Structural Study of Core Complex Cascade in the CRISPR-Cas Immune System

Clustered regularly interspaced short palindromic repeats (CRISPR) together with CRISPR-associated (cas) proteins form the CRISPR-Cas system to defend against foreign nucleic acids of bacterial and archaeal origin. In the I-E subtype CRISPR/Cas system, 11 subunits from five Cas proteins ($CasA_1B_2C_6D_1E_1$) assemble along a crRNA to form the Cascade complex. We reported on the 3.05 Å crystal structure of the 405-kDa *E. coli* Cascade complex, which provides molecular details beyond those available from earlier lower-resolution cryo-EM structures.

In recent years, a novel adaptive immune system called the CRISPR-Cas system has been found in approximately half of bacteria and almost all archaea [1, 2]. CRISPR-Cas immune systems depend the invading nucleic acid by three steps. The first step is spacer acquisition, in which new spacers are acquired from the foreign nucleic acid into the CRISPR locus. The second step is CRISPR-Cas expression, in which CRISPR arrays are transcribed and processed into small interfering crRNAs. The final step is interference, in which mature crRNAs assemble with Cas proteins into a surveillance complex that targets DNA for degradation, thereby preventing the propagation of viruses and plasmids. The hallmark of the type I CRISPR-Cas system is the assembly of the large Cascade surveillance complex that uses crRNA to recognize DNA targets. Previous studies indicated that Cascade is a 405-kDa complex packaged by five essential proteins with different numbers of copies ($CasA_1B_2C_6D_1E_1$) and a 61-nucleotide crRNA (Fig. 1a). Despite comprehensive functional and structural characterizations, the high-resolution structure of Cascade at the atomic level is needed to clarify the molecular mechanism of its reconstitution and targeting DNA.

We determined the crystal structure of *Escherichia coli* Cascade complex using diffraction data collected at the Photon Factory (Fig. 1b) [3]. This structure provides molecular details beyond those available from earlier lower-resolution cryo-electron microscopy structures. Overall, this complex exhibits a sea-horse-shaped architecture. Instead of forming a simple, compact structure, the 11 subunits are assembled into two structural layers. Six CasC proteins, together with CasD and CasE, tightly pack to form the outer layer. The inner layer consists of CasA and CasB dimer. With the perfect electron density map, we observed all 61 nucleotides of the crRNA and subsequently analyzed its interactions with different Cas proteins. Specifically, the 3'-end of crRNA adopts a stem loop-fold and the stem makes extensive contact with the positively charged area in the CasE C-terminal, whereas the 5'-end of crRNA takes on a hook-like shape, and is embedded in a "pocket" formed by CasD, CasA, and CasC6. The crRNA consists of a 32-nucleotide spacer flanked by two end repeats. Interestingly, the spacer fragment is not continuously stacked, with five kinks present at regular intervals, namely at the positions of the 6th, 12th, 18th, 24th, and 30th nucleotides. The kinked positions precisely co-localize and interact with the five beta-hairpins projecting from CasC2-CasC6. Consequently, the bases of the kinked nucleotides are flipped out and do not participate in DNA binding. Subsequent mutagenesis and biochemical experiments showed that the mutations of the kinked nucleotides had no obvious effect on target DNA recognition and confirmed the discovery from the structure.

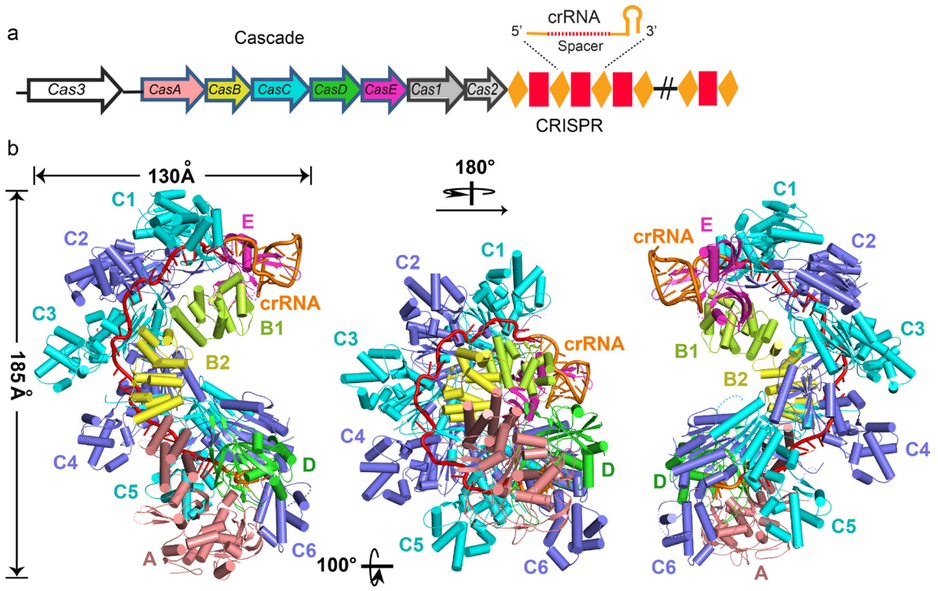

Figure 1: Crystal structure of the Cascade complex from *E. coli*. a, The I-E subtype CRISPR system in *E. coli* (K12) consists of eight Cas proteins and the CRISPR locus. b, Overall structure of the Cascade complex. Reprinted with permission from the Nature.

In conclusion, these structural studies have provided insights into both the assembly and target recognition of the Cascade complex. The results also revealed that crRNA plays an essential role not only in target recognition, but also in Cascade complex formation. Furthermore, the elongated beta-hairpin loop from Cas protein represents a multifunctional element that plays a critical role in spacer RNA stabilization, CasC helix formation, and CasA–CasD as well as CasC–CasA and CasC–CasB interactions.

REFERENCES

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