Published in final edited form as:

Nat Struct Mol Biol. 2016 September 06; 23(9): 765–766. doi:10.1038/nsmb.3287.

A Molecular Arms Race: New Insights into anti-CRISPR mechanisms

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Abstract

Bacteria and phage co-evolve in a molecular arms race. The crystal structure of the AcrF3-PaCas3 complex sheds light on how phages have found ways to suppress CRISPR-Cas immunity.

Bacteria are under constant threat of viral infection by phages, which are estimated to outnumber bacteria ten to one in most environments. Consequently, bacteria have evolved multiple immune responses to resist infection, which places selective pressure on phage populations. Phages then counter-evolve, putting selective pressure back onto the host. This cycle results in rapid co-evolution of the host and phage in what has been termed a molecular arms race. One of these immune responses is the CRISPR-Cas (Clustered Regularly Interspaced Short Palindromic Repeat - CRISPR associated) immune response. In keeping with the arms race, proteins that suppress the CRISPR-Cas immunity were recently discovered in phages that infect *Pseudomonas aeruginosa*. These anti-CRISPR proteins were found to bind and inactivate key components of the CRISPR-Cas systems. However, the molecular details of how this binding suppresses CRISPR-Cas immunity are unknown. In this issue Wang *et al.* present the crystal structure of a Cas protein in complex with an anti-CRISPR protein, providing the first molecular view of how an anti-CRISPR protein suppresses CRISPR-Cas immunity.

CRISPR-Cas systems are RNA-guided adaptive immune systems that protect a broad range of prokaryotes against invasion by phage or plasmids. Immunity is first acquired by integration of short DNA fragments from the invader into a CRISPR array. The CRISPR array is then transcribed and the resulting transcript processed into small CRISPR RNAs (crRNAs) that guide the immune system to the invader, which is subsequently degraded by Cas nucleases. As a likely consequence of the molecular arms race, CRISPR-Cas systems are extremely diverse and are currently separated into at least five different types. Type I systems, which are further divided into seven subtypes (A-F and U), are the most widespread and are mediated by the multi-subunit ribonucleoprotein complex Cascade (CRISPR-associated complex for antiviral defense) and the Cas3 helicase-nuclease. Guided by crRNA, Cascade binds to invading DNA, creating an R-loop. Completed R-loop formation serves as a signal to recruit Cas3, which then uses its helicase and nuclease activities to unwind and degrade the invading DNA. Recently, Bondy-Denomy and colleagues discovered that AcrF3, a protein encoded by the *P. aeruginosa* phage JBD5, specifically inhibits *P. aeruginosa* Type I-F Cas3 (PaCas3) by blocking its recruitment to Cascade (also called the Csy complex). To understand how AcrF3 inhibits the activities of

PaCas3, Wang *et al* determined the 2.6 Å crystal structure of the PaCas3 in complex with AcrF3.

Although sequences of Cas3 are highly diverse between type I systems, PaCas3 shares substantial structural similarity to the Type I-E Cas3s from *Thermobifida fusca* and *Thermobaculum terrenum*. All three Cas3 proteins contain a core superfamily-2 (SF2) helicase domain (composed of two tandem RecA-like subdomains, RecA1 and RecA2), flanked at the N-terminus by an HD nuclease domain and at the C-terminus by a long linker region and a C-terminal domain (CTD) (Fig. 1). In SF2 helicases binding of ATP to RecA1 induces rotation of RecA2 toward RecA1 to orient residues important for ATP hydrolysis. Following hydrolysis RecA2 rotates away from RecA1, releasing ADP and phosphate. This rotation couples ATP hydrolysis to DNA translocation and unwinding. The RecA subdomains of PaCas3 in the AcrF3 complex appear positioned in the closed state and are bound to an ADP molecule. ADP was not added during purification or crystallization, therefore the ADP likely co-purified with the complex from the cell. This suggests that AcrF3 may trap PaCas3 in a closed state, blocking release of ADP.

AcrF3 functions as a dimer to suppress PaCas3 activity. Size exclusion chromatography indicates that AcrF3 alone is a dimer in solution and the crystal structure of the complex reveals a dimer of AcrF3 bound to one molecule on PaCas3. This suggested that dimerization of AcrF3 is required for binding to PaCas3. Indeed, mutation of a valine residue (Val 14), which lies at the AcrF3 dimerization interface, to aspartate disrupts AcrF3-PaCas3 interaction and the ability of AcrF3 to inhibit the nuclease activity of PaCas3. The AcrF3 dimer binds on a concave surface of PaCas3 formed by the HD domain, RecA2 subdomain, the linker region and the CTD (Fig. 1). More than half of the assessable surface area of the AcrF3 dimer is buried at this interface. AcrF3 binds the HD domain via a protruding helix and its two connecting loops, which are unique to PaCas3. Indeed the majority of PaCas3 residues that contact AcrF3 are distinct from other Cas3s; hence these residues determine the specificity of AcrF3 for PaCas3. The recruitment and activation of Cas3 by Cascade relies on physical interaction between the linker region of Cas3 and Cascade, the interaction of Cas3 with the ssDNA generated at the R-loop and on the hydrolysis of ATP. Each of these requirements is suppressed by AcrF3. The AcrF3 dimer covers the linker region, blocks access to the DNA binding groove and traps AcrF3 in an ADP bound form.

Another contribution of this study is new insight into the interaction of Cas3 with the CRISPR adaptation machinery. Adaptation, mediated by the Cas1-Cas2 integrase complex, is the process whereby DNA fragments from invaders are incorporated into host CRISPR arrays. Uniquely in Type I-F systems Cas2 is fused to the N-terminus of Cas3, and in type I-E and I-F systems Cas3 has been suggested to have a role in adaptation. In the structure of PaCas3, the Cas2 domain interacts with the RecA1 subdomain, opposite the AcrF3 binding interface, and is connected to the HD domain by a disordered loop. This interaction is likely mirrored in the other type I systems, where Cas2 and Cas3 are encoded as separate proteins, but this awaits experimental validation. In addition, overlay of PaCas3 with the Cas1-Cas2 integrase complex via its Cas2 domain revealed no notable clashes and reveals the likely architecture of the Cas1-Cas2-Cas3 complex.

In summary, the present study provides the first picture of how an anti-CRISPR protein suppresses its target, in this case AcrF3 suppressing Cas3. This study represents the first of its kind but we expect more structural insights into the mechanisms of anti-CRISPR proteins will be forthcoming, as other anti-CRISPR proteins have been identified, for example AcrF1 and AcrF2, and undoubtedly more will be discovered.

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Figure 1.