CRISPR

Enzymatic anti-CRISPRs improve the bacteriophage arsenal

Bacteriophage-encoded anti-CRISPR (Acr) proteins were previously thought to inhibit CRISPR-mediated immunity by acting as physical barriers against the binding or cleavage of DNA. Two new studies report that recently discovered type V Acr proteins use enzymatic activities to shut down the Cas12a endonuclease, providing a multi-turnover 'off switch' for CRISPR-based immunity and technology.

Shravanti K. Suresh, Karthik Murugan and Dipali G. Sashital

Bacteria and phages are in a constant war for survival. In this fight, bacteria have developed an arsenal to defend against infections, including sophisticated CRISPR-Cas (clustered regularly interspaced short palindromic repeats–CRISPR associated) systems that enable adaptive immunity[1]. The basis of this immunity is the activity of CRISPR RNA (crRNA)-guided Cas effectors, which find and destroy nucleic acids from invader phages (Fig. 1). To thwart this attack, phages deploy anti-CRISPR (Acr) proteins that directly inhibit Cas effectors to shut down CRISPR-mediated defense[2–4]. Acr proteins can also be used as 'off switches' for CRISPR-based technology in which Cas effectors, such as Cas9 and Cas12a, are used for genomic manipulation[5,6]. Although Acr proteins directed against Cas9 have been extensively characterized[4], Acr proteins directed against Cas12a were discovered only recently[7,8]. In this issue, Knott et al.[9] and Dong et al.[10] provide the first mechanistic characterization of these type V-A Acr (AcrVA) proteins. Surprisingly, each study uncovers a unique AcrVA activity in which enzymatic cleavage of the crRNA or covalent modification of Cas12a renders the Cas effector complex inactive. These enzymatic inhibitors of CRISPR-Cas systems are the first of their kind to be identified and may provide a considerable advantage over previously characterized Acr mechanisms.

Previously identified Acr proteins generally inhibit CRISPR-Cas immunity by preventing either binding to the target or its cleavage by the Cas effector[3,4]. Attacking phages must cooperate to match each Cas effector already present in the host cell with a copy of an Acr protein to completely shut down immunity[11,12]. In contrast, it has been proposed that enzymatic Acr proteins would more effectively deactivate CRISPR-Cas immunity by turning over and neutralizing many Cas effectors per Acr copy[12] (Fig. 1).

In studies of recently discovered type V-A Acr proteins, Knott et al. and Dong et al. now demonstrate that such enzymatic Acr proteins do indeed exist[9,10]. By collectively characterizing three inhibitors of Cas12a, AcrVA1, AcrVA4 and AcrVA5, the authors discover that similar to other Acr proteins, all three AcrVA proteins block binding of DNA by such Cas12a. However, AcrVA1 and AcrVA5 act through novel mechanisms (Fig. 2).

Knott et al. discover that AcrVA1 truncates the 3′ end of the guide crRNA, which is required for binding to the DNA target through complementary base pairing[9] (Fig. 2). While AcrVA1 alone lacks RNA-cleavage activity, it robustly cleaves the crRNA within the effector complex. Intriguingly, when the authors test AcrVA1 activity at sub-stoichiometric concentrations, crRNA cleavage is still observed at maximal levels. These data demonstrate that AcrVA1 is a bona fide enzyme that releases the Cas12a–crRNA complex following RNA cleavage, allowing multi-turnover activity.

The authors demonstrate that neither residues in the Cas12a active site nor metal ions are required for the AcrVA1-dependent truncation of crRNA, although the exact mechanism of AcrVA1's activity remains unclear. Like most Acr proteins, AcrVA1 lacks conservation with any proteins of known function, obscuring the identification of a putative active site. The data presented by Knott et al. strongly suggest that AcrVA1's activity is linked to binding to a complete Cas12a–crRNA complex[9]. Notably, AcrVA1 has previously been shown to potently inhibit a broad range of Cas12a orthologs[7,8], suggesting that this activation can occur across several Cas effector sequences.

Dong et al. discover that AcrVA5 is an acetyltransferase that covalently modifies lysine residues within Cas12a[10] (Fig. 2). One particular target of this acetylation is the lysine residue involved in 'reading out' the PAM (protospacer-adjacent motif), a short sequence located next to the target that is required for binding to double-stranded DNA (dsDNA)[13,14]. Modification of this residue to acetyllysine nullifies its ability to interact with the PAM and thus renders Cas12a unable to unwind and bind dsDNA targets (Fig. 2). The authors show that AcrVA5's activity relies on acetyl coenzyme A (acetyl-CoA) and that its acetylation activity can be enhanced by the provision of excess acetyl-CoA in vitro. These results suggest an inherent multi-turnover capacity

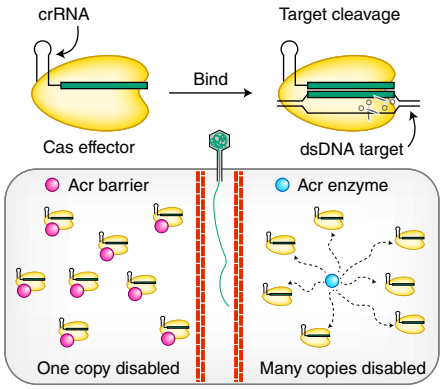


Fig. 1 | Alternative mechanisms for disabling Cas effectors. At top, Cas effector proteins (yellow) use crRNA guides to bind to complementary dsDNA targets, which leads to cleavage of the target. Below, previously characterized Acr proteins (left) act as physical barriers (magenta) to block binding or cleavage of DNA; this requires a 1:1 stoichiometry between Acr protein and Cas effector. Acr enzymes (right; blue) can theoretically inactivate multiple Cas effectors for each copy of Acr protein and increase the rate of CRISPR-Cas nullification.

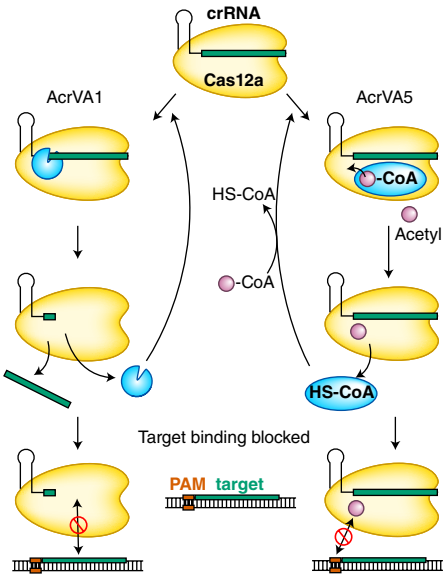


Fig. 2 | AcrVA1 and AcrVA5 are enzymatic inhibitors of Cas12a. AcrVA1 (left) cleaves the crRNA within the guide region (green) when the crRNA is bound to Cas12a. Following cleavage, the cleaved guide fragment and AcrVA1 are released. The shortened crRNA is not competent for binding dsDNA, as it lacks complementarity to the target region of the dsDNA. AcrVA1 can turn over and cleave crRNA in other copies of the Cas12a effector complex. AcrVA5 (right) uses an acetyl-CoA cofactor to add an acetyl group (purple) to the PAM-sensing lysine residue in the PAM-interacting domain of Cas12a. This chemical modification renders Cas12a unable to recognize the PAM (brown) or unwind dsDNA. Following replacement of the HS-CoA with a new acetyl-CoA cofactor, AcrVA5 can turn over and inactivate more copies of Cas12a.

of AcrVA5 within bacterial cells in which acetyl-CoA is abundant (Fig. 2).

Intriguingly, many Cas12a orthologs contain a PAM-interacting arginine rather than a lysine residue. Dong et al. show that this substitution renders AcrVA5 ineffective, enabling continued targeting of DNA by such Cas12a[10]. Surprisingly, AcrVA5's acetylation activity does not appear to be highly sequence specific, as many lysine residues within Cas12a are also acetylated. Although only a single acetylation of the PAM-sensing lysine is required for inhibition of Cas12a, it will be interesting in the future to determine whether other AcrVA5-mediated acetylation events have consequences on the bacterial cell.

The discovery of Acr enzymes continues to elucidate the evolutionary battle between prokaryotes and viruses for their survival. However, although enzymatic Acr proteins are theoretically advantageous, they seem to be relatively uncommon. Is the rarity of Acr enzymes a consequence of the complex set of requirements for the evolution of enzymatic activities? Do Acr proteins that act as physical blocks actually provide better long-term inhibition by remaining bound to the Cas effector? It is possible that the two enzymatic activities of AcrVA1 and AcrVA5 may provide only temporary inactivation of Cas12a. Recent studies have suggested that Cas12a can efficiently replace its guide to reset the complex, which could reduce the efficacy of the truncation of crRNA by AcrVA1[15]. Similarly, bacterial cells could employ a deacetylase to counter AcrVA5. Nevertheless, if Acr enzymes have evolved robust turnover kinetics, they may overcome such counteractive measures. With Acr enzymes now in hand, scientists can begin to determine whether these mechanisms truly provide phages a stronger weapon and whether humans can take advantage of these enzymes for tighter control of CRISPR technology. ❐

Shravanti K. Suresh, Karthik Murugan and Dipali G. Sashital*

Roy J. Carver Department of Biochemistry, Biophysics and Molecular Biology, Iowa State University, Ames, Iowa, USA.

**e-mail: sashital@iastate.edu*

Published online: 01 April 2019
https://doi.org/10.1038/s41594-019-0210-5

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Competing interests

The authors declare no competing interests.