Crystal Structure of a Flp Recombinase-DNA Holliday Junction Complex

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Flp, the best characterized eukaryotic member of the λ integrase family of site-specific recombinases, is a gene product of the 2 μm plasmid from yeast and is responsible for regulating the copy number of this plasmid.[1] Like the over one hundred members of its family, Flp catalyzes a recombination reaction using a highly conserved tyrosine in conjunction with several other positively charged residues that help activate the scissile phosphate on the target DNA. The recombination event goes through a protein tetramer-DNA complex intermediate known as a Holliday junction, which undergoes a stepwise process of forming and breaking 3'-phosphotyrosyl covalent linkages between the protein and DNA. Flp and another family member, Cre, are widely used in experiments to alter the genetic composition of a variety of organisms, as the recombination reaction catalyzed by these two proteins requires only the recombinases and their specific DNA target sequences. Despite the catalytic similarities Flp shares with other family members, Flp is unique not only in its primary sequence, but, more importantly, in that its active site consists of residues from two adjacent monomers, with the tyrosine donated in trans to attack the DNA bound by the neighboring protein, as shown by most biochemical studies.[2]

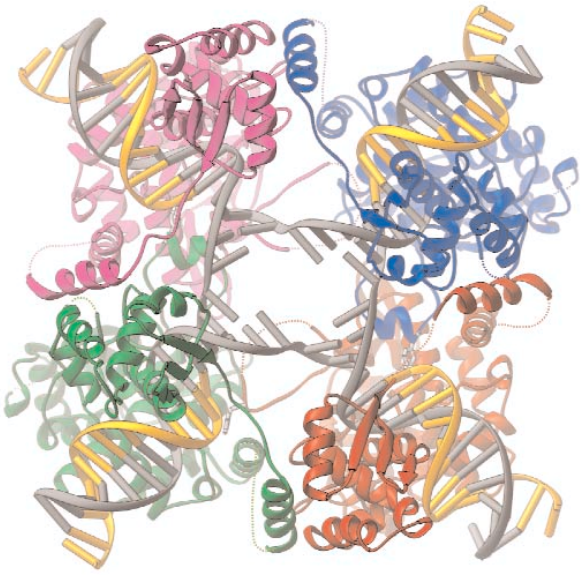

FIG. 1. "Top view" of the Flp-DNA complex with the N-terminal domain of the protein above the DNA. The trans-donated active site tyrosine is shown in gray. It is disordered in the purple monomer. Dotted lines represent connections not visible in the electron density.

To elucidate the structural details of Flp-mediated site-specific recombination, suicide DNA substrates were designed and used to trap the reaction intermediate. A Holliday junction complex was obtained and crystallized by the hanging drop method. The crystals reached their maximum size of 0.2 x 0.2 x 0.4 mm in 3-4 weeks and belong to the space group $P2_1$, with unit cell dimensions of 80 Å, 180 Å, 97 Å, 90°, 95.7°, and 90°. Multi-wavelength anomalous dispersion (MAD) and multiple isomorphous replacement with anomalous scattering (MIRAS) methods were used in structure determination, using crystals grown with brominated DNA substrates or those soaked in mercury salt solutions. The final model has been refined to a resolution of 2.65 Å, the working and free R values being 24.9% and 29.7%, respectively.

Our structure represents a Flp tetramer bound to a nicked DNA Holliday junction held in a nearly square-planar conformation[3] (Fig. 1). The complex displays a pseudo four-fold and an almost exact two-fold symmetry. Each monomer has nearly the same conformation as the one opposite to it but is slightly different from the two adjacent ones. Each protein consists of a relatively small N-terminal domain and a large C-terminal domain, forming a clamp-like bipartite structure around the DNA. Most of the DNA-binding specificity resides in the C-terminal domain, where the protein backbone and side chains make extensive contacts with the phosphates and bases of the DNA. The catalytic core of the C-terminal domain shows striking similarities to those of other family members, such as Cre, as well as proteins of a closely related family, the type IB topoisomerases.

The *trans* cleavage model of Flp has been confirmed by our structure. The catalytic tyrosine-containing helix is donated to its neighbor through flexible loop regions. Two states of the active site are also found in the complex, corresponding to the half-of-the-sites activity characteristic of the reaction and important for preventing double-strand DNA cleavage. In the functional state of the active site, the tyrosine is positioned and oriented properly to attack the scissile phosphate, while in the other state, the hydroxyl group of the tyrosine is placed approximately 10 Å away from its target. Close examination of the complex structure has revealed some clues as to why there are only two out of four recombinase monomers that are active at a specific step. Two types of protein-protein interfaces exist in the complex: one of them contains the functional catalytic site, with the C-terminal domains of the two monomers relatively close to each other; in the other, the two C-terminal domains are further apart from one another, making the distance too long for the *trans* peptide to stretch out to its neighbor and pulling the active tyrosine out of the proper place to attack DNA. The increase in the distance between the monomers also prevents the formation of the catalytic tyrosine-containing helix, whose contacts with the adjacent protein are important for correctly positioning the tyrosine for DNA cleavage.

Besides this nucleophilic tyrosine (Tyr343), Arg191, His305, and Arg308 have been shown by biochemical experiments to be

important for the reaction; two other residues, Lys223 and Trp330, have also been implicated to be involved in catalysis by our structure and by studies on other systems.[4] While their equivalent residues in some closely related proteins have been demonstrated to be essential for catalysis, no previous experiments have been conducted on these two residues of Flp to examine their roles during the recombination process.

The Flp-DNA complex structure has also shown that, aside from the tyrosine-containing helix, another *trans* helix is involved in protein-protein interactions. This helix is also connected to the rest of the protein through loops, thus increasing the overall flexibility of the whole complex. This might be one of the reasons why Flp is able to work on DNA substrates containing spacers of different lengths, while some other family members are fairly stringent regarding the number of bases in the spacer region.

Our structure of the Flp tetramer-DNA Holliday junction has resolved the disputes among previous biochemical studies as to whether Flp cleaves DNA in *trans* or in *cis*. It has also provided some answers to several interesting questions about Flp-mediated recombination, while raising other questions to be studied by future structural and biochemical experiments.

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