Mechanistic Insights into the Activation of Rad51-Mediated Strand Exchange from the Structure of a Recombination Activator: the Swi5-Sfr1 Complex

Rad51 forms a helical filament on single-stranded DNA and promotes strand exchange between two homologous DNA molecules during homologous recombination. The Swi5-Sfr1 complex interacts directly with Rad51 and stimulates strand exchange. We describe structural and functional aspects of the complex. Swi5 and the C-terminal domain of Sfr1 form an essential activator complex with a parallel coiled-coil heterodimer. The resultant coiled-coil is sharply kinked, generating an elongated crescent-shaped structure suitable for binding within the helical groove of the Rad51 filament. Our data suggest that the snug fit resulting from the complementary geometry of the heterodimer activates and stabilizes the Rad51 filament.

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 susceptibility to cancer. RecA-family DNA recombinases are key players in HR. In eukaryotes, there are two known classes of the 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. Recombinases bind to single-stranded DNA (ssDNA), and the resultant functional complex, termed a presynaptic filament, carries out DNA strand exchange; binding and strand exchange are the central reactions of HR. Auxiliary factors (referred to as mediators) are required to form/activate the presynaptic filament of eukaryotic recombinases.

The Swi5-Sfr1 complex from the fission yeast is one of the auxiliary proteins. Swi5 (85 residues) and Sfr1 (299 residues) are both widely conserved from yeast to humans. The Swi5-Sfr1 complex binds to the Rad51 presynaptic filament, directly stimulates Rad51-mediated displacement of RPA from ssDNA, and stabilizes the Rad51 filament that has already formed on the ssDNA. It is of great interest how Swi5-Sfr1 stimulates Rad51-driven strand exchange. To address this issue, we undertook structural analyses of the fission yeast Swi5-Sfr1 complex.

We produced a truncated Sfr1 lacking the N-terminal 180 residues, hereafter referred to as Sfr1C. Sfr1C forms a complex with Swi5. To gain structural insight into the mechanism of Swi5-Sfr1-mediated activation of the Rad51 filament, we determined the crystal structure of the Swi5-Sfr1C complex (2.3 Å resolution) [1]. Swi5 and Sfr1C form a parallel coiled-coil heterodimer and the Swi5-Sfr1C complex has an elongated shape (Fig. 1).

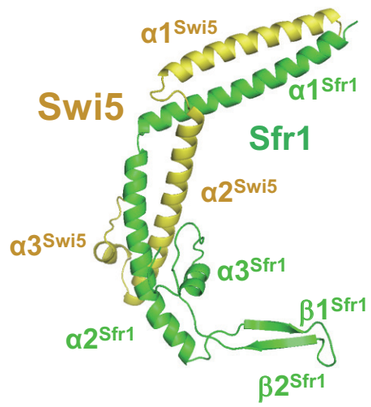

Figure 1: Ribbon representation of the Swi5-Sfr1C complex. Swi5 and Sfr1C are presented in yellow and green, respectively.

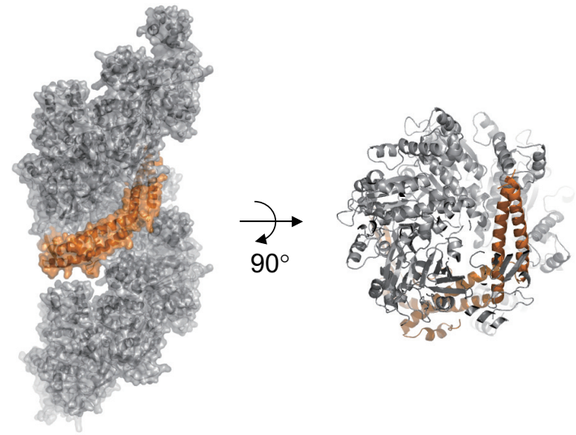

Figure 2: Docking model of the Swi5-Sfr1 complex (orange) into the Rad51 filament (gray) viewed from the side (left) and top (right). Molecules are shown as a ribbon drawing and transparent molecular surface (left). The Rad51 filament is modeled by a RadA structure (PDB id:2FPM).

The Swi5 and Sfr1C subunits of the heterodimers are firmly joined by two leucine zippers and a bundle structure at the bottom. The C-terminal regions in both Swi5 and Sfr1 fold back to the second leucine zipper and form a globular domain resembling a four-helix bundle that is stabilized primarily by van der Waals interactions. Hydrophobic residues from $\alpha 2^{sfr1}$, $\alpha 3^{sfr1}$, and the loop are buried in the interior of the bundle, where they pack together and stabilize the heterodimer.

Remarkably, the structure of the Swi5-Sfr1C complex is sharply kinked (130°). 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.

Residues participating in the interaction between the leucine zippers and the bundle are highly conserved across species. This conservation suggests that, in various species, Swi5 in complex with Sfr1C adopts a structural architecture similar to that observed in the S. pombe Swi5-Sfr1 complex.

The kinked structure of Swi5-Sfr1C fits snugly into the helical groove of the Rad51 filament, as revealed by molecular docking (Fig. 2). A single Swi5-Sfr1C complex seems to interact with up to four to five Rad51 molecules to bind into the filament. SAXS data revealed that the Swi5-SfrFL complex is approximately twice as long as the Swi5-Sfr1C [2]. This molecular "vine", composed of the Swi5-Sfr1 complex, seems to wind itself around the Rad51 filament, which is composed of up to 10 Rad51 molecules. This structural feature agrees well with the fact that the stimulation was most effective when the Swi5-Sfr1:Rad51 ratio was 1:10 to 1:20. We propose that Swi5-Sfr1 may transiently and repeatedly interact with the filament via the groove, inducing a local conformational change that activates the strand-exchange activity. Our present results provide the first exploration of the mechanistic aspects of the functional interactions between recombinases and their activators at atomic resolution.

REFERENCES

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