Crystal Structure of Human DNA Recombinase, Dmc1

Meiosis is a cell division process specifically occurring in germ cells (testis and ovary) of eukaryotes. During the cell division, homologous chromosomes pair and parts or all of the genes are shuffled between chromosomes, a process called homologous recombination (Fig. 1) [1]. Eukaryotes obtain genetic variation by this method. Homologous recombination is also essential for the repair of DNA damage, thus, for the cell to function properly, homologous recombination is important. We have focused on the protein that is responsible for the homologous recombination in germ cells, namely Dmc1, and have succeeded in crystallizing the full-length human protein [2]. The crystal was used to determine the three-dimensional structure of Dmc1 at atomic resolution. Based on this structure, we have studied the homologous recombination promoted by Dmc1.

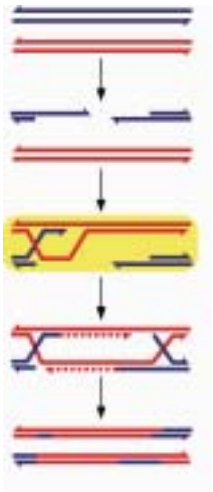

Fig. 1. The model of homologous recombination in meiosis.

The human Dmc1 protein, consisting of 340 amino acids, binds both single-stranded DNA and double-stranded DNA, and promotes the homologous-pairing reaction, a key step of homologous recombination. We overexpressed the Dmc1 protein in *Escherichia coli* and purified the Dmc1 protein using several chromatographic techniques. A single crystal (100 μm × 600 μm × 600 μm) of Dmc1 was obtained, and the crystal structure of Dmc1 was successfully determined using data collected at the synchrotron radiation of the RIKEN Structural Biology II beamline **BL44B2**.

The human Dmc1 protein is a homolog of the *E. coli* RecA protein. Studies have shown that the bacterial RecA protein forms a helical filament structure and promotes homologous pairing. Contrary to the extensive sequence similarity between Dmc1 and RecA, we show that Dmc1 functions as a double-ring structure. The ring consists of eight subunits, and two rings are stacked in a bipolar nature with a diameter of 130 Å and a height of 85 Å (Fig. 2).

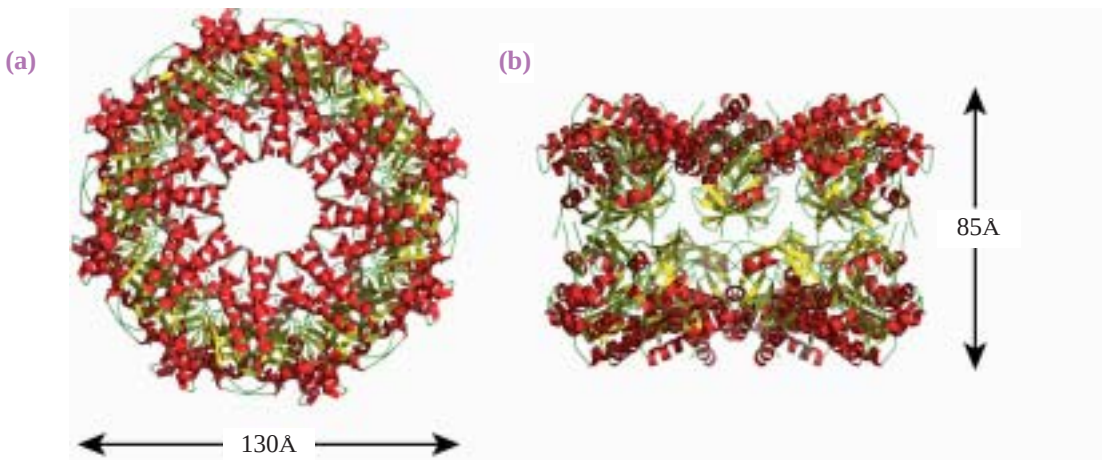

Fig. 2. Crystal structure of Dmc1. Dmc1 double-ring structure viewed from top **(a)** and side **(b)**.

The Dmc1 double-ring structure contains two open-ended passages: one at the center of the ring (about 30 Å in diameter) and the other between the stacked rings (about 15 Å in diameter). On the basis of alanine-scanning mutagenesis studies, the central channel was shown to bind double-stranded DNA, while the passage along the side of the ring that leads to the central channel was shown to bind single-stranded DNA (Fig. 3(a)). These results formed the basis of the model of the ternary complex containing Dmc1, single-stranded DNA, and double-stranded DNA. In this model, homologous pairing occurs at the center of the double ring, and the recombined DNA is spooled out of the passages (Fig. 3(b)). This mechanism contrasts with those proposed for the helical filament forming RecA and Rad51 proteins. Hence, the recent studies suggest that there are at least two recombination mechanisms utilized in germ cells. Such multiple mechanisms may be essential for cells to properly divide during meiosis.

Since homologous recombination is the mechanism underlying gene therapy and genetic engineering, understanding the structural and biochemical properties of eukaryotic recombinases is essential for improving these technologies. The present study may provide a foundation for modifying the activity of Dmc1 recombinase, leading to an increased recombination frequency in eukaryotic cells. Therefore, structural information on Dmc1 recombinase may contribute to the advancement of gene therapeutic and transgenic technologies.

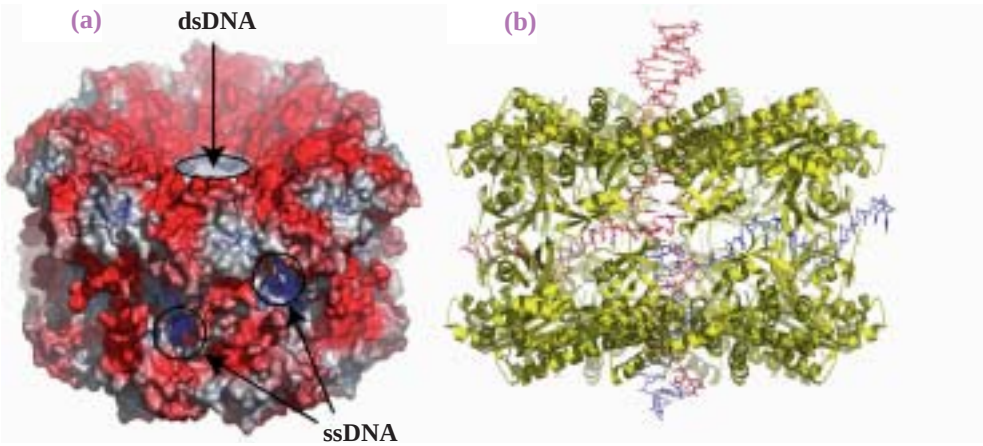

Fig. 3. Model of a homologous pairing intermediate complex of Dmc1, ssDNA, and dsDNA. **(a)** Important region for ssDNA and dsDNA binding on surface of double ring. **(b)** The Dmc1 double ring is a functional unit that could be essential for efficient binding to ssDNA and dsDNA. dsDNA is shown to pass through the central channel, while ssDNA is shown to pass through the channel formed as result of the stacking of two Dmc1 rings. The search and pairing of two homologous sequences could occur at the center of the Dmc1 double ring.

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