17A, NW12A/2011G087

Crystal structure of Swi5-Sfr1C complex from fission yeast

Naoyuki Kuwabara[1*], Hiroshi Hashimoto[2], Mamoru Sato[2] and Toshiyuki Shimizu[1]
[1]Grad. Sch. of Pharm. Sci., Univ. of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan
[2]Grad. Sch. of Nanobio., Yokohama City Univ., 1-7-29 Suehiro-cho, Tsuruku, Yokohama, Kanagawa 230-0045, Japan

1 Introduction

Homologous recombination (HR) plays a central role not only in genetic diversity but also in preserving genomic integrity, and thus defects in HR may result in cancer susceptibility. RecA-family DNA recombinases are key players in HR. In eukaryotes, there are two known classes of recombinases, Rad51 and Dmc1; the former functions generally in both mitotic and meiotic HR and HR-mediated DNA repair, and the latter acts specifically in meiosis. The Swi5-Sfr1 complex from the fission yeast *Schizosaccharomyces pombe* is a second class of auxiliary proteins. The complex binds directly and stimulates both Rad51- and Dmc1-mediated strand-exchange reactions. However, the mechanistic insight remains unclear.

Here we present the crystal structures of Swi5 and its complex with the C-terminal domain of Sfr1 (Sfr1C), which is more conserved among various species than the N-terminal domain.

2 Experiment

Diffraction data were collected at 100 K on beamline BL-17A at the Photon Factory (PF), Tsukuba, Japan using an ADSC Quantum 270 detector and on beamline NW12A at the Photon Factory Advanced Ring (PF-AR) using an ADSC Quantum 210r detector. Diffraction images were indexed, integrated and scaled using the *HKL*-2000 package

The structure of the Swi5-Sfr1C complex was solved by single-wavelength anomalous diffraction using the SeMet-labeled Swi5-Sfr1C complex crystal. Experimental phases were calculated with SHELX. Refinment and model building were performed using the programs Refmac5 and Coot, respectively.

3 Results and Discussion

The three-dimensional structure of the Swi5-Sfr1C complex shows an elongated shape (Fig.1). Swi5 and Sfr1 form a parallel coiled-coil heterodimer. In the complex, the long continuous α helix observed in the Swi5 structure alone is interrupted. The Swi5 and Sfr1C subunits of the heterodimer are firmly joined by two leucine zippers and a bundle structure at the bottom. The first leucine zipper, formed by $\alpha1^{swi5}$ and $\alpha1^{sfr1}$, shows a typical arrangement. The second leucine zipper, composed of $\alpha2^{swi5}$ and $\alpha2^{sfr1}$. Remarkably, the structure of the Swi5-Sfr1C complex is sharply kinked (130°) beginning just after the first leucine zipper at $Lys30^{swi5}$ and $Lys212^{sfr1}$. Beyond this kink, the structure is held together by the second leucine zipper. The kinked region is stabilized by several interactions. These interactions maintain the relative orientation between the two leucine zippers.

We demonstrated that the Swi5-Sfr1C possesses the function of a recombination activator of Rad51. These results of the present study, including structural features, and biochemical analyses strongly suggest that the elongated and sharply kinked Swi5-Sfr1 is wedged into the Rad51 filament to fix it in an active ATP-bound form. The docking model also shows that Swi5-Sfr1 fits well in the continuous groove created by the in-silico-reconstituted Rad51 filament (Fig.2).

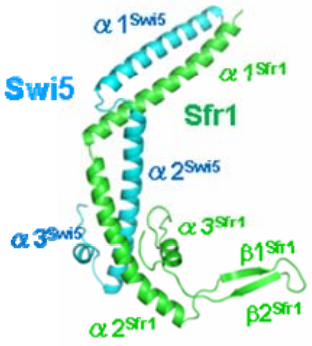

Fig. 1: Crystal structure of Swi5-Sfr1C

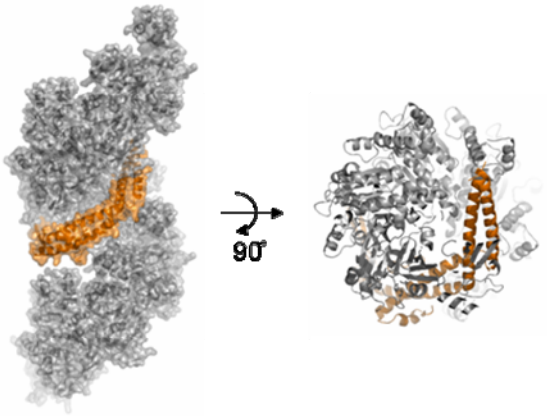

Fig. 2. Rad51 (gray) and Swi5-Sfr1C (orange) ternary complex model

References

[1] N. Kuwabara *et al.*, Acta Crystallogr. Sect. F **66** (2010) 1124.
[2] N. Kuwabara *et al.*, Structure. **20** (2012) 440.

* kuwanao@mol.f.u-tokyo.ac.jp