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Cas Protein Cmr2 Full of Surprises

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The Cmr complex carries out target RNA degradation in organisms possessing the CRISPR-Cas system. In this issue of *Structure*, Cocozaki et al. present the crystal structure of Cmr2, providing insight into the architecture of the Cmr complex.

The clustered regularly interspaced short palindromic repeats-CRISPR associated proteins (CRISPR-Cas) systems are recently discovered self-defense systems against invading genetic elements found in bacteria and archaea (Barrangou et al., 2007; Brouns et al., 2008). In a CRISPR-Cas system, the CRISPR locus is transcribed into a long primary transcript, which is then processed into a library of short CRISPR-derived RNAs (crRNAs). Each crRNA is loaded into a multiple protein complex (Cas complex) that carries out degradation of nucleic acids. From studies reported so far, the majority of the targets for degradation are DNAs (Garneau et al., 2010). However, a subset of the Cas complex, the Cas module RAMP (Cmr) complex, was shown to cleave target RNA in vitro and in vivo (Hale et al., 2012; Hale et al., 2009). From a structural point of view, significant progress has recently been made in understanding how the Cas complex recognizes DNA for degradation, exemplified by a recent EM structure of Cascade (Wiedenheft et al., 2011). On the other hand, little is known about target RNA recognition and degradation by the Cmr complex. In this issue of *Structure*, Cocozaki et al. (2012) presents the crystal structure of Cmr2 from *Pyrococcus furiosus* (Pf). The structure, combined with functional analyses, reveals a few surprises, sheds some light on the architecture of the Cmr complex, and raises a few questions regarding the mechanism of target RNA recognition and cleavage by the Cmr complex.

The Cmr complex from Pf (Pf Cmr) is composed of six proteins, Cmr1–6. Four of the six subunits are RNA-binding proteins of the repeat-associated mysterious proteins (RAMP) family. Pf Cmr2, which is not a RAMP protein, is the largest subunit in the complex (100 kDa, and the rest of the subunits range from 20 to 39 kDa). Cmr2 was predicted to have a nuclease domain of the histidine, aspartic acid (HD) family, a zinc ribbon domain, and a DNA polymerase-like domain (Makarova et al., 2002). Because of its size and, more importantly, the presence of the nuclease domain, Cmr2 was thought to be the catalytic subunit of the Cmr complex. However, the findings from Cocozaki et al. (2012) now tell a different story.

The first unexpected finding was from a functional assay using the Pf Cmr complex assembled from a truncated Pf Cmr2 that lacks the N-terminal HD nuclease domain (Pf Cmr2dHD). The complex with the truncated Pf Cmr2 cleaved target RNA as effectively as the one with the full-length protein in vitro, demonstrating that the element responsible for target RNA cleavage does not reside in the HD nuclease domain.

Having demonstrated that the N-terminal nuclease domain of Pf Cmr2 is not required for target RNA cleavage, Cocozaki et al. (2012) solved the crystal structure of Pf Cmr2dHD, which provides additional surprises. Instead of a polymerase-like fold, the structure of Pf Cmr2dHD shows a fold of two ferredoxin-like domains and two small helical domains. Furthermore, two CXXC motifs form two disulfide bonds instead of the predicted zinc ribbon domain. Structural search and comparison with known protein structures showed that the two ferredoxin-like domains resemble the adenylyl cyclase homodimer, implying that Pf Cmr2 might have adenylyl cyclase-like activity. Soaking crystals of Pf Cmr2dHD with ADP and divalent ions prior to data collection indeed produced a structure of Pf Cmr2dHD with ADP and metal ions bound. However, functional assays of Pf Cmr2dHD, either alone or in the Pf Cmr complex, failed to detect any enzymatic activity of Cmr2dHD to hydrolyze ATP. In addition, mutations of the conserved residues responsible for ADP and metal ion binding did not affect target RNA cleavage by the Pf Cmr complex assembled with the Pf Cmr2dHD mutants.

The structure of Pf Cmr2dHD by Cocozaki et al. (2012) provides insight into the architecture of the Cmr complex. Additional functional analyses indicate that Cmr2 is unlikely to be the catalytic subunit of the Cmr complex for target RNA cleavage. Thus, the studies raise two intriguing questions: (1) if Cmr2 is not responsible for target RNA cleavage, what is its role in the Cmr complex?, and (2) if Cmr2 is not the catalytic subunit, which subunit in the Cmr complex is responsible for target RNA cleavage?

Because Cmr2 is the largest subunit in the Cmr complex, it is reasonable to hypothesize that it serves as the structural foundation of the complex where other Cmr proteins interact. Such a role does not require enzymatic activity, consistent with the results from ATP hydrolysis and mutagenesis (Cocozaki et al., 2012). Studies of pair-wise protein-protein interactions of the six proteins constituting the Pf Cmr complex should shed some light on the validity of the hypothesis. Recently, Zhang et al. (2012) reported low resolution EM structures of the Cmr complex from *Sulfolobus solfataricus*. Sequence alignments indicate that the subunits of the Pf Cmr complex are homologous to their counterparts in the SsoCmr complex, although the degree of the sequence identities varies among different subunits. Therefore, docking the high-resolution crystal structure of Pf Cmr2dHD into the low-resolution EM structure of the SsoCmr complex may also shed light on the possible function of Cmr2.

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Due to the lack of effect of mutations on target RNA cleavage, Cocozaki et al. (2012) suggested that the ribonuclease activity might reside in other Pf Cmr proteins. This is entirely reasonable, as some RAMP proteins have been shown to possess nuclease activity. Cmr5 can immediately be ruled out, because the Pf Cmr complex lacking Pf Cmr5 was able to cleave target RNA (Hale et al., 2009). Among the remaining subunits (Cmr1, Cmr3, Cmr4, and Cmr6), Cmr3 appears to be the best candidate as the nuclease, because the EM structure showed that SsoCmr3 is located near the center of the SsoCmr complex, where the RNA cleavage is expected to occur (Zhang et al., 2012). However, the reported difference of the cleaved RNA products by the Cmr complexes from these two organisms complicates the hunt for the nuclease, but it also makes mechanistic study more interesting. While the target RNA cleaved by Pf Cmr complex produced 2′,3′-cyclic phosphate and 5′-OH (Hale et al., 2009), the cleavage products of the SsoCmr complex contained 3′-OH and 5′-phosphate (Zhang et al., 2012). It is difficult to envision a highly homologous subunit of the Cmr complex cleaving target RNA with two distinct mechanisms. Therefore, if the analyses of the ends of the cleaved RNA stand, the best candidate for the nuclease would be Cmr1, as it is the least conserved, both in amino acid sequence and size, among all subunits of the Cmr complex. Regardless of the final outcome, the hunt for the nuclease responsible for target RNA cleavage is shaping up to be an interesting one, and there are bound to be more surprises along the way.

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