Crystal Structure of the CRISPR-Cas RNA Silencing Complex

CRISPR-Cas constitutes a prokaryotic adaptive immune system against invading genetic elements. The crRNA and Cas protein(s) form an interference complex that degrades invading nucleic acid complementary to the crRNA guide. The type III Cmr interference complex comprises six Cas proteins and a crRNA, and degrades target RNA. The crystal structure of the Cmr complex bound to a target analog was determined. The complex recognizes the crRNA 5'-tag and deforms the guide-target duplex at 6-nt intervals. The structure reveals the periodic RNA cleavage mechanism by the Cmr complex, and provides insights into the evolution of the type I and III interference complexes.

The CRISPR-Cas system is a prokaryotic RNA-based defense system against mobile genetic elements [1]. The Cas proteins and the crRNA, which is derived from the CRISPR locus, form the interference complex for target degradation, in a base-complementary manner of the crRNA guide [2]. The interference complexes are classified into three major types (I, II, and III) [3]. The type I and II complexes target foreign DNA for degradation, and their mechanisms of action were revealed by crystallography [4, 5]. In contrast to these DNA-targeting complexes, the type III interference complex cleaves RNA [6]. However, the RNA degrading mechanism of the type III complex remains elusive, because of the lack of structural information.

We reconstituted the Cmr complex (one of the type III complexes) that comprises six Cas proteins (Cmr1–Cmr6) and a crRNA which has an eight-nucleotide (nt) tag at the 5'-region followed by a guide sequence. A subsequent biochemical experiment revealed that five protein subunits (Cmr2–Cmr6) and the crRNA are enough for the RNA cleavage activity despite the absence of Cmr1. To reveal the crRNA-guided RNA-silencing mechanism, we solved the crystal structure of the Cmr1-deficient Cmr complex (CmrΔ1) bound to the target analog (ssDNA complementary to the crRNA guide) by the MR-SAD method [7, 8].

The CmrΔ1 comprises eight protein subunits with their stoichiometry of Cmr2 : Cmr3 : Cmr4 : Cmr5 : Cmr6 = 1 : 1 : 3 : 2 : 1 (Fig. 1a). Cmr2 and Cmr3 form the base region of the complex, and contact the crRNA 5'-tag. The crRNA guide is located in the groove formed by the three Cmr4 and two Cmr5 molecules. Cmr6 caps the Cmr4 and Cmr5 stacks at the opposite end of the base region. The target analog is recognized by the crRNA guide in a base-complementary manner in the groove.

The crRNA 5'-tag is recognized by Cmr3, where its main-chain carbonyl and amide of Thr196 and Gly198, respectively, form specific hydrogen bonds with U2 of the crRNA (Fig. 1b). The importance of these interactions was confirmed by the U2A mutation of the crRNA. Furthermore, the sugar-phosphate moieties of the 5'-tag form extensive hydrogen bonds with the proteins. Intriguingly, the 5'-terminal OH group of the crRNA is recognized by the main chain atom of Gly58 from Cmr3 (Fig. 1b).

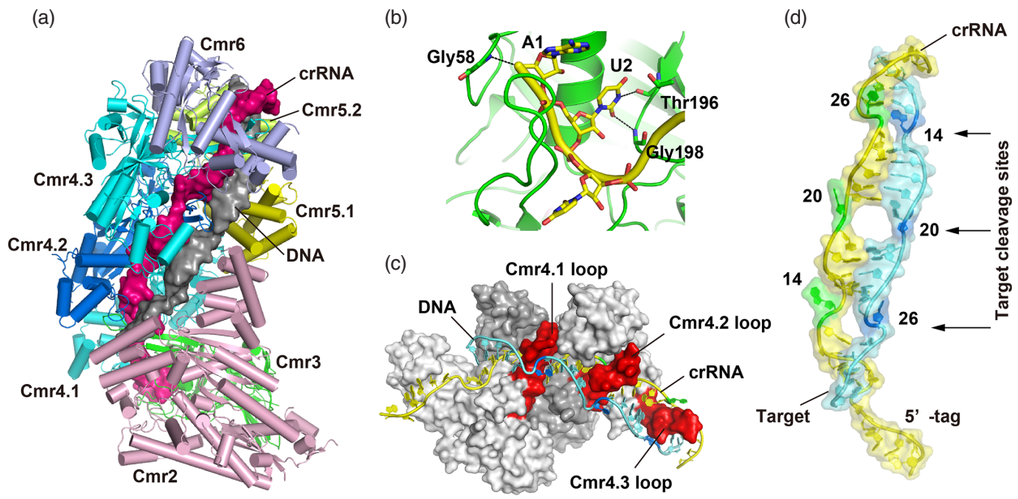

Figure 1: Crystal structure of the target analog bound Cmr complex. **(a)** Overall structure of the complex. **(b)** The mechanism of the crRNA 5'-tag recognition by Cmr3 in the complex. **(c)** Interaction between Cmr4 stack and crRNA-target duplex. **(d)** Structure of the crRNA-target duplex in the Cmr complex.

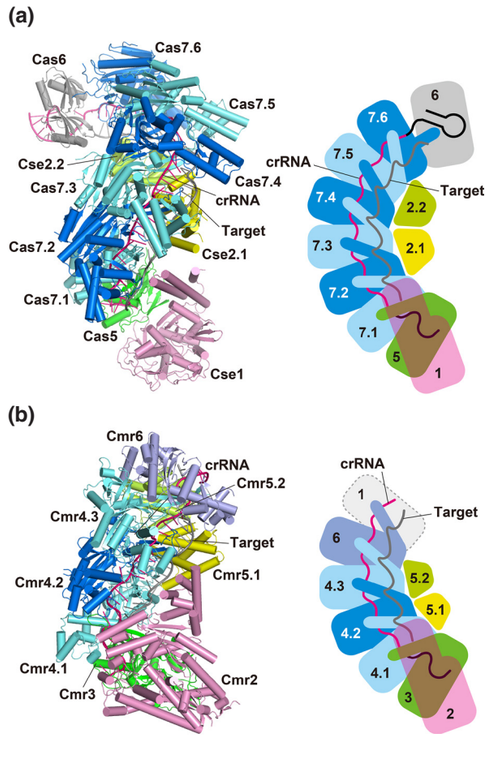

Figure 2: Structural similarity between the types I **(a)** and III **(b)** interference complexes. The overall structures (left panels) and the schematic diagrams (right panels) of each complex.

The crRNA guide is bound to the groove through the interaction mainly with three Cmr4 subunits in a non-sequence-specific manner. Although the crRNA guide and ssDNA form a duplex, they adopt an unwound ribbon-like structure instead of the canonical helix. This is caused by the intercalations of the idiosyncratic loops of three Cmr4 subunits into the duplex at 6-nt intervals (Fig. 1c). Therefore, the periodic intercalations deform the guide-target duplex at positions 14, 20, and 26 from the 5' end of the crRNA, suggesting the destabilization of these three sites in the target strand for cleavage reaction. Consistent with this, our biochemical experiment demonstrated that the Cmr complex cleaves the target RNA at these three sites deduced from the structure. We also identified the catalytic residue of Asp31 in Cmr4 as a possible general acid catalyst to protonate the 5' terminus of the cleavage product during the reaction. The Cmr complex recognizes the crRNA 5'-tag and defines the binding site for its 5' terminal group (Fig. 1b). Therefore, the Cmr complex degrades the target RNA by using the 5' ruler mechanism, with which the target cleavage sites in 6-nt intervals are strictly specified in length from the 5' end of the crRNA (Fig. 1d).

Structural comparison revealed that the overall structure of the type III Cmr complex resembles that of the type I interference complex [5] (Fig. 2). Intriguingly, the structural and functional roles of Cmr3 and Cmr4 in the type III complex are quite similar to those of Cas5 and Cas7, respectively, in the type I complex [5]. Therefore, these interference complexes interact with the crRNAs in a similar fashion, and the target strands are recognized in a similar mechanism by these interference complexes, despite the fact that these complexes target different kinds of nucleic acid, RNA and DNA, respectively. These findings show the occurrence of divergent evolution from a common ancestral complex in the CRISPR-Cas system.

In conclusion, this study revealed the mechanisms of Cmr complex assembly and target cleavage site specification, and also paves the way for understanding the molecular evolution of the CRISPR-Cas interference complex.

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