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REVIEW

Molecular mechanisms of CBASS-mediated bacteriophage defense

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Abstract The cyclic oligonucleotide-based anti-phage signaling system (CBASS) is an innate immune mechanism in bacteria that mediates phage defense through programmed cell death. The CBASS system utilizes cyclic oligonucleotides (cOs) synthesized by cGAS/DncV-like nucleotidyltransferases (CD-NTase) to activate CD-NTase-associated proteins (Caps). This process elicits three cytocidal effects: DNA degradation, membrane disruption and NAD^+ depletion. These effects collectively block phage replication and dissemination by causing infected cells to self-destruct. To achieve immune evasion from CBASS, phages have evolved anti-CBASS proteins that disrupt the binding of cOs to Caps effectors. This enables intracellular phage replication, assembly and propagation. This review systematically elucidates the multidimensional defense strategies of CBASS, with a focus on the molecular mechanisms underlying its anti-phage defensive functions. It provides novel insights into understanding the evolutionary dynamics of phage-host interactions.

Keywords CBASS, Programmed cell death, Anti-CBASS, CD-NTase, Immune evasion

INTRODUCTION

The cyclic oligonucleotide-based anti-phage signaling system (CBASS) is a widespread bacterial immune system found in around 14.4% of sequenced bacterial and archaeal species (Millman *et al.* 2020). CBASS exhibits striking evolutionary homology with the cyclic GMP-AMP synthase-stimulator of interferon genes (cGAS-STING) pathway (Cohen *et al.* 2019; Millman *et al.* 2020; Morehouse *et al.* 2020; Slavik and Kranzusch 2023). The cGAS-STING pathway is the primary immune mechanism in eukaryotes (Ishikawa and Barber 2008; Sun *et al.* 2013; Zhong *et al.* 2008). Upon binding to dsDNA, cGAS enzyme produces the second messenger 2'3'-cGAMP, which activates STING (Ablasser *et al.* 2013; Civril *et al.* 2013; Diner *et al.* 2013; Gao *et al.* 2013; Zhang *et al.* 2013). STING recognition of 2'3'-cGAMP initiates recruitment of the kinase TANK-binding kinase 1 (TBK1) and the transcription factors interferon regulatory factor 3 (IRF3) and non-canonical nuclear factor kappa-B (NF-κB), culminating in initiating type I interferon signaling cascades (Ouyang *et al.* 2012; Tanaka and Chen 2012). The core components of the CBASS system comprise CD-NTase (cGAS/DncV-like nucleotidyltransferases), Caps (CD-NTase-associated proteins), and associated auxiliary proteins, with CD-NTase activating Caps effectors through the synthesis of cyclic oligonucleotides (cOs) during phage infection (Cohen *et al.* 2019; Millman *et al.* 2020; Whiteley *et al.* 2019). CD-NTase and Caps mirror the functions of cGAS and STING, respectively, revealing a profound evolutionary connection between the immune systems of prokaryotes and eukaryotes (Cohen *et al.* 2019; Millman *et al.* 2020; Morehouse *et al.* 2020; Slavik and Kranzusch 2023). The cytocidal domains within Caps effectors execute three key functions: DNA degradation, membrane disruption and nicotinamide adenine dinucleotide (NAD^+) depletion – thereby initiating programmed cell death prior to the completion of phage replication (Cohen *et al.* 2019; Millman *et al.* 2020).

Previous articles have summarized the functions of CBASS effector proteins and phage proteins (Wang and

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Zhang 2023). This review supplements the newly identified CBASS effectors and phage proteins and provides an integrated analysis of their interactions. This review details the three phage defense strategies of CBASS, highlighting the molecular mechanisms of effector proteins involved in DNA degradation, membrane damage, and NAD^+ depletion. Moreover, we integrate the corresponding phage-encoded anti-CBASS proteins and their mechanisms, providing novel insights into the evolutionary arms race between phages and host cells.

REGULATION OF CD-NTASE ACTIVITY

In the CBASS system, CD-NTase acts as a second messenger generator, initiating downstream immune responses by synthesizing cOs (Whiteley *et al.* 2019). CD-NTase plays a central role in CBASS systems (Cohen *et al.* 2019; Whiteley *et al.* 2019), and its activity is regulated by accessory proteins.

Research demonstrates that Cap2 activates CD-NTase activity through a ubiquitin transferase-like mechanism (Jenson *et al.* 2023). Cap2 conjugates the C-terminus of CD-NTase to a target molecule, priming CD-NTase and boosting the production of signaling molecules (Ledvina *et al.* 2023). Recent research has revealed that Cap2-mediated conjugation of CD-NTase to PspA and Cap3-mediated deconjugation maintain a dynamic equilibrium, preventing spontaneous activation of CD-NTase (Krüger *et al.* 2024). Under non-infectious conditions, Cap2 specifically tethers CD-NTase to phage shock protein A (PspA), thereby keeping the system quiescent (Krüger *et al.* 2024). Following phage infection, the dissociation factor Cap3 disrupts the binding of CD-NTase-PspA, releasing CD-NTase into an active state and initiating cOs synthesis (Krüger *et al.* 2024).

Furthermore, CD-NTase activity is regulated by HORMA domain proteins and Trip13-like ATPases (Ye *et al.* 2020). These domains undergo conformational switching between open and closed states; the closed conformation binds to CD-NTase, activating its cAAA synthesis function (Ye *et al.* 2020). Conversely, ATPase Pch2/Trip13 mediates the disassembly of the HORMA-CD-NTase complex.

Studies have shown that a single E2 enzyme (ubiquitin-conjugating enzyme homolog) in prokaryotes can mimic the eukaryotic ubiquitination cascade, independently executing functions that require coordinated action of E1, E2, E3 ligases, proteases, and receptor proteins in eukaryotic systems (Yan *et al.* 2024). This E2 enzyme processes the C-terminus of cGAS, conjugating it to cysteine or lysine residues to form poly-cGASylation modifications (Yan *et al.* 2024). This modification ultimately activates cGAS to synthesize the immune signaling molecule cGAMP, triggering programmed cell death to defend against phage infection. This research demonstrates that prokaryotes may achieve immune regulation through a simplified ubiquitination-like mechanism, suggesting that the eukaryotic ubiquitin system may have originated from more ancient bacterial defense mechanisms (Yan *et al.* 2024).

The latest research has revealed that the activity regulation mechanism of CD-NTase in the type IV CBASS system involves two key auxiliary proteins: Cap9 and Cap10. Cap9 generates 7-amido-7-deazaguanine (NDG) and attaches it to the N-terminus of CD-NTase (*e.g.*, CdnD). Cap10 then stabilizes the covalent conjugation of NDG to CD-NTase (Wassarman *et al.* 2025). This activates the enzymatic activity of CD-NTase and drives its synthesis of cyclic nucleotide signaling molecules, which trigger programmed cell death (Wassarman *et al.* 2025). When Cap10 binds to the N-terminus of CD-NTase, the latter is rendered inactive, which effectively inhibits premature activation and prevents abnormal signal transduction (Wassarman *et al.* 2025). This regulatory mechanism reveals the molecular switch principle by which the type IV CBASS system dynamically balances CD-NTase activity through auxiliary proteins. This provides new insights into the precise control of bacterial immune defense systems.

Furthermore, the transcription of the CBASS operon and the expression of CD-NTase are indirectly regulated by the transcription factor CapW. CapW maintains low levels of CD-NTase expression in uninfected cells to prevent cytotoxicity (Blankenchip *et al.* 2022).

TRIPARTITE ANTI-PHAGE MECHANISMS OF THE CBASS SYSTEM

The development of CBASS began in 2012, when Davies *et al*. first identified the cGAS homolog gene *DncV* in *Vibrio cholerae*, demonstrating its role in synthesizing cyclic dinucleotides (*e.g.*, c-di-GMP) to regulate bacterial virulence, laying the foundation for subsequent research (Davies *et al.* 2012). In 2015, the Burroughs' team proposed through comparative genomics that the CD-NTase family might play a central role in microbial antiviral defense by linking cyclase activity to immune functions (Burroughs *et al.* 2015). In 2017, Kazlauskiene *et al.* discovered a cyclic oligoadenylate

signaling pathway in type III CRISPR-Cas systems, revealing the universality of cyclic nucleotide signaling in antiphage defense and providing clues for studying the interplay between CBASS and other defense systems (Kazlauskiene *et al.* 2017). In 2018, Severin's team experimentally confirmed that cGAMP directly activates the phospholipase effector CapV in *Vibrio cholerae*, leading to membrane degradation and highlighting the critical role of cyclic nucleotide signaling in bacterial immunity (Severin *et al.* 2018).

2019 marked a milestone in CBASS research: Cohen *et al*. formally named the CBASS system, elucidating its antiphage mechanism – where cGAS generates cGAMP to activate phospholipase-induced cell death – and validating its widespread distribution across microbes through genomic analysis (Cohen *et al.* 2019). That same year, Whiteley *et al*. revealed the diversity of CBASS signaling molecules by demonstrating that CD-NTase synthesize various cyclic nucleotides (*e.g*., cUA, cAAA) (Whiteley *et al.* 2019). In 2020, Millman *et al*. conducted a comprehensive taxonomic analysis of CBASS systems, proposing a classification framework based on effector types (Types I–IV) and highlighting differences in signal perception and effector mechanisms across categories (Millman *et al.* 2020). Subsequent studies further resolved the substrate cleavage mechanisms of nuclease effectors (*e.g*., Cap5, CAP4, NucC, Cap18), the membrane-disrupting mechanisms of membrane-damaging effectors (*e.g*., CapE, CapV, Cap15), and the metabolic interference mechanisms of NAD^+ depletion (*e.g*., TIR-STING, TIR-SAVED).

Nuclease effectors

CBASS nuclease effectors, including Cap5, Cap4, NucC and Cap18, induce programmed cell death by cleaving both the host and phage genomes non-specifically. This ultimately blocks phage replication (Chang *et al.* 2023; Fatma *et al.* 2021; Lau *et al.* 2020; Liang *et al.* 2022).

The Cap5 monomer consists of an N-terminal HNH (histidine-asparagine-histidine) nuclease domain and a C-terminal SAVED (SMODS-associated and fused to various effector domains) signal-sensing domain (Fatma *et al.* 2021; Millman *et al.* 2020). The signaling molecule 3',2'-cGAMP activates Cap5 for *in vivo* DNA degradation. In its inactive state, Cap5 exists as a homodimer formed by two HNH domains stacked side-by-side (Fatma *et al.* 2021). Recent studies have revealed that binding of the second messenger to the PsCap5 dimer triggers a transition from an open to a closed state of the SAVED domains within each dimer (Rechkoblit *et al.* 2024). This provides an interface for assembly of a PsCap5 tetramer (two dimers joined together). This conformational change in the SAVED domains is then propagated to the HNH nuclease domains, converting two of them to a catalytically competent state and positioning them in close proximity to each other for coordinated DNA degradation (Rechkoblit *et al.* 2024) (Fig. 1). Cap5 degrades DNA in a non-sequence-specific and complete manner, resulting in degradation products that are small fragments (less than 100 bp) or mononucleotides that are undetectable by gel electrophoresis (Fatma *et al.* 2021; Rechkoblit *et al.* 2024).

The Cap4 protein contains an N-terminal nuclease domain and a C-terminal SAVED domain that recognizes nucleotide second messengers (Chang *et al.* 2023; Lowey *et al.* 2020). The SAVED domain of Cap4 comprises two CRISPR-associated Rossmann fold (CARF) subunits that specifically recognize both 3'-5' and 2'-5' phosphodiester linkages (such as 3'3'3'-cAAG and 2'3'3'-cAAA) (Chang *et al.* 2023; Lowey *et al.* 2020). In the absence of signal molecules, Cap4 remains in an inactive monomeric state. Upon activation, Cap4 oligomerizes into a higher-order complex where the catalytic sites of its endonuclease domains are juxtaposed, significantly expanding its cleavage capacity toward both host and phage genomic DNA (Chang *et al.* 2023) (Fig. 1). In its activated state, Cap4 can degrade various DNA substrates and exhibits no apparent sequence preference. When EcCap4 degrades genomic or plasmid DNA, the resulting fragment sizes tend to cluster around 17 bp (Chang *et al.* 2023; Lowey *et al.* 2020).

The NucC monomer adopts a restriction endonuclease-like fold (Lau *et al.* 2020; Millman *et al.* 2020). NucC activation depends on the upstream signal 3',3',3'-cAAA. In its inactive state, NucC forms a homotrimer with three monomers arranged in a triangular configuration (Lau *et al.* 2020). When 3',3',3'-cAAA binds to the trimer, it interacts with the allosteric site via hydrogen bonds and π-stacking interactions. This induces a conformational rearrangement of the NucC trimer. This process drives oligomerization into a functional hexamer (Lau *et al.* 2020) (Fig. 1). The activated NucC hexamer increases the efficiency of DNA cleavage per monomer, enabling effective DNA degradation and suppressing phage replication (Lau *et al.* 2020). NucC does not have strict sequence specificity for DNA. High-throughput sequencing analysis revealed a weak sequence preference at cleavage sites. NucC cleaves DNA nonspecifically, generating ~44 bp fragments that trigger chromosomal degradation and cell death in bacterial immunity (Lau *et al.* 2020).

Unlike the endonucleases described above, Cap18

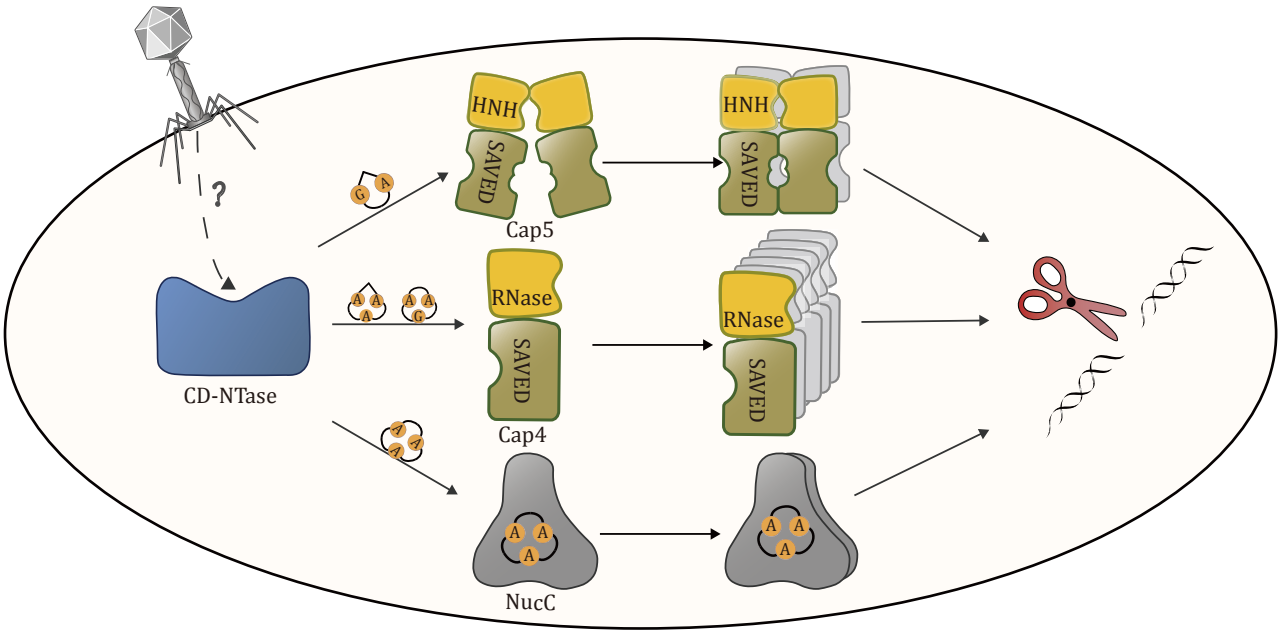

Fig. 1 Schematic of the nuclease effector mechanism. The Cap5 homodimer binds 3',2'-cGAMP, triggering a structural rearrangement of the HNH domain to activate its nuclease function. Cap4 oligomerizes into an active high-order complex after binding upstream signaling molecules, enabling DNA degradation. Upon binding to the upstream signal cAAA, the NucC trimer undergoes oligomerization to form a functional hexamer, which exhibits DNA cleavage activity

forms an exonuclease-like homodimer. It directly degrades single-stranded DNA (ssDNA) and RNA (ssRNA) through its non-specific 3'-5' exonuclease activity without requiring activation by signal molecules (Liang *et al.* 2022; Millman *et al.* 2020). Cap18 exhibits a strong preference for ssDNA, while showing minimal activity toward ssRNA (Liang *et al.* 2022). Cap18 functions as a regulatory component of CBASS. The Cap18 homodimer coexists with CBASS transcription factors (CapW or the CapH–CapP complex), maintaining CBASS homeostasis by degrading transcription factor-associated ssDNA (Liang *et al.* 2022).

Notably, Cap5, Cap4 and NucC all rely on second messenger-regulated oligomerization to control enzymatic domain activation, a mechanism that underscores the evolutionary conservation of CBASS while highlighting its functional efficiency and specificity in bacterial immunity (Chang *et al.* 2023; Fatma *et al.* 2021; Lau *et al.* 2020). Cap5, Cap4, and NucC directly degrade host and phage genomic DNA, whereas Cap18 clears ssDNA via its exonuclease activity. None of these nucleases (Cap5, Cap4, NucC, or Cap18) exhibits discernible sequence preference in their DNA degradation functions (Chang *et al.* 2023; Fatma *et al.* 2021; Lau *et al.* 2020; Liang *et al.* 2022). Future studies could further investigate the DNA cleavage sequences of these enzymes. Notably, Cap4 typically generates DNA cleavage products of ~17 bp, while NucC produces fragments of ~44 bp (Chang *et al.* 2023; Lau *et al.* 2020; Lowey *et al.* 2020).

Acb4 and Acb2 subvert nuclease effectors

Acb4 is a phage-encoded protein that suppresses CBASS immunity by binding to nucleotide immune signals and blocking activation of downstream Cap effector proteins (Table 1). The crystal structure of the Acb4-3'3'-cGAMP complex reveals that Acb4 assembles into a “windmill-shaped” tetramer to form a 4:4 Acb4-3'3'-cGAMP assembly. Acb4 exhibits broad-spectrum binding to cOs such as 3'3'-cGAMP, 2'3'-cGAMP, 3'2'-cGAMP, 3'3'-cUA, and 2'3'-cUA. This blocks Caps effector activation and facilitates phage immune evasion (Chang *et al.* 2023) (Fig. 2). For instance, experiments have confirmed that Acb4 inhibits Cap5 nuclease activity activated by 3'3'-cGAMP in a dose-dependent manner *in vitro* (Chang *et al.* 2023). This indicates that Acb4 suppresses the CBASS system by inhibiting Cap5 activity.

Acb2 inhibits the CBASS pathway primarily by sequestering signaling molecules, thereby preventing their interaction with effector proteins (Table 1). Crystallographic analysis reveals that the Acb2 monomer consists of an N-terminal short helix and

Table 1 Mechanism of bacteriophage proteins against the CBASS system

Blockade level	Protein name	Inhibition mechanism	References
Second messenger sequestration	Acb4	Binds cOs to prevent activation of Cap effectors	Chang *et al.* 2023
	Acb2	High-affinity binding depletes cOs	Cao *et al.* 2024; Huiting *et al.* 2023
	Acb1	Metal-independent hydrolysis of cOs	Hobbs *et al.* 2022
TIR-STING pathway subversion	NTase	Synthesizes decoy ligands (*e.g.*, 3'3'-cGAMP)	Ho *et al.* 2023
	Tad1/Tad2	Simultaneously binds CTNs/CDNs	Li *et al.* 2024
Direct enzyme inhibition	Acb3	Blocks catalytic site and ligand-binding pocket of CD-NTase	Yirmiya *et al.* 2025

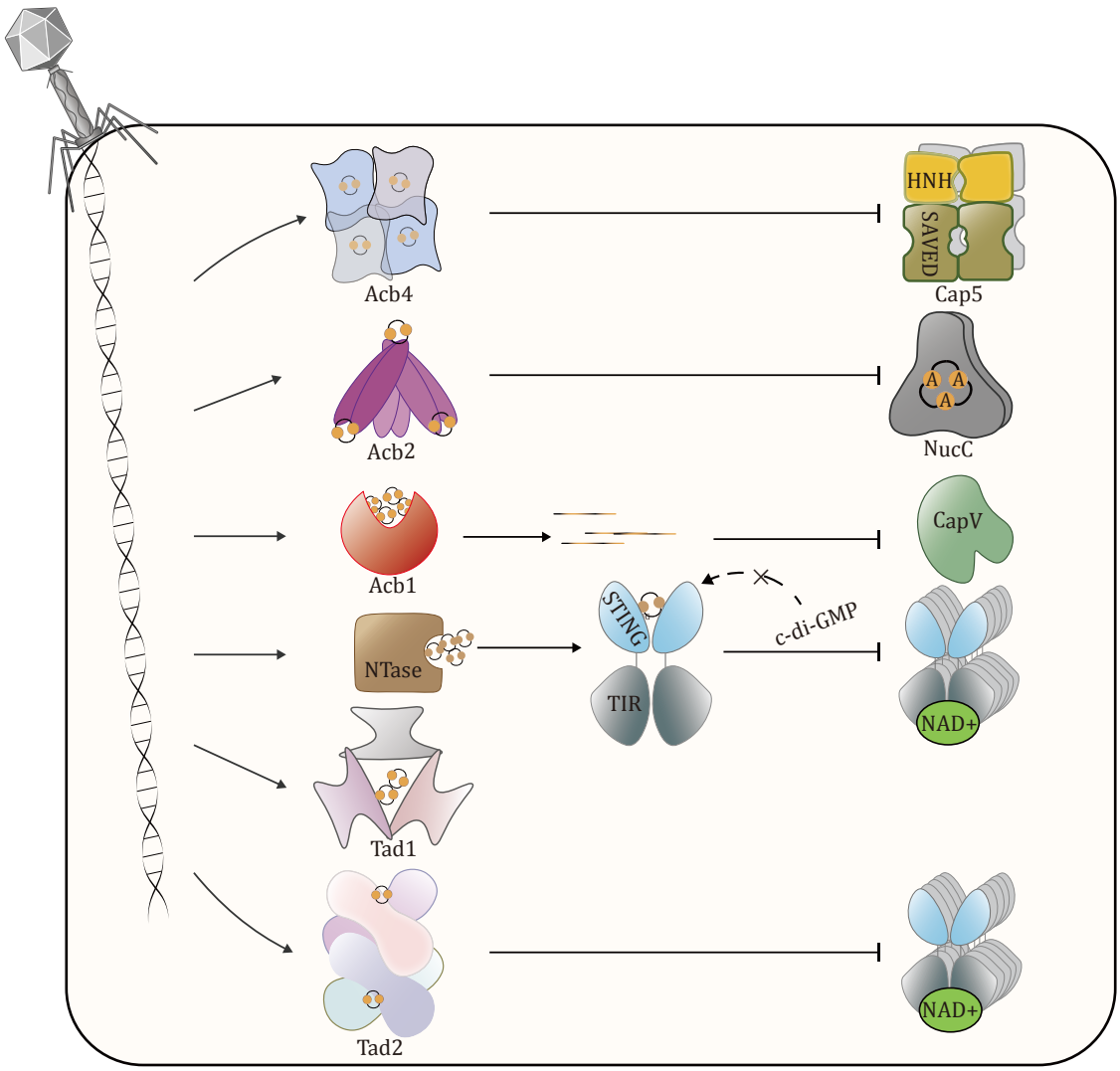

Fig. 2 Molecular strategies of bacteriophage antagonism against CBASS immunity. The anti-CBASS proteins Acb4, Acb2 and Acb1 degrade or sequester cOs signaling molecules directly, thereby suppressing the activation of Caps effectors. Phage-encoded NTase subverts the TIR-STING pathway to block the initiation of host immune defenses. Phage Tad1 and Tad2 neutralize CBASS immunity by sequestering signaling molecules with high affinity, thereby blocking their activation of downstream effectors

antiparallel helices (Huiting *et al.* 2023). Adjacent monomers assemble into a homotypic hexamer through tight interfacial contacts. Each Acb2 hexamer binds three 3'3'-cGAMP molecules at conserved pockets located between two head-to-head oriented monomers (Huiting *et al.* 2023) (Fig. 2). Experiments demonstrate that Acb2 exhibits broad-spectrum binding to cOs such as 2'3'-cGAMP, cAA, 3'3'-cGAMP, cUU and cUA. For example, the Acb2 protein can inhibit the effector activity of NucC in the CBASS system. *In vitro* experiments showed that Acb2 significantly suppresses the DNA cleavage activity of NucC activated by cAAA, whereas mutation of its CTN-binding site (R67A/T74A) abolished this inhibitory capacity (Cao *et al.* 2024).

Membrane-damaging effectors

CBASS membrane-damaging effectors, including CapE, CapV, and Cap15, disrupt inner membrane integrity to mediate collapse of the proton gradient and cause osmotic imbalance, ultimately triggering programmed cell death and blocking phage replication (Cohen *et al.* 2019; Millman *et al.* 2020).

Recent studies have revealed that in its inactive state, CapE derived from Escherichia coli exists as a dimer with a closed substrate channel. Activation of CapE depends on the second messenger cUA. Upon cUA binding, the CapE dimers undergo head-to-tail linear stacking to form a filamentous supramolecular complex (Wang *et al.* 2025) (Fig. 3). Cryo-electron microscopy (cryo-EM) analysis demonstrated that this self-assembly opens the substrate channel and exposes the catalytic sites, thereby activating phospholipase activity to efficiently lyse the bacterial inner membrane (Wang *et al.* 2025). cUA-induced filamentous assembly significantly increases the binding of CapE to liposomes. The filamentous structure has cUA-binding sites on one side and exposes the hydrophobic substrate channel on the other (Wang *et al.* 2025). This configuration allows for the targeted binding and hydrolysis of membrane phospholipids, which results in liposome dissolution, increased membrane permeability, and ultimately, cell lysis, thereby preventing phage propagation (Wang *et al.* 2025).

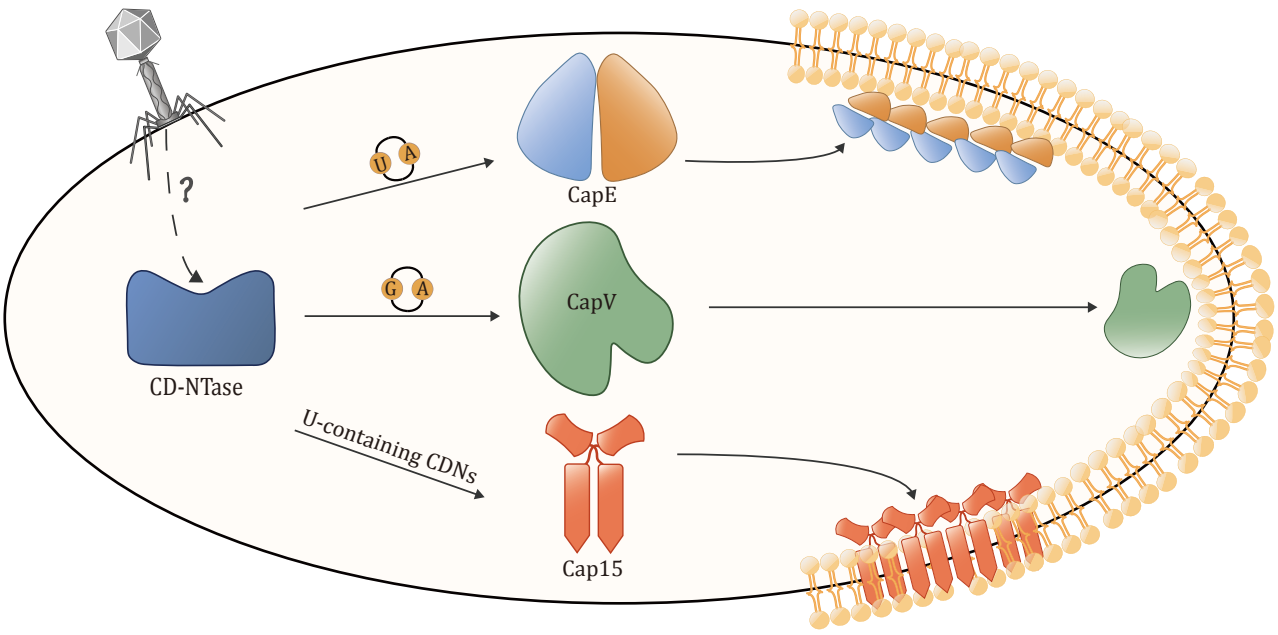

Fig. 3 Schematic of the membrane damage effector mechanism. Upon cUA binding, CapE dimers undergo head-to-tail linear stacking to form a filamentous supramolecular complex that efficiently lyses the bacterial inner membrane. Activated by binding to 3',3'-cGAMP, CapV mediates phospholipid degradation in the cell membrane. Cap15 effector oligomerizes to form pore structures that directly disrupt the integrity of the inner membrane

In *Vibrio cholerae*, CapV is a phospholipase effector activated by the signal molecule 3',3'-cyclic GMP-AMP (Severin *et al.* 2018). After binding to 3'3'-cGAMP, CapV's phospholipase activity is activated, which

degrades the inner membrane during phage infection (Cohen *et al.* 2019; Severin *et al.* 2018) (Fig. 3). The activated enzyme selectively hydrolyzes inner membrane phospholipids phosphatidylethanolamine (PE) and phosphatidylglycerol (PG), thereby inducing cell death and effectively blocking phage propagation (Cohen *et al.* 2019).

Sequencing of the CBASS genome reveals that more than 40% of CBASS operons encode transmembrane (TM) effectors (Burroughs *et al.* 2015; Millman *et al.* 2020). Cap15 is composed of an N-terminal TM domain and a C-terminal β-barrel domain containing a nucleotide-binding pocket (Duncan-Lowey *et al.* 2021). Cap15 is activated by uracil-containing cyclic dinucleotide signals (*e.g.*, 3'3'-c-di-UMP, 3'3'-cUMP-AMP). Upon binding of these signaling molecules to its β-barrel domain, Cap15 undergoes further oligomerization, forming high-order complexes that disrupt inner membrane integrity (Duncan-Lowey *et al.* 2021) (Fig. 3). The activation of Cap15 specifically triggers the collapse of the bacterial inner membrane, which leads to cytoplasmic condensation. Disruption of the inner membrane causes it to shrink away from the outer membrane and cell wall. This ultimately results in complete membrane rupture and cell death (Duncan-Lowey *et al.* 2021). The CBASS system also encodes other TM effectors, including Cap13, Cap14, and Cap16 (Duncan-Lowey *et al.* 2021).

CapE, CapV, and Cap15 depend on nucleotide signaling to activate and disrupt the integrity of the bacterial inner membrane. This disruption collapses the osmotic balance, ultimately triggering programmed cell death and aborting phage propagation. CapE and CapV degrade phospholipids through enzymatic catalysis at their active sites (Cohen *et al.* 2019; Wang *et al.* 2025), while Cap15 damages membrane structure mechanically via ligand-induced oligomerization (Duncan-Lowey *et al.* 2021). Together, these effectors form a multi-layered defense strategy that targets the inner membrane. Notably, TM effectors are present in over 40% of CBASS operons, demonstrating their broad applicability across bacterial hosts.

Acb1 disables membrane effector signaling

The Acb1 protein evades CBASS immunity by degrading signaling molecules, such as 3'3'-cGAMP, thereby preventing the activation of downstream effector proteins (Hobbs *et al.* 2022) (Table 1). Acb1 adopts a 2H phosphodiesterase fold, forming a U-shaped ligand-binding pocket. Through a metal-ion independent catalytic reaction, Acb1 degrades cyclic signaling molecules into linear products (Hobbs *et al.* 2022) (Fig. 2). Acb1 can degrade various cyclic nucleotide signaling molecules, primarily including 3'3'-cGAMP, cUA, cAAA, and cAAG (Hobbs *et al.* 2022). Experiments confirmed that CapV, when activated by 3'3'-cGAMP, degraded the fluorescently labeled phospholipid substrate and enhanced the fluorescent signal. However, when the signal was pretreated with Acb1, the fluorescent signal remained unchanged, indicating that Acb1 completely blocked CapV activity (Hobbs *et al.* 2022).

NAD^+ depletion

The CBASS NAD^+-degrading effectors TIR-STING and TIR-SAVED disrupt redox homeostasis and central metabolic pathways by degrading the cellular metabolite NAD^+, ultimately inducing programmed cell death (Cohen *et al.* 2019; Doron *et al.* 2018; Essuman *et al.* 2018; Morehouse *et al.* 2020). The TIR domain is an enzyme capable of degrading NAD^+ (Horsefield *et al.* 2019; Wan *et al.* 2019).

TIR-STING is a fusion protein consisting of an N-terminal TIR domain and a C-terminal STING domain. The upstream signaling molecule c-di-GMP activates the NAD^+ hydrolase activity of TIR-STING. In its inactive state, TIR-STING exists as a non-oligomerized dimer (Morehouse *et al.* 2022). When c-di-GMP binds to the central cavity formed at the dimer interface of STING domains, it triggers parallel stacking of STING domains, driving further assembly into a filamentous architecture (Morehouse *et al.* 2022). This filamentous structure forces the TIR domains into proximity, thereby activating their NAD^+ hydrolase activity (Fig. 4). The TIR domains cleave NAD^+ into nicotinamide (NAM) and ADP-ribose (ADPR) (Morehouse *et al.* 2022). TIR-STING achieves phage defense by depleting cellular NAD^+, thereby triggering cell growth arrest to block viral propagation.

TIR-SAVED consists of the SAVED domain, a nucleotide sensor, and the TIR domain (Hogrel *et al.* 2022). Binding of the signaling molecule 3',3',3'-cAAA to the SAVED domain triggers the assembly of the protein into a superhelical filamentous polymer via head-to-tail stacking (Hogrel *et al.* 2022) (Fig. 4). Structural analyses using cryo-EM reveal that the complex adopts a right-handed superhelical architecture, with each helical turn comprising 17 TIR-SAVED monomers (Hogrel *et al.* 2022). The helical assembly forces the BB and DE loops of adjacent TIR domains to interact (Hogrel *et al.* 2022). This exposes the substrate-binding pocket and enables NAD^+ hydrolysis. Furthermore, the oligomerization of TIR-SAVED domains is essential for establishing the

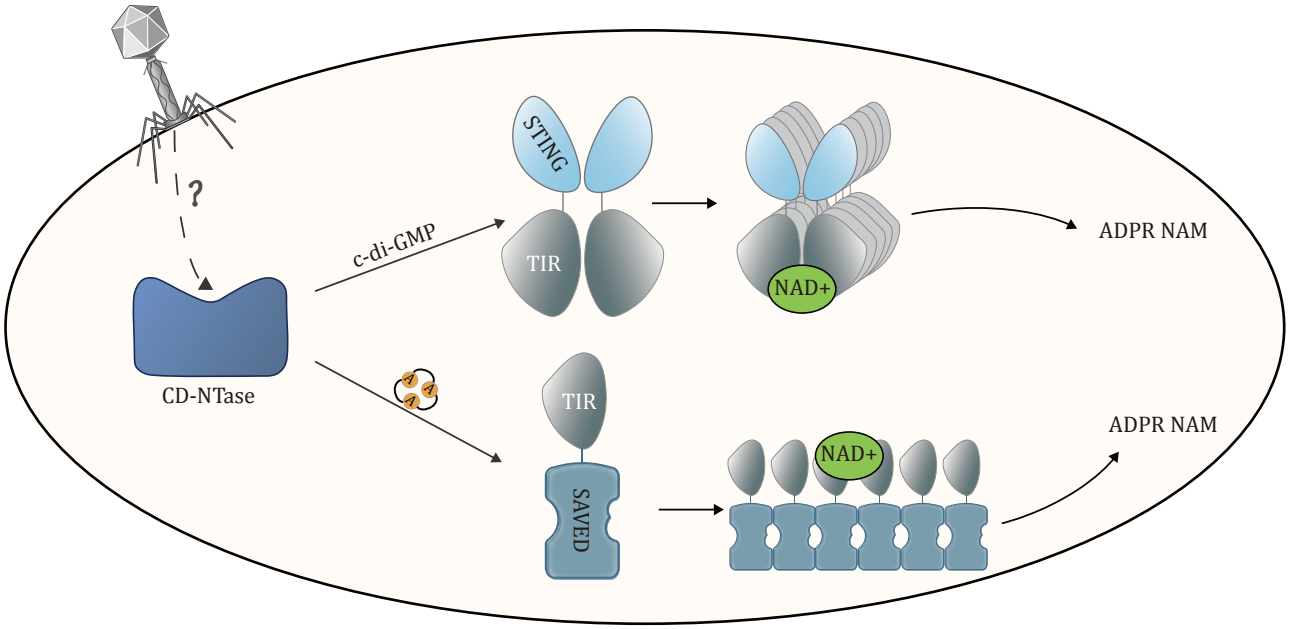

Fig. 4 Schematic of the NAD⁺ depletion effector mechanism. TIR-STING interacts with c-di-GMP through parallel stacking, assembling into analogous fibrous structures to execute NAD⁺ hydrolysis. Upon binding to 3'3'3'-cAAA, the TIR-SAVED complex oligomerizes into filamentous assemblies that mediate NAD⁺ hydrolase activity

structural framework necessary for NAD⁺ hydrolase activity (Hogrel *et al.* 2022).

Both TIR-STING and TIR-SAVED contain a TIR domain and are activated by ligand-induced oligomerization, which triggers cell death via NAD⁺ hydrolysis. TIR-STING forms a linear, double-stranded filament structure through parallel stacking of STING domains. In contrast, TIR-SAVED mediates NAD⁺ hydrolysis activity through head-to-tail stacking of SAVED domains into a right-handed superhelical solenoid (Hogrel *et al.* 2022; Morehouse *et al.* 2022). These mechanisms elucidate not only the NAD⁺-degrading function of CBASS systems, but also provide evolutionary and functional insights into eukaryotic TIR pathways.

NTase, Tad1 and Tad2 sequester NADase effector activation

The study revealed that bacteriophage nucleotidyltransferase (NTase) synthesizes competitive cyclic nucleotide ligands (*e.g.*, 3'3'-cGAMP), which bind to the host STING domain and competitively block its natural activating ligand cGG, thereby inhibiting the activation of the TIR-STING effector protein (Ho *et al.* 2023) (Fig. 2) (Table 1). *In vitro* enzymatic activity assays confirmed that incubating the TIR-STING protein with the fluorescent substrate ε-NAD, adding activating ligands and the phage NTase, and real-time monitoring of the ε-NAD cleavage rate revealed that the NTase concentration-dependently inhibited the initial NADase rate (Ho *et al.* 2023). This demonstrates that the phage NTase effectively inhibits TIR-STING.

Research demonstrates that bacteriophage anti-Thoeris proteins Tad1 and Tad2 suppress the CBASS system by sequestering broad-spectrum CDNs and CTNs (Leavitt *et al.* 2022; Li *et al.* 2024; Yirmiya *et al.* 2024). Tad1 assembles into a hexamer with two independent CTN-binding pockets. By binding to CBASS signaling molecules, Tad1 prevents the activation of effector proteins (Li *et al.* 2024) (Table 1, Fig. 2). Beyond its two dedicated CTN-binding pockets, Tad1 additionally contains CDN-binding sites that are structurally identical to and co-shared with gcADPR-binding pockets. Tad1 can sequester multiple CBASS signaling molecules, including CTNs (such as cAAA, cAAG) and CDNs (such as 2',3'-cGAMP, 3',2'-cGAMP) (Li *et al.* 2024). In contrast, Tad2 forms a tetramer with two distinct CTN-binding pockets that block the CBASS pathway by sequestering signaling molecules (Li *et al.* 2024) (Fig. 2, Table 1). Tad2 can sequester multiple signal molecules of the CBASS system (such as cGG, 3',3'-cGAMP, cUG, 3',2'-cGAMP). *In vitro* assays confirmed that while the NADase activity of TIR-STING from *Sphingobacterium faecium* DSM 11690 is activated by cGG, this activity is abolished when Tad2 is

pre-incubated with cGG, demonstrating that the phage Tad2 protein effectively suppresses TIR-STING function (Li *et al.* 2024).

Recent research utilizing a structure-guided screening approach identified Acb3, a phage-encoded protein that antagonizes the CBASS system (Yirmiya *et al.* 2025). Structural analysis of the predicted complex reveals that Acb3 inhibits CBASS by wrapping around its CD-NTase enzyme and forming extensive interactions, including hydrogen and ionic bonds, with the catalytic active site and ligand-binding pocket (Yirmiya *et al.* 2025) (Table 1). This mechanism directly blocks the enzymatic function of CD-NTase, preventing activation of the CBASS immune defense pathway.

DISCUSSION

As a highly efficient anti-phage immune system, CBASS activates downstream effectors via cOs signals synthesized by CD-NTase to initiate programmed cell death and block phage dissemination (Cohen *et al.* 2019; Millman *et al.* 2020). CBASS employs three cytocidal effectors – nuclease activity, membrane damage and NAD^+ depletion – that work together to execute cell death programs (Cohen *et al.* 2019; Millman *et al.* 2020). The CBASS system not only retains broad-spectrum killing capability (such as the direct destructive effects of NucC and CapV), but also achieves precise regulation through specific signal recognition (including Cap4 subtype identification and TIR-SAVED ligand binding). In contrast, CRISPR-Cas and Thoeris systems rely on singular effector mechanisms: CRISPR-Cas utilizes nucleases, while Thoeris depletes NAD^+ (Garneau *et al.* 2010; Ka *et al.* 2020; Patel *et al.* 2022). By integrating diverse effectors (nuclease, membrane damage and NAD^+ depletion), cOs signals (*e.g.*, cGAMP and cAAA), and auxiliary proteins, CBASS establishes multilayered defence hierarchies, making it nearly impossible for phages to evade by using a single countermeasure. Consequently, CBASS exhibits a marked evolutionary advantage over functionally restricted systems like CRISPR-Cas and Thoeris (Wang and Zhang 2023). The CBASS system has broad application potential. First, it is feasible to design CBASS-targeted antimicrobial agents. Since CBASS effector proteins, such as CapV, require cOs for activation (Severin *et al.* 2018), stable cOs analogs could be engineered to enhance endogenous CBASS activity. This offers a promising strategy for treating antibiotic-resistant bacterial infections. Second, novel diagnostic tools could be developed. The cOs released upon CBASS activation can serve as biomarkers of bacterial infection. A rapid diagnostic kit based on cOs detection could enable the specific identification of drug-resistant pathogens. Third, phage-encoded anti-CBASS proteins (*e.g.*, Acb1, Acb2) evade immunity by degrading or sequestering cOs (Hobbs *et al.* 2022; Huiting *et al.* 2023). Developing small-molecule inhibitors that target their active sites could mitigate their interference with CBASS-mediated killing. Combining these inhibitors with antibiotics could enhance bactericidal efficacy and delay the evolution of resistance, providing a theoretical framework for combinatorial therapies.

CD-NTase initiates downstream immune cascades by synthesizing cOs, with its activity regulation mechanisms reflecting the sophistication and diversity of bacterial defense systems. Current studies reveal that CD-NTase function is dynamically regulated at multiple levels: (1) Ubiquitination-like modification: Type II CBASS activates CD-NTase through E2 enzyme-mediated polycGASylation (Yan *et al.* 2024), demonstrating that prokaryotes may achieve precise immune regulation via simplified ubiquitin-like mechanisms. This suggests the eukaryotic ubiquitin system might originate from bacterial defense components. (2) Protein interaction networks: The Cap2-Cap3-PspA complex (Krüger *et al.* 2024) and HORMA-ATPase system (Ye *et al.* 2020) dynamically balance CD-NTase activity, ensuring rapid phage invasion response while preventing abnormal cell death caused by basal activity. (3) Cap9-Cap10 synergistic regulation: In Type IV CBASS, Cap9 generates NDG, which covalently modifies CD-NTase. Cap10 binds to CD-NTase, collectively fine-tuning its enzymatic activation and immune signaling output (Wassarman *et al.* 2025). (4) Transcriptional regulation: The WYL-domain transcription factor CapW (Blankenchip *et al.* 2022) coordinates CBASS operon expression to maintain homeostasis and temporal synchronization of immune responses. These regulatory mechanisms, combined with CBASS's tripartite effectors, constitute a complete "sensing-signal amplification-effector execution" defense pathway.

Bacteriophages counteract the CBASS immune system through multi-layered molecular strategies, revealing the highly dynamic and complex nature of host-phage arms races: (1) Second Messenger Sequestration: Phage-encoded Acb4, Acb2, and Acb1 block effector activation by degrading or chelating CBASS signaling molecules. Their structural diversity and widespread conservation indicate this is a key strategy against cGAS-like immunity. (2) TIR-STING Pathway Subversion: Phages disrupt this pathway through two mechanisms: Synthesizing competitive cOs to occupy TIR-STING binding sites. Utilizing "molecular sponges" like Tad1/Tad2 (Li *et al.* 2024) to sequester gcADPR and cGAMP, thereby inhibiting NAD^+ depletion-mediated host suicide. Structural analysis of the

predicted complex reveals that Acb3 blocks the enzymatic activity of CD-NTase by encapsulating its catalytic active site, thereby preventing activation of the CBASS immune defense pathway (Yirmiya *et al.* 2025). The synergistic effects of these strategies highlight phages' evolutionary optimization for maximal escape probability. Their precision in targeting CBASS pathways (*e.g.*, Acb1's specific cleavage of 3'3'-cGAMP (Hobbs *et al.* 2022) and broad-spectrum suppression (*e.g.*, Tad1/Tad2 cross-kingdom inhibition of Thoeris and CBASS (Li *et al.* 2024) reflect continuous innovation of "anti-bacterial immune tools" under evolutionary pressure.

A central challenge in the field of CBASS lies in deciphering the pattern recognition mechanism of CD-NTase, and establishing whether they detect phage DNA signatures, specific proteins or metabolic products. In mammals, the cGAS-STING pathway is activated by cytosolic DNA (Stetson and Medzhitov 2006). When cGAS detects an increase in intracellular DNA levels, it binds to DNA and undergoes a conformational change to its active state, synthesizing the second messenger cGAMP to activate the cGAS-STING pathway (Wu *et al.* 2013). In *Drosophila melanogaster*, cGAS-like receptors (cGLRs) are activated by dsRNA. Activated cGLRs synthesize 3'2'-cGAMP and 2'3'-cGAMP to trigger the STING-dependent immune pathway (Holleufer *et al.* 2021; Slavik *et al.* 2021). Due to the structural and functional evolutionary homology between CD-NTase and cGAS, it is possible that phages employ strategies to counteract CD-NTase during infection that are similar to those observed in eukaryotic systems (Kranzusch 2019). Recent studies have revealed that *Staphylococcus* phages transcribe a bacteriophage RNA (cabRNA) that activates CBASS during infection (Banh *et al.* 2023). This cabRNA specifically binds to and activates oligonucleotide cyclases, triggering CBASS immunity, suggesting RNA sensing as a potential mechanism for CBASS activation (Banh *et al.* 2023). These findings expand the diversity of potential CBASS activation mechanisms. While mammalian cGAS relies on DNA sensing and *Drosophila* cGLR is activated by RNA, this suggests that CD-NTase may employ dual DNA/RNA sensing modes to detect phage infection. Furthermore, CD-NTase likely enhances antiphage efficiency through multi-target sensing capabilities (*e.g.*, DNA, RNA, or metabolites), enabling broad-spectrum defense against diverse phage strategies.

Future research could employ comparative genomics to identify homologous systems of CD-NTase regulatory elements (*e.g.*, WYL and HORMA domains) in archaea and eukaryotes, thereby reconstructing the evolutionary pathway from bacterial immunity to eukaryotic innate immunity. By determining whether phages share anti-CBASS and anti-CRISPR regulatory modules to evade coordinated elimination by host multi-layered immune systems. By elucidating phage anti-CBASS strategies, this approach would not only advance the theoretical framework of host-pathogen interactions but also provide: Molecular targets for novel antimicrobial development (*e.g.*, designing cOs analogs to disrupt phage escape mechanisms). Strategic insights for optimizing phage therapy (*e.g.*, screening phages harboring specific resistance genes).

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Compliance with Ethical Standards

Conflict of interest Yao Ge, Ang Gao, and Yalan Zhu declare that they have no conflict of interest.

Human and animal rights and informed consent This article does not contain any studies with human or animal subjects performed by the any of the authors.

References

Ablasser A, Goldeck M, Cavlar T, Deimling T, Witte G, Röhl I, Hopfner K-P, Ludwig J, Hornung V (2013) cGAS produces a 2'-5'-linked cyclic dinucleotide second messenger that activates STING. Nature 498(7454): 380–384

Banh DV, Roberts CG, Morales-Amador A, Berryhill BA, Chaudhry W, Levin BR, Brady SF, Marraffini LA (2023) Bacterial cGAS senses a viral RNA to initiate immunity. Nature 623(7989): 1001–1008

Blankenchip CL, Nguyen JV, Lau RK, Ye Q, Gu Y, Corbett KD (2022) Control of bacterial immune signaling by a WYL domain transcription factor. Nucleic Acids Res 50(9): 5239–5250

Burroughs AM, Zhang D, Schäffer DE, Iyer LM, Aravind L (2015) Comparative genomic analyses reveal a vast, novel network of nucleotide-centric systems in biological conflicts, immunity and signaling. Nucleic Acids Res 43(22): 10633–10654

Cao X, Xiao Y, Huiting E, Cao X, Li D, Ren J, Fedorova I, Wang H, Guan L, Wang Y, Li L, Bondy-Denomy J, Feng Y (2024) Phage anti-CBASS protein simultaneously sequesters cyclic trinucleotides and dinucleotides. Mol Cell 84(2): 375–385. e7

Chang J-J, You B-J, Tien N, Wang Y-C, Yang C-S, Hou M-H, Chen Y (2023) Specific recognition of cyclic oligonucleotides by Cap4 for phage infection. Int J Biol Macromol 237: 123656. https://doi.org/10.1016/j.ijbiomac.2023.123656

Civril F, Deimling T, de Oliveira Mann CC, Ablasser A, Moldt M, Witte G, Hornung V, Hopfner K-P (2013) Structural mechanism of cytosolic DNA sensing by cGAS. Nature 498(7454): 332–337

Cohen D, Melamed S, Millman A, Shulman G, Oppenheimer-Shaanan Y, Kacen A, Doron S, Amitai G, Sorek R (2019) Cyclic GMP–AMP signalling protects bacteria against viral infection. Nature 574(7780): 691–695

Davies BW, Bogard RW, Young TS, Mekalanos JJ (2012) Coordinated regulation of accessory genetic elements produces cyclic Di-nucleotides for *V. cholerae* virulence. Cell 149(2): 358–370

Diner EJ, Burdette DL, Wilson SC, Monroe KM, Kellenberger CA, Hyodo M, Hayakawa Y, Hammond MC, Vance RE (2013) The innate immune DNA sensor cGAS produces a noncanonical cyclic dinucleotide that activates human STING. Cell Rep 3(5): 1355–1361

Doron S, Melamed S, Ofir G, Leavitt A, Lopatina A, Keren M, Amitai G, Sorek R (2018) Systematic discovery of antiphage defense systems in the microbial pangenome. Science 359(6379): eaar4120. https://doi.org/10.1126/science.aar412

Duncan-Lowey B, McNamara-Bordewick NK, Tal N, Sorek R, Kranzusch PJ (2021) Effector-mediated membrane disruption controls cell death in CBASS antiphage defense. Mol Cell 81(24): 5039–5051. e5

Essuman K, Summers DW, Sasaki Y, Mao X, Yim AKY, DiAntonio A, Milbrandt J (2018) TIR domain proteins are an ancient family of NAD$^+$-consuming enzymes. Curr Biol 28(3): 421–430. e4

Fatma S, Chakravarti A, Zeng X, Huang RH (2021) Molecular mechanisms of the CdnG-Cap5 antiphage defense system employing 3', 2'-cGAMP as the second messenger. Nat Commun 12(1): 6381. https://doi.org/10.1038/s41467-021-26738-2

Gao P, Ascano M, Wu Y, Barchet W, Gaffney BL, Zillinger T, Serganov AA, Liu Y, Jones RA, Hartmann G, Tuschl T, Patel DJ (2013) Cyclic [G(2', 5')pA(3', 5')p] is the metazoan second messenger produced by DNA-activated cyclic GMP-AMP synthase. Cell 153(5): 1094–1107

Garneau JE, Dupuis M-È, Villion M, Romero DA, Barrangou R, Boyaval P, Fremaux C, Horvath P, Magadán AH, Moineau S (2010) The CRISPR/Cas bacterial immune system cleaves bacteriophage and plasmid DNA. Nature 468(7320): 67–71

Ho P, Chen Y, Biswas S, Canfield E, Abdolvahabi A, Feldman DE (2023) Bacteriophage antidefense genes that neutralize TIR and STING immune responses. Cell Rep 42(4): 112305. https://doi.org/10.1016/j.celrep.2023.112305

Hobbs SJ, Wein T, Lu A, Morehouse BR, Schnabel J, Leavitt A, Yirmiya E, Sorek R, Kranzusch PJ (2022) Phage anti-CBASS and anti-Pycsar nucleases subvert bacterial immunity. Nature 605(7910): 522–526

Hogrel G, Guild A, Graham S, Rickman H, Grüschow S, Bertrand Q, Spagnolo L, White MF (2022) Cyclic nucleotide-induced helical structure activates a TIR immune effector. Nature 608(7924): 808–812

Holleufer A, Winther KG, Gad HH, Ai X, Chen Y, Li L, Wei Z, Deng H, Liu J, Frederiksen NA, Simonsen B, Andersen LL, Kleigrewe K, Dalskov L, Pichlmair A, Cai H, Imler J-L, Hartmann R (2021) Two cGAS-like receptors induce antiviral immunity in *Drosophila*. Nature 597(7874): 114–118

Horsefield S, Burdett H, Zhang X, Manik MK, Shi Y, Chen J, Qi T, Gilley J, Lai J-S, Rank MX, Casey LW, Gu W, Ericsson DJ, Foley G, Hughes RO, Bosanac T, von Itzstein M, Rathjen JP, Nanson JD, Boden M, Dry IB, Williams SJ, Staskawicz BJ, Coleman MP, Ve T, Dodds PN, Kobe B (2019) NAD$^+$ cleavage activity by animal and plant TIR domains in cell death pathway. Science 365(6455): 793–799

Huiting E, Cao X, Ren J, Athukoralage JS, Luo Z, Silas S, An N, Carion H, Zhou Y, Fraser JS, Feng Y, Bondy-Denomy J (2023) Bacteriophages inhibit and evade cGAS-like immune function in bacteria. Cell 186(4): 864–876. e21

Ishikawa H, Barber GN (2008) STING is an endoplasmic reticulum adaptor that facilitates innate immune signalling. Nature 455(7213): 674–678

Jenson JM, Li T, Du F, Ea C-K, Chen ZJ (2023) Ubiquitin-like conjugation by bacterial cGAS enhances anti-phage defence. Nature 616(7956): 326–331

Ka D, Oh H, Park E, Kim J-H, Bae E (2020) Structural and functional evidence of bacterial antiphage protection by Thoeris defense system via NAD$^+$ degradation. Nat Commun 11(1): 2816. https://doi.org/10.1038/s41467-020-16703-w

Kazlauskiene M, Kostiuk G, Venclovas Č, Tamulaitis G, Siksnys V (2017) A cyclic oligonucleotide signaling pathway in type III CRISPR-Cas systems. Science 357(6351): 605–609

Kranzusch PJ (2019) cGAS and CD-NTase enzymes: structure, mechanism, and evolution. Curr Opin Struct Biol 59: 178–187

Krüger L, Gaskell-Mew L, Graham S, Shirran S, Hertel R, White MF (2024) Reversible conjugation of a CBASS nucleotide cyclase regulates bacterial immune response to phage infection. Nat Microbiol 9(6): 1579–1592

Lau RK, Ye Q, Birkholz EA, Berg KR, Patel L, Mathews IT, Watrous JD, Ego K, Whiteley AT, Lowey B, Mekalanos JJ, Kranzusch PJ, Jain M, Pogliano J, Corbett KD (2020) Structure and mechanism of a cyclic trinucleotide-activated bacterial endonuclease mediating bacteriophage immunity. Mol Cell 77(4): 723–733. e6

Leavitt A, Yirmiya E, Amitai G, Lu A, Garb J, Herbst E, Morehouse BR, Hobbs SJ, Antine SP, Sun Z-YJ, Kranzusch PJ, Sorek R (2022) Viruses inhibit TIR gcADPR signalling to overcome bacterial defence. Nature 611(7935): 326–331

Ledvina HE, Ye Q, Gu Y, Sullivan AE, Quan Y, Lau RK, Zhou H, Corbett KD, Whiteley AT (2023) An E1–E2 fusion protein primes antiviral immune signalling in bacteria. Nature 616(7956): 319–325

Li D, Xiao Y, Fedorova I, Xiong W, Wang Y, Liu X, Huiting E, Ren J, Gao Z, Zhao X, Cao X, Zhang Y, Bondy-Denomy J, Feng Y (2024) Single phage proteins sequester signals from TIR and cGAS-like enzymes. Nature 635(8039): 719–727

Liang Q, Richey ST, Ur SN, Ye Q, Lau RK, Corbett KD (2022) Structure and activity of a bacterial defense -associated 3'-5 ' exonuclease. Protein Sci 31(7): e4374. https://doi.org/10.1002/pro.4374

Lowey B, Whiteley AT, Keszei AFA, Morehouse BR, Mathews IT, Antine SP, Cabrera VJ, Kashin D, Niemann P, Jain M, Schwede F, Mekalanos JJ, Shao S, Lee ASY, Kranzusch PJ (2020) CBASS immunity uses CARF-related effectors to sense 3'–5'- and 2'–5'-linked cyclic oligonucleotide signals and protect bacteria from phage infection. Cell 182(1): 38–49. e17

Millman A, Melamed S, Amitai G, Sorek R (2020) Diversity and classification of cyclic-oligonucleotide-based anti-phage signalling systems. Nat Microbiol 5(12): 1608–1615

Morehouse BR, Govande AA, Millman A, Keszei AFA, Lowey B, Ofir G, Shao S, Sorek R, Kranzusch PJ (2020) STING cyclic dinucleotide sensing originated in bacteria. Nature 586(7829): 429–433

Morehouse BR, Yip MCJ, Keszei AFA, McNamara-Bordewick NK, Shao S, Kranzusch PJ (2022) Cryo-EM structure of an active bacterial TIR–STING filament complex. Nature 608(7924): 803–807

Ouyang S, Song X, Wang Y, Ru H, Shaw N, Jiang Y, Niu F, Zhu Y, Qiu W, Parvatiyar K, Li Y, Zhang R, Cheng G, Liu Z-J (2012)

Structural analysis of the STING adaptor protein reveals a hydrophobic dimer interface and mode of cyclic di-GMP binding. Immunity 36(6): 1073–1086

Patel DJ, Yu Y, Jia N (2022) Bacterial origins of cyclic nucleotide-activated antiviral immune signaling. Mol Cell 82(24): 4591–4610

Rechkoblit O, Sciaky D, Kreitler DF, Buku A, Kottur J, Aggarwal AK (2024) Activation of CBASS Cap5 endonuclease immune effector by cyclic nucleotides. Nat Struct Mol Biol 31(5): 767–776

Severin GB, Ramliden MS, Hawver LA, Wang K, Pell ME, Kieninger A-K, Khataokar A, O'Hara BJ, Behrmann LV, Neiditch MB, Benning C, Waters CM, Ng W-L (2018) Direct activation of a phospholipase by cyclic GMP-AMP in El Tor *Vibrio cholerae*. Proc Natl Acad Sci USA 115(26): E6048–E6055

Slavik KM, Morehouse BR, Ragucci AE, Zhou W, Ai X, Chen Y, Li L, Wei Z, Bähre H, König M, Seifert R, Lee ASY, Cai H, Imler J-L, Kranzusch PJ (2021) cGAS-like receptors sense RNA and control 3'2'-cGAMP signalling in *Drosophila*. Nature 597(7874): 109–113

Slavik KM, Kranzusch PJ (2023) CBASS to cGAS-STING: the origins and mechanisms of nucleotide second messenger immune signaling. Annu Rev Virol 10: 423–453

Stetson DB, Medzhitov R (2006) Recognition of cytosolic DNA activates an IRF3-dependent innate immune response. Immunity 24(1): 93–103

Sun L, Wu J, Du F, Chen X, Chen ZJ (2013) Cyclic GMP-AMP synthase is a cytosolic DNA sensor that activates the type I interferon pathway. Science 339(6121): 786–791

Tanaka Y, Chen ZJ (2012) STING specifies IRF3 phosphorylation by TBK1 in the cytosolic DNA signaling pathway. Sci Signal 5(214): ra20. https://doi.org/10.1126/scisignal.2002521

Wan L, Essuman K, Anderson RG, Sasaki Y, Monteiro F, Chung E-H, Nishimura EO, DiAntonio A, Milbrandt J, Dangl JL, Nishimura MT (2019) TIR domains of plant immune receptors are NAD^+-cleaving enzymes that promote cell death. Science 365(6455): 799–803

Wang J, Li Z, Lang H, Fu W, Gao Y, Yin S, Sun P, Li Z, Huang J, Liu S, Zhu Y, Sun F, Li D, Gao P, Gao A (2025) Cyclic-dinucleotide-induced filamentous assembly of phospholipases governs broad CBASS immunity. Cell 188(14): 3744–3756. e16

Wang L, Zhang L (2023) The arms race between bacteria CBASS and bacteriophages. Front Immunol 14: 1224341. https://doi.org/10.3389/fimmu.2023.1224341

Wassarman DR, Pfaff P, Paulo JA, Gygi SP, Shokat KM, Kranzusch PJ (2025) Deazaguanylation is a nucleobase-protein conjugation required for type IV CBASS immunity. bioRxiv. https://doi.org/10.1101/2025.04.06.647259

Whiteley AT, Eaglesham JB, de Oliveira Mann CC, Morehouse BR, Lowey B, Nieminen EA, Danilchanka O, King DS, Lee ASY, Mekalanos JJ, Kranzusch PJ (2019) Bacterial cGAS-like enzymes synthesize diverse nucleotide signals. Nature 567(7747): 194–199

Wu J, Sun L, Chen X, Du F, Shi H, Chen C, Chen ZJ (2013) Cyclic GMP-AMP is an endogenous second messenger in innate immune signaling by cytosolic DNA. Science 339(6121): 826–830

Yan Y, Xiao J, Huang F, Xian W, Yu B, Cheng R, Wu H, Lu X, Wang X, Huang W, Li J, Oyejobi GK, Robinson CV, Wu H, Wu D, Liu X, Wang L, Zhu B (2024) Phage defence system CBASS is regulated by a prokaryotic E2 enzyme that imitates the ubiquitin pathway. Nat Microbiol 9(6): 1566–1578

Ye Q, Lau RK, Mathews IT, Birkholz EA, Watrous JD, Azimi CS, Pogliano J, Jain M, Corbett KD (2020) HORMA domain proteins and a Trip13-like ATPase regulate bacterial cGAS-like enzymes to mediate bacteriophage immunity. Mol Cell 77(4): 709–722. e7

Yirmiya E, Leavitt A, Lu A, Ragucci AE, Avraham C, Osterman I, Garb J, Antine SP, Mooney SE, Hobbs SJ, Kranzusch PJ, Amitai G, Sorek R (2024) Phages overcome bacterial immunity via diverse anti-defence proteins. Nature 625(7994): 352–359

Yirmiya E, Hobbs SJ, Leavitt A, Osterman I, Avraham C, Hochhauser D, Madhala B, Skovorodka M, Tan JMJ, Toyoda HC, Chebotar I, Itkin M, Malitsky S, Amitai G, Kranzusch PJ, Sorek R (2025) Structure-guided discovery of viral proteins that inhibit host immunity. Cell 188(6): 1681–1692. e17

Zhang X, Shi H, Wu J, Zhang X, Sun L, Chen C, Chen ZJ (2013) Cyclic GMP-AMP containing mixed phosphodiester linkages is an endogenous high-affinity ligand for STING. Mol Cell 51(2): 226–235

Zhong B, Yang Y, Li S, Wang Y-Y, Li Y, Diao F, Lei C, He X, Zhang L, Tien P, Shu H-B (2008) The adaptor protein MITA links virus-sensing receptors to IRF3 transcription factor activation. Immunity 29(4): 538–550

