence of non-homologous ssDNA in presence of 10 mM of Mg$^{2+}$ or Ca$^{2+}$ is also shown in the figure; (C) Extent of strand exchange activity of with 3.0 µM of OsDmc1A in the presence of increasing concentrations (0 to 10 mM) Mg$^{2+}$ or Ca$^{2+}$. Absolute fluorescence intensity values were given as strand exchange activity]
Discussion

In homologous recombination (HR), formation of nucleoprotein filament is an important step. Ca\textsuperscript{2+} is found to stimulate the strand exchange activity of hDmc1 and hRad51, as well as ScDmc1. Though hRad51 and hDmc1 are structurally and functionally similar, they differ in the mechanism of activation by Ca\textsuperscript{2+}, probably due to small structural variations in the two proteins. Interestingly, in case of yeast recombinases, only ScDmc1 is found to be stimulated by Ca\textsuperscript{2+}, while ScRad51 is not stimulated by Ca\textsuperscript{2+}. Thus, Ca\textsuperscript{2+} stimulation appears to distinguish small structural differences in the two recombinases (Dmc1 and Rad51) that probably occur during evolution\textsuperscript{7}.

In electrophoretic mobility shift assays, OsDmc1A protein showed a protein concentration-dependent DNA binding activity (Figs 1 and 2) like other recombinases Rad51 and RecA. Though OsDmc1A showed good binding to ssDNA, very little binding to dsDNA was observed. This was in good agreement with the OsDmc1A protein from japonica rice\textsuperscript{13,14}. It is reported that the recombinases bind to the both the forms of DNA and the pre-synaptic filament formed by ssDNA is thought to be the main substrate in the recombination process\textsuperscript{5}. As human Dmc1 has shown to stimulate recombination activity at lower Ca\textsuperscript{2+} concentration\textsuperscript{7}, we analyzed the DNA binding activity at lower Ca\textsuperscript{2+} or Mg\textsuperscript{2+} concentrations (Figs 1 and 2). Unlike hDmc1, we did not observe the stimulation of ssDNA and dsDNA binding activity of OsDmc1A in the presence of Ca\textsuperscript{2+}.

Since DNA binding activity did not show any difference between the Ca\textsuperscript{2+} and Mg\textsuperscript{2+}, we further analyzed the renaturation and strand exchange activity, which are more relevant activities of the recombinase OsDmc1A. OsDmc1A promoted the renaturation activity of in the presence of 10 mM of Mg\textsuperscript{2+} or Ca\textsuperscript{2+} to the same extent (Fig. 3B). Under the same conditions, spontaneous renaturation was found to be very low, suggesting that renaturation activity was indeed mediated by OsDmc1. It was interesting to test the renaturation activity at lower concentrations of Mg\textsuperscript{2+} or Ca\textsuperscript{2+}, as hDmc1 protein recombinase activity is shown to be stimulated\textsuperscript{7} at lower concentrations of Ca\textsuperscript{2+}. We tested a range of Mg\textsuperscript{2+} or Ca\textsuperscript{2+} concentrations in the reaction and even at low concentration, no difference was observed between the renaturation activities of the protein. As the Ca\textsuperscript{2+} or Mg\textsuperscript{2+} concentration increased, activity of OsDmc1A was also increased. Interestingly, OsDmc1A required 2.5 to 5.0 mM of Mg\textsuperscript{2+} or Ca\textsuperscript{2+} for its optimal recombinase activity. The exact mechanism of how the Mg\textsuperscript{2+} or Ca\textsuperscript{2+} are playing a role in promoting pre-synaptic events, which in turn will mediate the renaturation activity, is unknown.

As strand exchange reaction represents the actual recombination function in double-strand break repair by HR process, we further compared strand exchange activity of OsDmc1A in the presence of Mg\textsuperscript{2+} and Ca\textsuperscript{2+}. We again employed the FRET assay for monitoring the ongoing strand exchange assay. We made a pre-synaptic filament with unlabeled oligo and strand exchange reaction was initiated by its FRET paired duplex partner, leading to the synaptic and post synaptic events for the generation of strand exchange products. Only a slight simulation of strand exchange was observed in the initial few minutes (Fig. 4B). In case of hsDmc1 and ScDmc1, Ca\textsuperscript{2+} is reported to stimulate strand exchange activity by 5-6 folds\textsuperscript{7,8}. In our study, the assay mixtures containing Ca\textsuperscript{2+} and Mg\textsuperscript{2+} reached same end point after 10 min of incubation.

Additionally, we also studied the strand exchange activity at low concentrations of Mg\textsuperscript{2+} or Ca\textsuperscript{2+} (Fig. 4C). Even in absence of ions, a basal level strand exchange activity was observed. As concentration of ions increased, a corresponding increase in the strand exchange activity was observed. Like renaturation activity, strand exchange activity of OsDmc1A was also dependant on Mg\textsuperscript{2+} or Ca\textsuperscript{2+} for its optimal activity. However, we did not observe any difference between Mg\textsuperscript{2+} and Ca\textsuperscript{2+} containing reaction at all the concentrations of the ions tested. This indicated that Ca\textsuperscript{2+} did not stimulate the recombinase function of OsDmc1A as observed for hsDmc1 or ScDmc1. This might be due to preponderance of filaments formation by OsDmc1A with DNA molecules.

Previously, it is reported that rice DMC1 proteins show robust strand exchange activities and form filaments with ssDNA\textsuperscript{11}. This is in contrast to the octameric rings shown for hDMC1, where in presence of Mg\textsuperscript{2+}, 90% of ScDmc1 binds to ssDNA as protein rings and 10% is able to form filament\textsuperscript{13,14}. The stimulation of ScDmc1 activity by Ca\textsuperscript{2+} has been attributed to the stimulation of helical filament formation by Ca\textsuperscript{2+} as against rings, thereby increasing the active form required for strand exchange\textsuperscript{8}. It is interesting to point out that the E. coli recombinases RecA protein binds to ssDNA and stretches its length to 1.5-times and bases are...
thought to be exposed for proper homology search and subsequent strand exchange. In case of human Rad51\(^{15}\), Ca\(^{2+}\) keeps the ssDNA bases in proper orientation, which is thought to be the reason for the stimulatory effect of Ca\(^{2+}\).

Further we did not observe any difference in OsDmc1A-ssDNA or OsDmc1A-DsDNA filaments under electron microscope in the presence of Ca\(^{2+}\) and Mg\(^{2+}\) (Fig. 5). Since there were no differences between the ssDNA-OsDmc1A filaments, which are thought to be the recombinogenic active filaments, the renaturation and strand exchange activities of the protein were also the same in presence of Mg\(^{2+}\) or Ca\(^{2+}\). However, in absence of DNA, OsDmc1 showed more ring-like structures in presence of Ca\(^{2+}\) than in presence of Mg\(^{2+}\) (Fig. 5). The exact mechanism of how Ca\(^{2+}\) promotes ring formation of OsDmc1A is not known and additional structural and mechanistic studies may provide more information about this process. The dendrogram in Fig. 6 shows the divergence of plant Dmc1 proteins from mammalian and fungal Dmc1 proteins. The minor differences among Dmc1 protein from these eukaryotes may be contributing their response to Mg\(^{2+}\) and Ca\(^{2+}\).

The HR frequencies are very high in fungi as compared to mammals and plants, with plants having the lowest recombination frequencies. Though these species have the structurally identical proteins involved in the formation of similar nucleoprotein filaments, they differ in the frequency of HR. The data demonstrating the differences among Dmc1 proteins from human, yeast and plant systems in response to Mg\(^{2+}\) or Ca\(^{2+}\) might be responsible for differences in HR frequencies. Additionally, distinct set of interacting proteins may be responsible for different rates of HR among these eukaryotes. Understanding the intricate structural differences in the Dmc1 proteins from plant sources as against human and yeast may help decipher some of these reasons. The information would be useful to comprehend the process of meiosis in the plants.

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