Structural and Functional Mechanism of a New Protein Complex Promoting the Stabilization of Genome during Homologous Recombination

Precise maintenance of DNA/genome is an essential requirement for all living systems. During homologous recombination, Rad51 assembles into a nucleoprotein filament on single-stranded DNA to catalyze homologous pairing and DNA-strand exchange with a homologous template in budding yeast. We identified a new Rad51 mediator complex, PCSS, comprising budding yeast Psy3, Csm2, Shu1 and Shu2 proteins, which binds to recombination sites and is required for Rad51 assembly and function during meiosis. The crystal structure of the Psy3-Csm2 complex suggests that this complex constitutes a core sub-complex with DNA-binding activity and stabilizes Rad51 filament formation.

Precise maintenance of DNA/genome, which is a genetic blueprint for life, is an essential requirement for all living systems. If the information is not maintained precisely, it will cause serious diseases such as cancer, or death. DNA can easily be damaged by various factors such as UV irradiation, reactive oxygen species (ROS) produced by internal metabolisms, ionizing radiation and so on. All living things have various mechanisms for repairing DNA to maintain the genetic information in order to survive. Homologous recombination plays a key role not only in maintaining genome stability, but also in generating genetic diversity. Recognition and exchange between two homologous DNAs are key steps for homologous recombination. These processes are catalyzed by RecA/Rad51 family proteins. Rad51 is a yeast homologue of a eukaryotic RecA protein. Rad51 assembles into a nucleoprotein filament on single-stranded DNA (ssDNA) to form a right-handed helical filament and catalyzes homologous repair and DNA strand exchange with a homologous template.

This Rad51 nucleoprotein filament is protein machinery essential for homology search and DNA exchange. The assembly and disassembly of Rad51 filaments is highly regulated by various positive and negative factors with dynamic processes. *In vivo*, Rad51 filament assembly necessitates the displacement of replication protein-A (RPA) from ssDNA. Brca2 (Breast cancer responsible gene 2), a product of tumor suppressor gene, is known to stabilize the Rad51 filament by binding to one end of the filament, and it promotes formation of the filament.

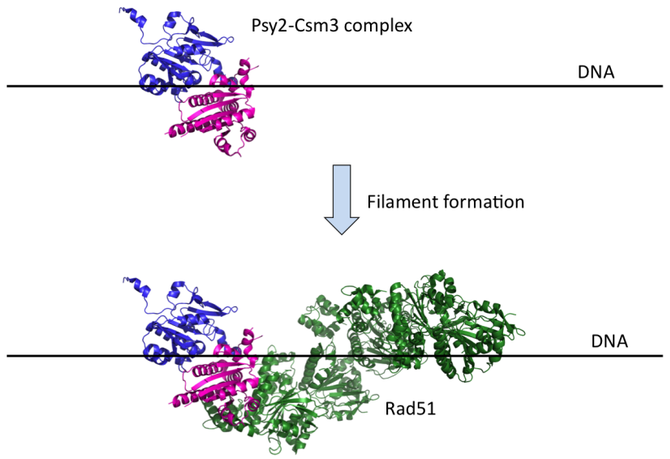

Figure 1: A model of Psy3-Csm2 to promote Rad51 filament formation.

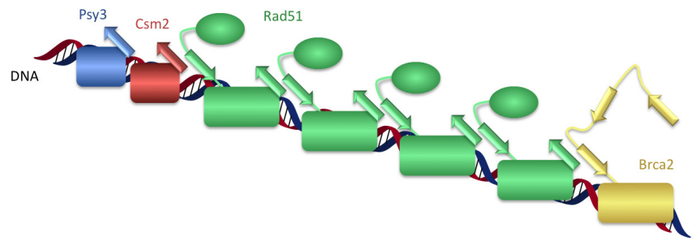

Figure 2: A schematic model of stabilization of Rad51 filament by Psy3-Csm2 complex and Brca2. The inferred site of interaction between Csm2 (red) and Rad51 (green) is completely opposite to the Rad51 interface with Brca2 (yellow).

We have identified a new protein complex that mediates Rad51 filament formation. The PCSS complex, comprising Psy3, Csm2, Shu1 and Shu2 proteins, binds to recombination sites and is required for Rad51 assembly and function during meiosis as well as mitosis in budding yeast, *Saccharomyces cerevisiae*. Within the hetero-tetramer, Psy3-Csm2 constitutes a core sub-complex with DNA-binding activity. We also revealed the high-resolution atomic structure of the Psy3-Csm2 complex using diffraction data collected at the Photon Factory at 1.75 Å resolution. The crystal structure of the Psy3-Csm2 complex showed that it resembles the dimeric structure of Rad51. This structure suggests that this complex constitutes a core sub-complex with DNA-binding activity and stabilizes Rad51 filament formation (Fig. 1). We confirmed that the Psy3-Csm2 complex acts as a core for filament formation by Rad51 by molecular biological approaches [1].

This filament model suggests that Psy3-Csm2, and by inference the PCSS complex, may interact with only one end of the Rad51 filament. It is interesting to note that the inferred site of interaction between Csm2 and Rad51 is completely opposite to the Rad51 interface with Brca2. Like the Psy3-Csm2 dimer, Brca2 is known to stabilize the Rad51 filament, possibly by binding to one end (Fig. 2).

The PCSS complex is a novel mediator of Rad51 nucleoprotein filament assembly by binding the 5'-end of the filament. The mechanism of PCSS action as a Rad51 mediator is distinct from that of other characterized mediators, such as Rad52, Rad55-Rad57 and Brca2.

The results suggest a paradigm for the mechanism of other Rad51 paralogues.

REFERENCE

[1] H. Sasanuma, M.S. Tawaramoto, J.P. Lao, H. Hosaka, E. Sanda, M. Suzuki, E. Yamashita, N. Hunter, M. Shinohara, A. Nakagawa and A. Shinohara, *Nature Commun.* **4**, 1676 (2013).

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