Crystal Structure of Cascade

Immune pathways protect all organisms from infection by genetic invaders such as viruses. It was recently discovered that prokaryotes protect against invasion by bacteriophages via an RNA based adaptive immune system, called the CRISPR system (1, 2). By acting as a barrier to the exchange of genetic information, a major route for the acquisition of antibiotic-resistance and virulence factors, the CRISPR system modulates the evolution of pathogenic bacteria.

The CRISPR system incorporates short fragments of the invading DNA between the repeat sequences of clusters of regulatory interspaced short palindromic repeats (CRISPRs). CRISPR transcripts are then processed into individual CRISPR RNAs (crRNAs) that guide CRISPR-associated (Cas) complexes to destroy invading DNA. Based on the presence of a signature gene, the CRISPR system has been divided into three types (3). In type I and type III systems, crRNA and Cas proteins assemble into large multisubunit complexes (2). In *Escherichia coli*, the Cas complex from its type I system is called Cascade (CRISPR-associated complex for antiviral defense). Cascade is a 405-kDa complex consisting of eleven subunits of five Cas proteins ($Cse1_1$, $Cse2_2$, $Cas7_6$, $Cas5_1$ and $Cas6e_1$) and a 61-nucleotide (nt) crRNA (1, 2). Cascade binds invading DNA if it contains a region complementary to the crRNA guide and a small 3 base pair (bp) sequence element called a protospacer adjacent motif (PAM) (1). CRISPR arrays lack a PAM sequence, enabling discrimination between self and non-self DNA. Once bound to DNA Cascade recruits a trans-acting helicase-nuclease, Cas3 that proceeds to unwind and degrade the DNA (1).

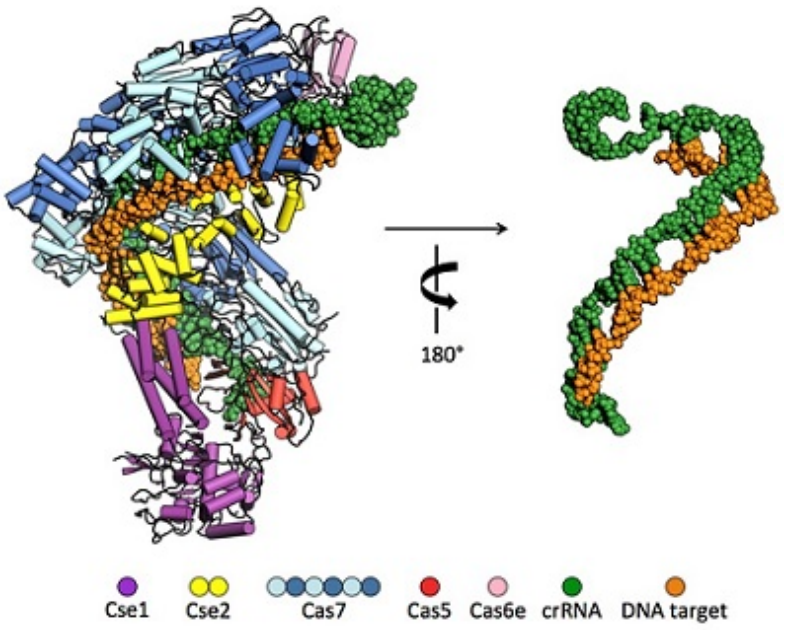

Figure 1: Structure of ssDNA-bound Cascade. Left: cartoon representation of the overall structure. Right: structure of the underwound crRNA-DNA hybrid.

To gain insights into the structural organization of Cascade and into target recognition, we determined the crystal structure of *E. coli* Cascade bound to a single-stranded DNA (ssDNA) target (**Figure 1**). X-ray diffraction data were collected at beam line 12-2 at the Stanford Synchrotron Radiation Lightsource (SSRL). The micro-focus beam coupled with a Pilatus detector were essential for measuring high quality data, in particular for measuring the anomalous signal from selenomethionine and mercury labeled crystals.

The 3.03 Å Cascade structure reveals a seahorse architecture, previously observed by cryo-EM (4). Perhaps the most striking feature of the complex is that the strands of the crRNA and target ssDNA do not twist around each other to form a helix but instead adopt an

underwound ribbon-like structure (**Figure 1**). This structure is facilitated by rotation of nucleotides out of the duplex region at six base pair intervals and stabilized by the highly interlocked organization of protein subunits. This underwound structure explains how Cascade avoids the topological problem of winding the crRNA around its DNA target. Formation of the crRNA-DNA hybrid initiates at the PAM and proceeds along the crRNA guide (2). The structure suggests that unwinding is characterized by 5-bp increments separated by 1-bp gaps. Incremental binding of the target likely increases the fidelity of target recognition by a mechanism similar to that used by RecA (5-7).

Primary Citation

S. Mulepati, A. Héroux and S. Bailey, "Crystal Structure of a CRISPR RNA-guided Surveillance Complex Bound to a ssDNA Target", *Science* **345**, 1479 (2014), DOI: 10.1126/science.1256996

References

1. R. Sorek, C. M. Lawrence, and B. Wiedenheft, "CRISPR-mediated Adaptive Immune Systems in Bacteria and Archaea", *Annu. Rev. Biochem.* **82,** 237 (2013).
2. J. van der Oost, E. R. Westra, R. N. Jackson, and B. Wiedenheft, "Unravelling the Structural and Mechanistic Basis of CRISPR-Cas Systems", *Nat. Rev. Microbiol.* **12,** 479 (2014).
3. K. S. Makarova *et al.,* "Evolution and Classification of the CRISPR-Cas Systems", *Nat. Rev. Microbiol.* **9,** 467 (2011).
4. B. Wiedenheft *et al.,* "Structures of the RNA-guided Surveillance Complex from a Bacterial Immune System", *Nature* **477,** 486 (2011).
5. Y. Savir and T. Tlusty, "RecA-Mediated Homology Search as a Nearly Optimal Signal Detection System", *Mol Cell* **40**, 388 (2010).
6. Z. Chen, H. Yang, N. P. Pavletich, "Mechanism of Homologous Recombination from the RecA ssDNA/dsDNA Structures", *Nature* **453**, 489 (2008).
7. S. C. Kowalczykowski, "Structural Biology: Snapshots of DNA Repair", *Nature* **453**, 463 (2008).

Contact

Scott Bailey, Johns Hopkins University, Bloomberg School of Public Health

SSRL is primarily supported by the DOE Offices of Basic Energy Sciences and Biological and Environmental Research, with additional support from the National Institutes of Health, National Institute of General Medical Sciences.