Bacteria SAVED from viruses

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Abstract
Increasingly, cyclic nucleotide second messengers are implicated in antiviral defence systems in bacteria and archaea as well as eukaryotes. Lowey et al. describe SAVED - a widespread, uncharacterised cyclic nucleotide sensor protein domain that activates cell defence systems. The structure of SAVED reveals links to the CRISPR system, which also generates cyclic nucleotides in response to viral infection.

Just like humans, microbes are frequently infected by viruses. Consequently, a wide range of defence systems, including restriction-modification and CRISPR, are employed to prevent infection spreading in a microbial population. Cyclic nucleotide second messengers are increasingly implicated in a wide range of antiviral defence systems in bacteria and archaea. These are collectively known as CBASS ("cyclic-oligonucleotide-based antiphage signalling systems") (Cohen et al., 2019), in a nod towards the eukaryotic cGAS antiviral signalling system. The diversity of CBASS is remarkable, arising from a wide range of cGAS/DncV-like Nucleotidyltransferases (CD-NTases) that synthesise a diverse collection of cyclic nucleotide second messengers, in turn activating a variety of effector proteins to effect immunity. Most of these systems remain uncharacterised, but Kranzusch and colleagues take a significant step forward in this issue by elucidating the structure and function of the enigmatic SAVED (Burroughs et al., 2015), found in many CBASS (Lowey, 2020). SAVED stands for "SMODS-associated and fused to various effector domains" – seldom was an acronym more welcome.

The authors focussed on a CBASS from the bacterium *Enterobacter cloacae*, comprising the CD-NTase CdnD and three associated genes (*cap2*, *cap3* and *cap4*). Previously, it was demonstrated that CdnD synthesises the cyclic trinucleotide second messenger 3',3',3'-cAAG (Whiteley et al., 2019). Here they show that the second messenger binds to the C-terminal SAVED domain of Cap4. This potentiates a structural rearrangement, resulting in activation of a potent N-terminal endonuclease that degrades DNA. The system provides immunity against infection by T2 and T5 (but intriguingly not T7) bacteriophage *in vivo*.

The structure of Cap4 in complex with a cyclic trinucleotide reveals that SAVED domains are distant family members of the CARF (CRISPR associated Rossmann fold) domain superfamily, confirming recent predictions (Shmakov et al., 2018). CARF proteins act as sensors for the cyclic oligoadenylate second messengers generated by the Cas10 cyclase domain of type III CRISPR systems (Kazlauskiene et al., 2017), and like SAVED domains are normally fused to effector proteins such as nucleases (Koonin and Makarova, 2018). While CARF domains are dimeric and bind oligoadenylate ligands with 2-fold symmetry, the monomeric SAVED domain is capable of recognising a diverse range of asymmetric ligands with different nucleobases and phospho-diester linkages. Moreover, SAVED domains are fused to a wide variety of effector domains, including nucleases, phospholipases, proteases and pore-forming proteins. In some bacteria, SAVED domain proteins are found in association with type III CRISPR systems, suggesting their ligand is the cyclic-triadenylate (cA_3) product of the Cas10 cyclase. This is also the ligand for the evolutionarily unrelated endonuclease NucC, a CBASS effector that binds cA_3 directly, resulting in hexamerization and nuclease activation (Lau et al., 2020). The diversity of cyclic nucleotide second messengers and effectors utilized by CBASS and CRISPR systems may have evolved as a response to pressure from viruses, which frequently

encode proteins that degrade second messengers and inhibit effector enzymes to suppress cellular immunity.

Elucidation of the structure and function of SAVED represents a significant advance in our understanding of CBASS. However, a number of important questions remain. Firstly, the mechanism of activation of CD-NTases is not understood. These enzymes tend to be constitutively active *in vitro*, and CBASS can be transplanted between different bacteria to provide effective anti-phage defence, but we don't yet know how the system is activated. Simple detection of viral DNA is unlikely to be straightforward for prokaryotes that cannot compartmentalise their genomes. Secondly, it is striking that many CBASS encode ubiquitin-like conjugation and protease machinery – in this case represented by the *cap2* and *cap3* genes of the operon. So far, there is no clue as to the function of this aspect of CBASS, but we can expect rapid progress and further surprises. Finally, by activating a broad specificity endonuclease for defence, cells with a Cap4 effector may, rather than being "saved", be signing their own death warrant. Most CBASS likely function using an Abortive Infection (Abi) model, committing altruistic suicide to prevent completion of the bacteriophage replication cycle (Cohen et al., 2019). Although perhaps counter-intuitive for a unicellular organism, this approach can be favoured by evolution if neighbours sharing genetic material are spared viral infection (Hampton et al., 2020).

To conclude, the discovery that SAVED and CARF domains are evolutionarily related and share common functions in the detection of cyclic nucleotides, leading to activation of fused effector proteins for anti-viral defence, represents a signficant advance in our understanding of these complex systems.

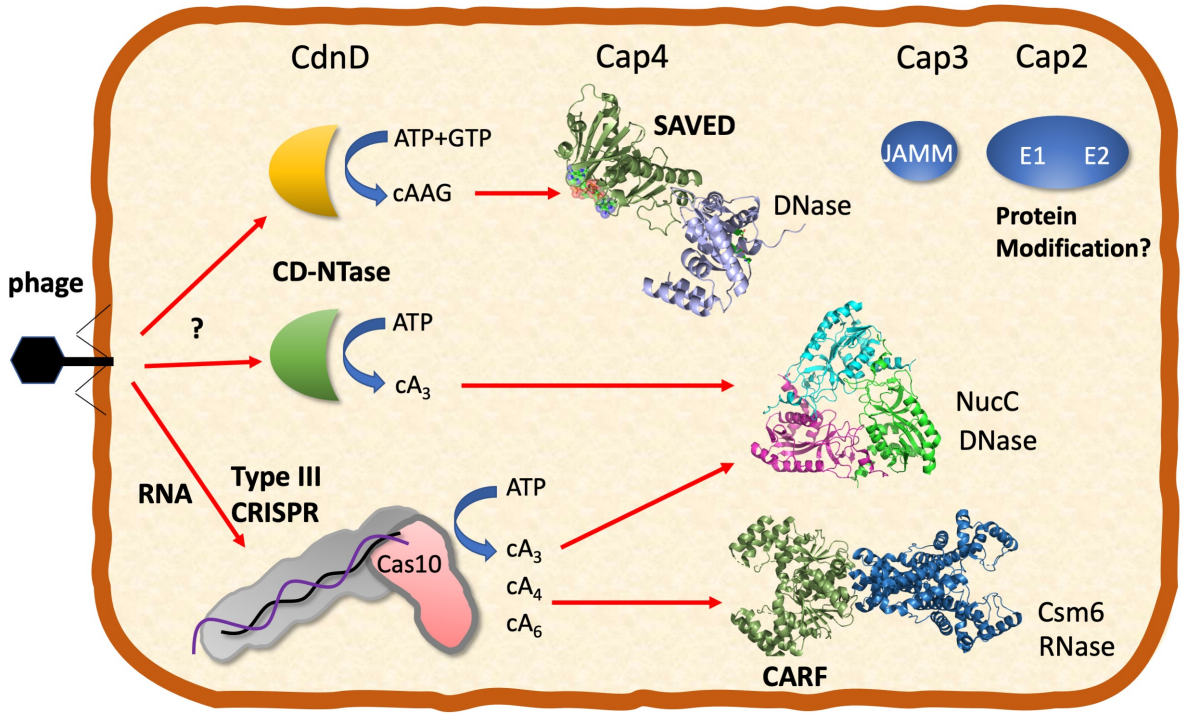

Figure 1. CBASS and CRISPR pathways for anti-phage defence. Phage infection activates CBASS pathways by an unknown mechanism. The nucleotide cyclase CdnD generates a cyclic-AAG second messenger that binds to the SAVED domain of Cap4, activating the N-terminal DNA nuclease domain. Alternative CBASS systems generate different second messengers and

activate diverse effectors such as the NucC endonuclease (Lau et al., 2020) that are thought to kill cells, preventing phage replication. CBASS systems are frequently found in association with Ubiquitin-like modification machinery (JAMM, E1 and E2) of unknown function. Type III CRISPR systems detect viral RNA and synthesise cyclic oligoadenylates, activating defence enzymes such as NucC and the extensive CARF family of effector proteins. CARF and SAVED domains are now understood to be related.

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