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Cyclic Nucleotide Signaling in Phage Defense and Counter-Defense

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Abstract

Advances in our understanding of prokaryotic antiphage defense mechanisms in the past few years have revealed a multitude of new cyclic nucleotide signaling molecules that play a crucial role in switching infected cells into an antiviral state. Defense pathways including type III CRISPR (clustered regularly interspaced palindromic repeats), CBASS (cyclic nucleotide-based antiphage signaling system), PYCSAR (pyrimidine cyclase system for antiphage resistance), and Thoeris all use cyclic nucleotides as second messengers to activate a diverse range of effector proteins. These effectors typically degrade or disrupt key cellular components such as nucleic acids, membranes, or metabolites, slowing down viral replication kinetics at great cost to the infected cell. Mechanisms to manipulate the levels of cyclic nucleotides are employed by cells to regulate defense pathways and by viruses to subvert them. Here we review the discovery and mechanism of the key pathways, signaling molecules and effectors, parallels and differences between the systems, open questions, and prospects for future research in this area.

1. INTRODUCTION

CRISPR: clustered regularly interspaced palindromic repeats

CBASS: cyclic nucleotide-based antiphage signaling system

Over the past 15 years, the discovery and elucidation of the clustered regularly interspaced palindromic repeats (CRISPR) system for prokaryotic antiviral defense have represented major advances in molecular biology, particularly as the ramifications for genome editing and a host of other applications became clear. More recently, discoveries of a multitude of antiphage defense pathways in bacteria have highlighted the diversity of cellular defense systems against mobile genetic elements (MGEs). Likewise, we are only beginning to appreciate how MGEs utilize or subvert these defense systems for their own ends. This is a large and rapidly growing field, and many excellent reviews already cover aspects of this in detail. Here, we focus on the role of cyclic nucleotide signaling in antiviral defense and in particular on the links and parallels between type III CRISPR and cyclic nucleotide-based antiphage signaling system (CBASS) pathways (**Figure 1**). We aim to draw out some common themes and signpost areas where important questions remain unanswered.

2. TYPE III CRISPR DEFENSE

2.1. Classification and Structure

There are six main types (I–VI) of CRISPR adaptive immune defense systems, which have little in common besides the requirement for a CRISPR RNA (crRNA) derived from transcription

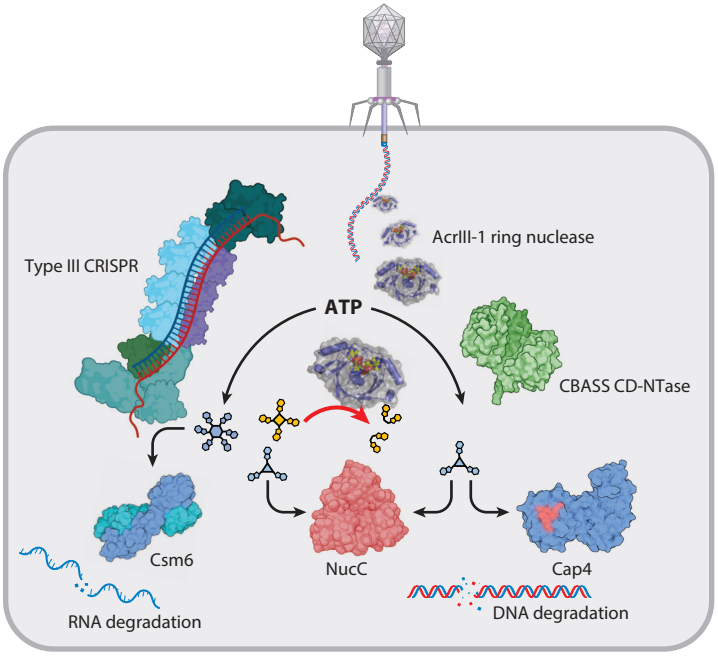


Figure 1

Cyclic nucleotide signaling in prokaryotic antiviral CRISPR and CBASS defense. Viral infection results in the generation of cyclic nucleotide second messengers that activate diverse effector proteins, leading to viral clearance or cell death/abortive infection. Although the sensors and cyclases used by type III CRISPR and CBASS are unrelated, they share second messengers and effector proteins. Csm6 is a ribonuclease activated by cA_6. The viral AcrIII-1 degrades cA_4 to neutralize CRISPR defense. Abbreviations: AcrIII-1, anti-CRISPR III-1; Cap4, cGAMP-activated phospholipase 4; CBASS, cyclic nucleotide-based antiphage signaling system; CRISPR, clustered regularly interspaced palindromic repeats; Csm6, Cas subtype Mtube 6; NucC, nuclease, CD-NTase associated. Figure adapted from images created with BioRender.com.

of a CRISPR locus, and an adaptation process centered on the CRISPR-associated (Cas) proteins Cas1 and Cas2 to capture new viral DNA samples (reviewed in 1). Here we focus on the type III systems, which use large multi-subunit effector complexes with a catalytic Cas10 subunit. Six type III CRISPR-Cas subtypes have been identified to date: Subtype III-A, III-D, III-E, and III-F systems are also known as Csm (Cas subtype Mtube), and subtype II-B and III-C systems are also known as Cmr (Cas module RAMP) (2, 3). Like type I systems, the backbone of type III effectors, which bind the bulk of the guide RNA, is composed of repeating Cas7 subunits, with a secondary helical protein filament provided by a small Cas11 subunit. Thus, type I and III systems can be considered distant cousins that have diverged from a common ancestor. This has been reviewed extensively (1, 4–7) and is briefly covered here. The major points of difference are seen in the large subunit of the complex (Cas8 for type I and Cas10 for type III), the type of nucleic acid recognized [double-stranded (ds) DNA and single-stranded (ss) RNA, respectively], and the enzymes used to degrade nucleic acids on activation (Cas3 for type I and, as we shall see, a complex picture for type III systems). Indeed, recent studies of the type I-D system have revealed that it represents a halfway house between type I and III systems, utilizing a Cas10d large subunit fused to a Cas3-like HD nuclease domain that affects target dsDNA degradation in conjunction with a Cas3′ helicase domain (8, 9).

The first activity ascribed to type III effectors was the specific degradation of target RNA (10), with subsequent studies assigning this activity to a conserved active site in the Cas7-family (Csm3/Cmr4) backbone subunit (11–13). As Cas7 is a repeating subunit, RNA cleavage occurs with a characteristic 6-nucleotide spacing (10). Although this specific RNA cleavage activity can knock down the level of messenger RNA (mRNA) transcripts effectively (13–15), it is not generally thought to be the primary means of type III CRISPR defense. This is the responsibility of the Cas10 subunit, which was predicted from the early days of the CRISPR field to possess HD nuclease and PALM polymerase active sites (16, 17).

2.2. The Role of the Cas10 HD Nuclease Domain

Several type III CRISPR systems are known to have ssDNA-specific DNA degradation activity, activated by target RNA binding, associated with the HD nuclease domain (18–22). However, the link between target RNA recognition and the DNase activity of the HD nuclease domain remains controversial. Several studies have demonstrated that type III CRISPR immunity is dependent on viral mRNA transcription (13, 15, 23), and type III CRISPR systems have been found to target transcriptionally active regions of DNA (15, 23, 24). Transcription-dependent targeting was found to conditionally tolerate lysogenization by temperate phage (15), which may confer fitness advantages to the host. A model has been proposed whereby tethering of the Csm/Cmr complex to nascent mRNA, by crRNA:target base pairing, triggers activation of the HD nuclease domain to cleave ssDNA exposed at the transcription bubble (15, 20, 22). However, recent cryogenic electron microscopy studies of the *Thermus thermophilus* Csm complex, tethered to an RNA target at a transcription elongation complex, revealed that the Csm complex may be positioned distant from the predicted path of the nontemplate DNA strand (25). There are obvious questions over the limited time available to detect and intercept a rapidly elongating viral transcript quickly enough to engage and cleave the DNA in the transcription bubble. The key question remains whether DNase activity is specific for transcribing viral DNA targets or largely nonspecific in nature, a question we return to shortly.

2.3. The Role of the Cas10 PALM Polymerase Domains

Some type III systems have Cas10 orthologs that lack an HD nuclease domain altogether (1, 12), suggesting that the HD nuclease activity is not the sole means of defense by Cas10. Meanwhile,

cOA: cyclic oligoadenylate

the conserved Palm polymerase domains of Cas10 remained enigmatic until two independent studies in 2017 revealed that they are activated by target RNA binding to polymerize ATP into a novel set of cyclic nucleotides known collectively as cyclic oligoadenylate (cOA) (26, 27). In vitro, *Streptococcus thermophilus* Csm was found to synthesize several cOA species consisting of between 3 and 6 AMP subunits (cA_n $n = 3–6$), and cyclic triadenylate (cA_3) was the predominant species (27), while *Staphylococcus epidermidis* Csm produced only cA_6 (26). cOA is formed in the two Palm polymerase domains of Cas10, and the conserved GGDD motif in Palm2 was found to be crucial for ATP binding. The proposed chemical mechanism for cOA formation involves a nucleophilic attack by the 3′-hydroxyl of one ATP on the α-phosphate of a second ATP molecule to generate the first pppApA intermediate, then extended by sequential rounds of polymerization (26, 27). Linear intermediates, containing between 3 and 6 adenosines, are thought to be cyclized by an intramolecular nucleophilic attack to form the final 3′-5′ bond. Since the discovery of cOA signaling by type III CRISPR systems, considerable evidence has accumulated that this is often the most important of the three enzymatic activities for antiviral defense (21, 23, 28–30).

2.4. Collateral Versus Specific Cleavage in CRISPR Defense

CRISPR systems are now well understood as adaptive immune systems that defend prokaryotic cells against invading nucleic acids from MGEs, directed by crRNA molecules transcribed from a CRISPR locus. Although CRISPR systems are often viewed as defense systems that are highly specific for the nucleic acid of an invading MGE, this is strictly true only for the minority of CRISPR types. Type II (Cas9) does directly bind and cleave target DNA based on guide RNA specificity, and type I (Cascade) loads the degradative helicase-nuclease Cas3 onto targeted DNA using an analogous approach. However, Cas12 (type V) and Cas13 (type VI) both have nonspecific, collateral cleavage modes (for ssDNA and RNA, respectively) once activated by target recognition (31–33) (**Figure 2**). Both of these effectors thus target host nucleic acid in addition to the invading entity that activated these defenses.

For type III (Cas10) systems, as we have seen, the HD nuclease domain of Cas10 is activated to cleave ssDNA on target RNA recognition, but there is still debate on the specificity or otherwise of this nuclease activity. Whether or not the HD domain of Cas10 is a promiscuous ssDNA nuclease, the second Cas10 activity, cOA synthesis, clearly functions via collateral cleavage (**Figure 2**). cOA synthesis involves an intrinsic signal amplification, with binding of one target RNA resulting in synthesis of more than 1,000 molecules of cOA (34, 35). These signaling molecules are likely to quickly diffuse through cells and build up to quite significant concentrations, of the order of 5–50 μM, on detection of a small number of foreign RNA molecules (35). cOA activates a number of ancillary defense proteins, which are described in the following section.

2.5. Ancillary Proteins of Type III CRISPR Defense

A wide range of genes are associated with type III CRISPR loci, many of which are thought to have an ancillary function in type III CRISPR defense. Here, we briefly review the ancillary proteins that have been characterized experimentally.

2.5.1. Csx1/Csm6 ribonucleases. cOA molecules function as second messengers by binding to and activating a range of type III CRISPR ancillary proteins, of which the best characterized are the cardiac-specific homeobox 1 (Csx1)/Csm6 ribonuclease family (**Figure 3**). Early genetic studies identified the association of Csx1 and Csm6 CRISPR ancillary proteins with type III CRISPR immunity; however, no physical interactions were detected with Csm/Cmr effector complexes (23, 30). It was first identified that *csx1* deletion compromised plasmid immunity conferred by Cmr-α

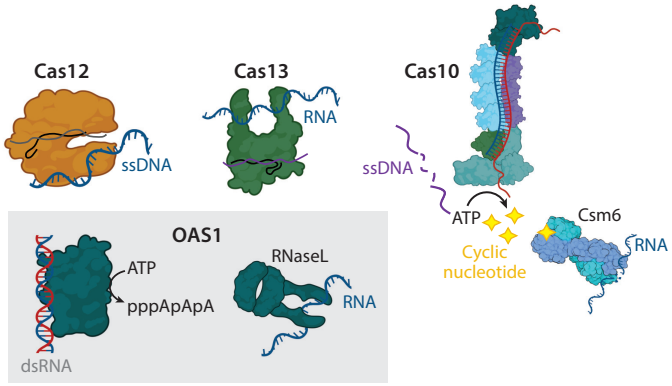


Figure 2

Collateral cleavage in CRISPR systems. The Cas12 and Cas13 enzymes have both specific, guide RNA targeted and promiscuous cleavage modes (for ssDNA and RNA, respectively). Type III (Cas10) CRISPR systems cleave target RNA specifically and have nonspecific ssDNA and cOA-activated collateral cleavage activity. These antiviral activities are analogous to that of eukaryotic OAS1, a dsRNA-sensing nucleotide polymerase (homologous to cGAS) that generates short linear nano-RNA second messengers that bind to and activate the RNaseL protein, resulting in nonspecific cleavage of RNA in infected cells. Abbreviations: Cas, CRISPR-associated; cGAS, cyclic GMP-AMP synthase; cOA, cyclic oligoadenylate; CRISPR, clustered regularly interspaced palindromic repeats; Csm6, Cas subtype Mtube 6; dsRNA, double-stranded RNA; OAS1, oligoadenylate synthase 1; ssDNA, single-stranded DNA. Figure adapted from images created with BioRender.com.

in *Sulfolobus islandicus*, which was restored upon *csx1* complementation of the deletion strain (23). Likewise, *csm6* deletion in *S. epidermidis* was found to compromise immunity from *Staphylococcal* conjugative plasmids (30). The first link between the Cas10 subunit and antiplasmid immunity was made when mutations in the Cas10 Palm polymerase domains prevented CRISPR immunity without hampering crRNA expression or effector complex formation (30). Deletion of *csm6* was found to permit accumulation of viral mRNA transcripts of late-expressed genes during phage infection, further highlighting a crucial role in interference (28).

Csx1/Csm6 enzymes comprise an N-terminal CRISPR-associated Rossmann fold (CARF) domain and a C-terminal higher eukaryotes and prokaryotes, nucleotide binding (HEPN) domain (36). HEPN domains function as essential RNase components of toxin-antitoxin and abortive infection (Abi) systems in prokaryotes (37) and also within the innate immune response of eukaryotes (38). Structural studies identified an electropositive pocket within dimeric N-terminal CARF domains for a putative allosteric effector (36, 39, 40). Once cOA was identified as the allosteric activator of CARF-fused effector domains, it became clear that Csm6/Csx1-family enzymes were specifically activated by cOAs to become potent ribonucleases (26, 27, 34). Further studies following this discovery demonstrated that, once activated by cOA, the nonselective activity of Csm6 caused growth arrest in cells until infection was cleared (41). Interestingly, Csm6 was dispensable for immunity if targets were well expressed, presumably because the HD nuclease of the cognate type III effector could compensate by degrading DNA (41). However, other studies using several microorganisms have found that Csm6/Csx1 is crucial for plasmid elimination, independent of DNA targeting by the type III effector complex (29, 42).

CARF: CRISPR-associated Rossmann fold

HEPN: higher eukaryotes and prokaryotes, nucleotide binding

2.5.2. Can1 and Can2/Card1 nucleases. Analysis of diverse CRISPR loci identified genetic association between type III CRISPR systems and a wide variety of CARF-family proteins, which included those containing putative DNA binding helix-turn-helix domains, toxin PIN and RelE

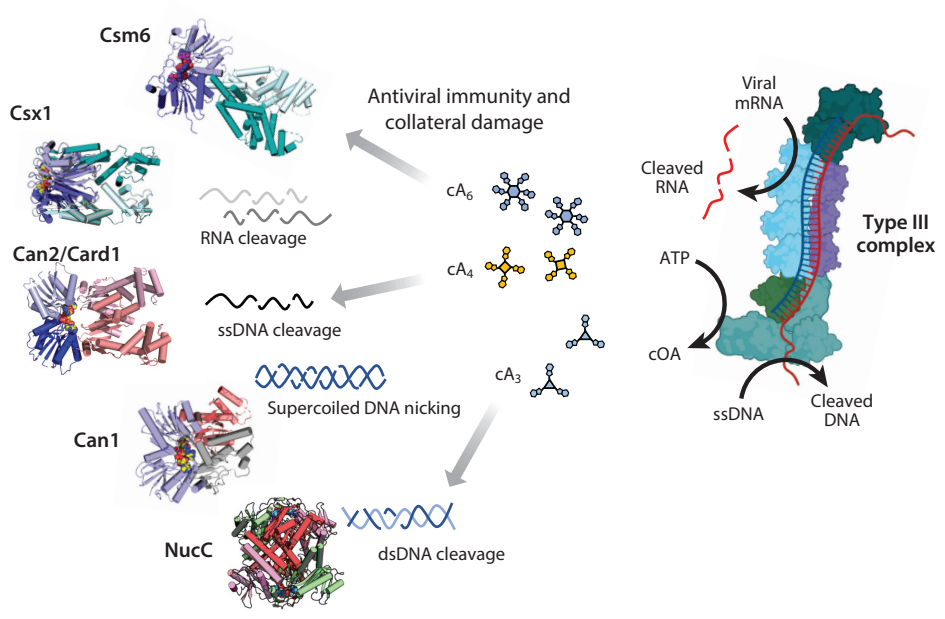


Figure 3

Type III CRISPR systems and ancillary effectors. Type III CRISPR systems detect foreign RNA using a crRNA guide, activating the Cas10 subunit HD nuclease (when present) and PALM polymerase domains. The latter generate large quantities of cOA second messengers of characteristic ring size, which in turn bind to and activate effector proteins that cleave both viral and host nucleic acids. Cleavage of viral RNA by the Cas7 subunit returns Cas10 to an inactive state. Abbreviations: Can, CRISPR ancillary nuclease; Card1, cyclic-oligoadenylate-activated single-stranded ribonuclease and single-stranded deoxyribonuclease 1; Cas, CRISPR-associated; cOA, cyclic oligoadenylate; CRISPR, clustered regularly interspaced palindromic repeats; crRNA, CRISPR RNA; Csm6, Cas subtype Mtube 6; Csx1, cardiac-specific homeobox 1; dsDNA, double-stranded DNA; mRNA, messenger RNA; NucC, nuclease, CD-NTase associated; ssDNA, single-stranded DNA. Figure adapted from images created with BioRender.com.

RNase domains, proteases, and PD-D/ExK DNase domains (43). Many of these remain uncharacterized biochemically, but recent work has shed light on some effector families. The first of these was CRISPR ancillary nuclease 1 (Can1), a CRISPR-associated protein found in the CRISPR locus of *T. thermophilus* (44). Unusually, Can1 is a monomer with two CARF domains encoded by a single polypeptide along with nuclease-like domains (44). Can1 bound to cA_4 revealed a structure reminiscent of a fused dimer, with the two CARF domains adopting a conformation similar to the dimeric arrangement seen in Csm6/Csx1 proteins. Can1 is activated by cA_4 and nicks supercoiled DNA nonspecifically to generate products with ligatable ends. Such an activity may be reasonably well tolerated by the host genome while impacting more significantly on rapidly replicating viral genomes, potentially causing replication fork collapse (44).

Can2, which is related to Can1, is a more conventional dimeric effector with an N-terminal CARF domain fused to a PD-DExK-family nuclease (45). Can2 is distributed more widely than Can1, and the latter may be an unusual, fused derivative as the overall folds of the two proteins are clearly closely related. Can2 has an unusual cA_4-activated nuclease activity that targets both supercoiled DNA and RNA in a nonspecific manner (45). Can2 is also known as cyclic-oligoadenylate-activated single-stranded ribonuclease and single-stranded deoxyribonuclease 1 (Card1), and some orthologs also target ssDNA, resulting in dormancy of infected host cells (46).

Such a wide specificity for different nucleic acid species in a nuclease is rarely found in nature. This may reflect the role of Can2/Card1, which is to be activated in emergencies and to cause collateral damage to nucleic acids of both the invader and host with the aim of slowing down infection kinetics.

2.5.3. NucC endonuclease. Nuclease, CD-NTase associated (NucC) is a trimeric nuclease that is activated by cyclic trinucleotide binding, whereupon it assembles into an active hexameric form (47). NucC is a potent endonuclease specific for dsDNA, which it degrades into short fragments by introduction of dsDNA breaks. NucC is unusual in being an effector found associated with both CRISPR and CBASS (48, 49), which can both generate cyclic trinucleotides (cA_3 in the case of CRISPR, and a wider range in the case of CBASS) in response to viral infection. In the context of CBASS immunity, the activation of NucC results in cell death by fragmentation of the genome (47), consistent with the paradigm that CBASS functions via Abi (50). Recently, a type III CRISPR system with a NucC effector from *Vibrio metoecus* has been studied (49). When activated by target RNA binding, the type III complex generates cA_3 and remains active for an extended period due to very slow target RNA cleavage. The associated NucC effector has a very high affinity for cA_3 and a robust nuclease activity, which has been harnessed for the development of a novel assay that can be programmed for specific RNA detection (48).

Although not yet analyzed in vivo, this has the hallmarks of a CRISPR system functioning via Abi/cell death rather than viral clearance/cell dormancy. Perhaps significantly, the CRISPR system is encoded by a prophage integrated into the genomes of *V. metoecus* and *Vibrio cholerae* hosts and appears to be a hybrid type III-B/I-F system (51). The prophage may use this system for inter-MGE conflict where the host is a convenient battleground, in which case the evolutionary pressures that influence the most beneficial outcome of activation may be more slanted toward cell death, but further work is required to explore these possibilities.

2.6. Ring Nucleases: Off Switches for cOA-Based Defense

The observation that a few viral RNA molecules could result in a large signal amplification, flooding cells with cOA second messengers and activating ancillary nucleases (34, 35) prompted the question: Is this a one-way ticket to cell death, or is there a way to switch off the signal? Although target RNA degradation by type III complexes deactivates the Cas10 polymerase and HD nuclease activities, extant cOA in the cell will persist unless actively turned over, leaving defense pathways activated. This would be acceptable for pathways providing immunity by Abi, but most CRISPR defense systems are not thought to operate in this manner. The first enzyme identified as a ring nuclease specific for degradation of cOA was Crn1 (CRISPR ring nuclease 1), purified using classical biochemical techniques from the archaeon *Sulfolobus solfataricus* (**Figure 4**). Crn1 is a minimal dimeric CARF domain protein that binds and converts cA_4 to linear A_2 products with relatively slow reaction kinetics (52). Recent structures have revealed a close-and-twist conformational change on cA_4 binding and cleavage that may be key to catalysis (53). Crn1 has a very narrow phylogenetic distribution, but in the past three years it has been joined by the unrelated Crn2 enzyme (54, 55), which has a wider distribution, and Crn3/Csx3, a distant cousin with a divergent CARF domain that has an unusual catalytic mechanism involving the formation of tetramers that sandwich the cA_4 substrate (56, 57). Crn2 is sometimes observed as a domain fused to a Csx1-family effector, providing an in-built off switch that does not use the cA_4-binding CARF domain (55). Further putative members of the ring nuclease family have been suggested based on bioinformatic and guilt-by-association approaches (58).

In addition to dedicated ring nucleases, a subset of the Csm6/Csx1-family ribonucleases are now understood to auto-deactivate by slowly degrading the cA_4 or cA_6 activator bound in the

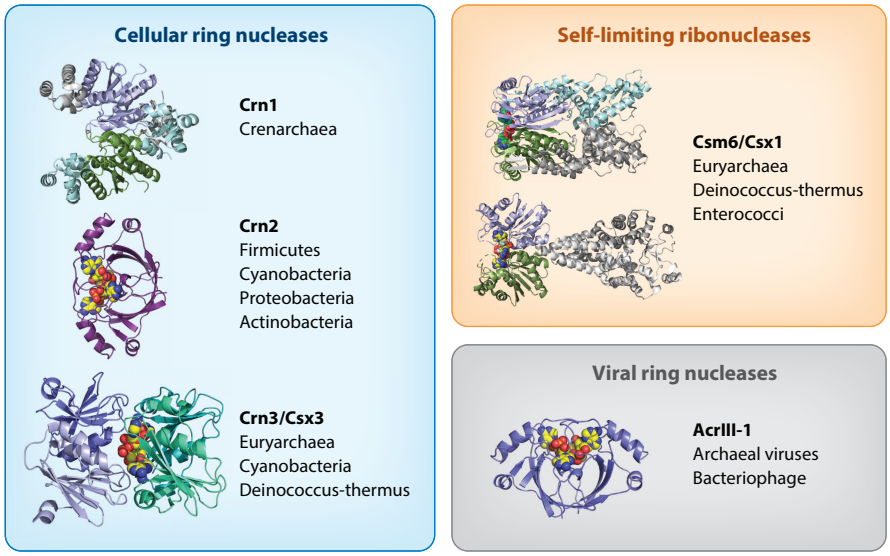


Figure 4

The ring nucleases. Three families of dedicated ring nucleases (Crn1, 2, and 3) have been experimentally confirmed, while a subset of the Csm6/Csx1 effector ribonucleases also use their CARF domains to hydrolyze cOA. The viral ring nuclease AcrIII-1, which is homologous to Crn2, is found in many archaeal viruses as well as some MGEs and bacteriophage. Abbreviations: AcrIII-1, anti-CRISPR III-1; CARF, CRISPR-associated Rossmann fold; cOA, cyclic oligoadenylate; CRISPR, clustered regularly interspaced palindromic repeats; Crn, CRISPR ring nuclease; Csm6, Cas subtype Mtube 6; Csx, cardiac-specific homeobox; MGE, mobile genetic element.

CARF domains using a similar mechanism to Crn1 (59–62) (**Figure 4**). The emerging paradigm is that the majority of type III CRISPR systems that operate via cyclic nucleotide signaling have a means to remove extant cOA from the cell once viral infection is cleared, with the result that Abi is not an inevitable consequence of the activation of these pathways (reviewed in 63).

2.7. Viral Ring Nucleases Subvert cOA-Based Defense

Following the discovery of cellular ring nucleases, it did not take long for the first viral ring nuclease to be identified. By revisiting a hypothetical protein from the *Sulfolobus* virus *S. islandicus* rod-shaped virus 1, whose structure we had solved some time ago (64) and which was known to be important for efficient viral infection (65), we uncovered an anti-CRISPR (Acr) enzyme that degrades cA_4 about 100 times faster than the cellular Crn1 enzyme (54). The enzyme, AcrIII-1, allows viruses to overcome type III CRISPR defense in vivo by degrading the cyclic nucleotide second messenger and thus deactivating the defense enzymes before they can mount a serious attack against viral replication (54). AcrIII-1 is homologous to the Crn2 family of ring nucleases and does not use a CARF domain for cA_4 recognition, suggesting that ring nucleases have evolved at least twice (**Figure 4**). By targeting a signaling molecule rather than a protein, AcrIII-1 can have an effective broad host range, explaining its conservation in many archaeal viruses as well as several bacteriophage genomes (54).

Notably, AcrIII-1 is encoded by the *yddf* gene of the MGE ICE*Bs1*, which is integrated in the genome of *Bacillus subtilis* (66). Orthologs of *yddf* are found in many *Bacillus* genomes, and it is apparent that these genomes lack a type III CRISPR defense system (M.F. White, unpublished observation). One interpretation of this observation is that, once the *acrIII-1* gene gets into a

genome, type III CRISPR systems are redundant and thus subsequently lost from that genome. As AcrIII-1 is specific for cA_4, an alternative approach for the cell would be to switch to a different signaling molecule such as cA_6 or cA_3. It may not be a coincidence that type III CRISPR systems based on the cA_3 activated NucC effector appear to be common in the *Bacilli*. So far there are no known cA_3-specific ring nucleases, but given the widespread use of this signaling molecule by CRISPR and CBASS, it would be surprising if bacteriophage have not evolved such an enzyme for counter-defense.

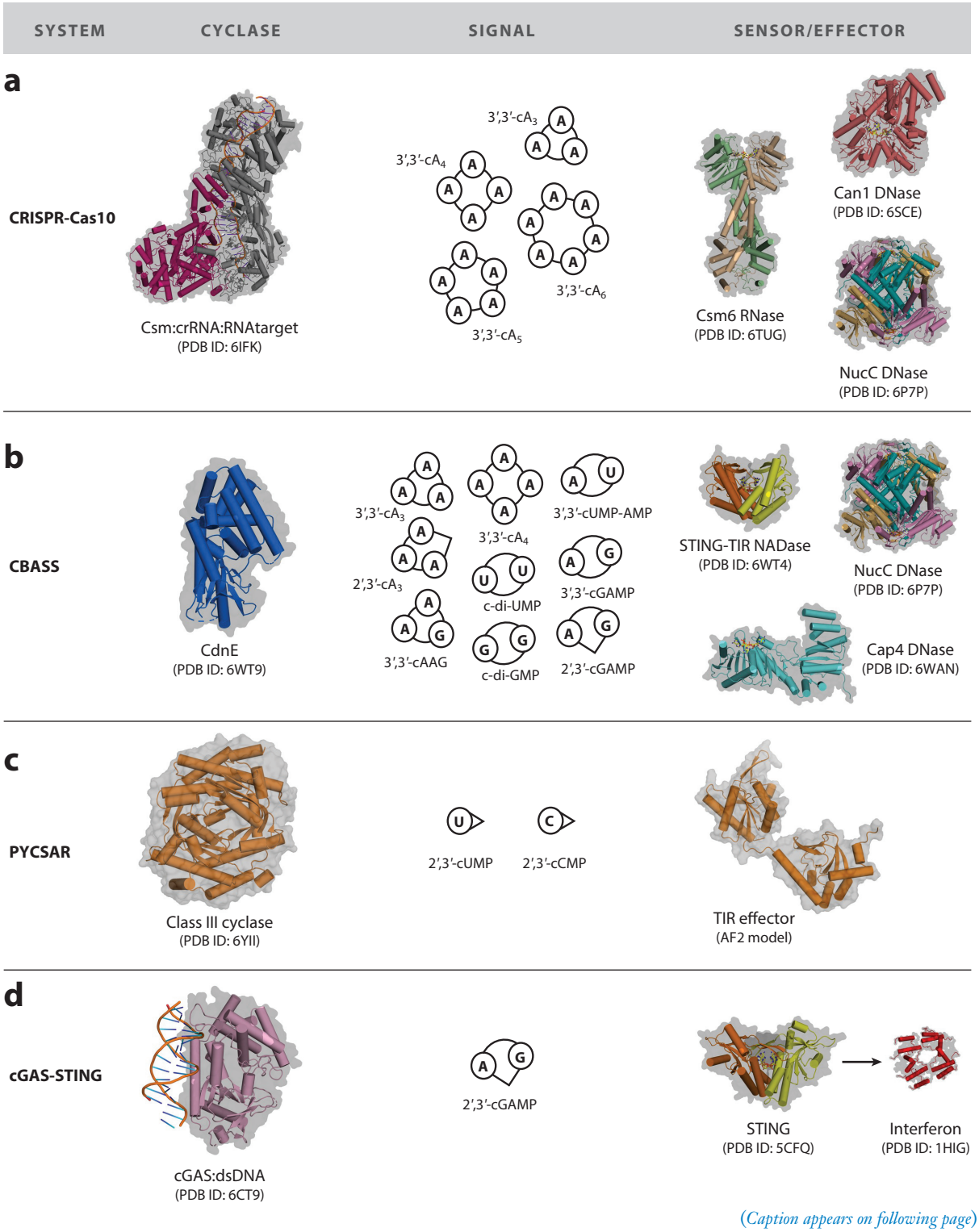
TIR: Toll/interleukin-1 receptor

PYCSAR: pyrimidine cyclase system for antiphage resistance

3. CBASS

In eukaryotes, the cyclic GMP-AMP synthase (cGAS)-stimulator of interferon genes (STING) pathway is activated by viral infection and cGAS functions as a nucleotide cyclase to generate 2′3′-cGAMP (cyclic GMP-AMP with 2′-5′/3′-5′ linkages), which binds to the STING protein, potentiating the innate immune response of the interferon pathway (67–69). Recent studies in bacteria have revealed an astonishing diversity of bacterial nucleotide cyclases, all related to cGAS, that produce a wide range of cyclic nucleotide signaling molecules, including cyclic dipurines, dipyrimidines, mixed purine/pyrimidine dinucleotides, and trinucleotides, in response to phage infection (50, 70, 71) (**Figure 5**). The founding member of this family is the *Vibrio cholerae* cyclase DncV, which generates 3′,3′-cGAMP (72), leading to the collective term CD-NTase (cGAS/DncV-like nucleotidyltransferase) for the family. The alternative name SMODS (second messenger oligonucleotide or dinucleotide synthetases) was proposed by Aravind and colleagues in their groundbreaking bioinformatic analyses of these systems (73). These cyclases in turn activate a plethora of effector proteins including DNA nucleases such as NucC and cGAMP-activated phospholipase (Cap) 4, phospholipases such Cap in *Vibrio* (74), catalytic Toll/interleukin-1 receptor (TIR) domains (75), and membrane disruption proteins (76). There is even a bacterial homolog of STING, which senses cyclic dimeric (c-di)-GMP generated by a CD-NTase and multimerises, resulting in activation of a fused TIR effector domain (71, 77) that degrades NAD+ (77). It is found only in the *Bacteroidetes*—a bacterial lineage that does not utilize c-di-GMP as a housekeeping second messenger.

cGAS and bacterial CD-NTases act as signal amplifiers in much the same way as the Cas10 cyclase of type III CRISPR systems, allowing one cyclase to activate potentially hundreds of effector proteins. The CBASS acronym, proposed by Sorek and colleagues (71), is a nod to the links with eukaryotic cGAS (and perhaps also an implicit recognition that a catchy name never hurts). There are major unanswered questions, the most important of which is what is the mechanism of activation of the nucleotide cyclases that somehow sense phage infection and generate the cyclic nucleotides that activate cellular defenses? These cyclases tend to be at least partially active as recombinant enzymes in vitro (70, 78), but the activity assays often require nonphysiological high pH buffers, and it is not yet known whether this reflects their full activity in vivo. CD-NTases must be held in an inactive state in vivo until required, but it is still unclear whether they are activated by an activation or de-repression mechanism. Activation could be linked to the changes in the transcriptome or metabolome, or direct detection of phage components on infection. Some CBASSs [and also pyrimidine cyclase system for antiphage resistance (PYCSAR) systems, described next] are found in operons with ubiquitin-like protein conjugation and deconjugation machinery, whose function is still not understood but which may provide a means to switch the system on and/or off (50, 73, 79). CBASSs are a vast and exciting new field of antiphage defense that we cannot do justice to here (reviewed in 50, 80), so we focus below on the links and parallels between CBASS and CRISPR.



(*Caption appears on following page*)

Figure 5 (*Figure appears on preceding page*)

Cyclic nucleotide-based antiviral signaling systems. (*a*) Type III CRISPR systems generate cyclic oligoadenylates which activate CARF-family and NucC effectors. (*b*) CBASS cyclases synthesize a wide range of cyclic nucleotides, associated with diverse effector proteins. (*c*) The PYCSAR system involve a class III-like nucleotide cyclase that generates cUMP or cCMP, activating effectors with TIR domains or predicted membrane disruption proteins. (*d*) In eukaryotes, cGAS synthesizes 2′,3′-cGAMP, which binds to STING, activating the innate immune interferon pathway. PDB codes of representative structures are indicated; the PYCSAR *Pseudomonas aeruginosa* TIR effector was modeled using AF2 (99) on the ColabFold server (100). Abbreviations: AF2, Alphafold2; c-di, cyclic dimeric; cAAG, cyclic AMP-AMP-GMP; Can, CRISPR ancillary nuclease; Cap, cGAMP-activated phospholipase; CARF, CRISPR-associated Rossmann fold; Cas, CRISPR-associated; CBASS, cyclic nucleotide-based antiphage signaling system; CdnE, CD-NTase clade E; cGAMP, cyclic GMP-AMP; cGAS, cyclic GMP-AMP synthase; CRISPR, clustered regularly interspaced palindromic repeats; crRNA, CRISPR RNA; Csm, Cas subtype Mtube; dsDNA, double-stranded DNA; NucC, nuclease, CD-NTase associated; PDB, Protein Data Bank; PYCSAR, pyrimidine cyclase system for antiphage resistance; STING, stimulator of interferon genes; TIR, Toll/interleukin-1 receptor.

4. PYCSAR AND THOERIS

Writing in 2020, Burroughs and Aravind (79), who had previously provided the first bioinformatic description of the system now known as CBASS along with colleagues (73), reported the identification of a distinct system that they predicted might utilize cyclic mononucleotide signaling molecules to activate effectors for antiphage defense. This was confirmed experimentally in 2021 by the Sorek and Kranzusch labs (81), who reported the mechanism of a group of antiphage defense enzymes that function via cyclic mononucleotide second messengers. This system, named PYCSAR, uses a cyclase of the class III adenylate/guanylate family distinct from CBASS-family cyclases (**Figure 5**). Unexpectedly, when activated by phage infection, PYCSAR cyclases generate cCMP or cUMP molecules (81). These cyclic mononucleotides have been detected in a variety of organisms, but their role has been a subject of controversy, with few definitive clues to function (reviewed in 82). The effector proteins have sensor domains that bind the cyclic mononucleotide second messenger, fused to TIR domains that degrade NAD+ or domains predicted to disrupt cell membranes (81), highly reminiscent of the already characterized CBASS. They are thus expected to function via an Abi mechanism, and this has been confirmed for one family member (81). As for the other CBASS cyclases, PYCSAR cyclases are active in vitro, suggesting that there is a mechanism to maintain them in an inactive state in the cell until viral infection is detected. Their association in some genomes with CBASS-like ubiquitin conjugation machinery (79) emphasizes the links between the two systems.

The Thoeris antiphage defense system (83) also utilizes a cyclic nucleotide signaling molecule and a catalytic TIR domain, but it differs from CBASS and PYCSAR in many crucial aspects. Viral infection is detected by the ThsB protein (the mechanism is not yet understood), leading to activation of a catalytic TIR domain in the protein that converts NAD+ into a variant cyclic ADP ribose (cADPR) molecule (84). This second messenger binds to the SMF/DprA-LOG domain of ThsA, resulting in activation of the Sirtuin domain, which depletes NAD+ leading to growth arrest and Abi (84, 85). The bacterial Thoeris system has clear links with TIR domain-containing plant defense systems, and elements are interchangeable (84). Here the TIR domain has switched roles from an effector to a sensor while retaining its core catalytic activity.

5. LINKS AND PARALLELS BETWEEN CBASS AND CRISPR

5.1. Shared Signals and Effectors

CBASS and type III CRISPR overlap in their ability to synthesize cA_3 in response to phage infection (27, 42, 47). It follows that there is the potential for shared effector proteins that sense cA_3 to activate the immune response, and we have already discussed the endonuclease NucC, which

SAVED: SMODS-associated and fused to various effector domains

is found associated with both systems, in this regard. A second important sensor of cA_3 is the SMODS-associated and fused to various effector domains (SAVED) domain, first described by the Aravind lab (73). The SAVED domain is a distant relative of the CARF domain (58), and they share the ability to bind cyclic nucleotides (75). While CARF domains are generally homodimers, SAVED domains are monomers that may have arisen by gene duplication of a CARF-encoding gene, as seen in the Can1 protein. As dimers, CARF domains appear constrained to recognize ligands with twofold symmetry such as cA_4 and cA_6. In contrast, SAVED domains suffer no such constraint, and examples binding specifically to a range of cyclic di- and trinucleotides have been characterized (75, 86). The best studied is the Cap4 protein of CBASS, which uses a cA_3 or cyclic AMP-AMP-GMP-sensing SAVED domain fused to a DNA endonuclease (75). Nucleotide binding causes head-to-tail multimerization of the SAVED domain, resulting in activation of the associated nuclease to bring about Abi in a mechanism reminiscent of NucC. Another, Cap5 from *Lactococcus lactis*, has a SAVED domain that senses 3′,2′-cGAMP fused to an HNH-family nuclease, demonstrating that SAVED domains are not restricted to cyclic trinucleotide second messengers (86). SAVED domains are found in approximately 30% of CBASS and are fused to a variety of effectors including nucleases, peptidases, and catalytic TIR domains (50, 75). SAVED fusions with enzymatic domains, such as Lon protease and PD-D/ExK nuclease, are also found associated with type III CRISPR systems (58). Thus, it is predicted that 3′-5′ linked cOA made by type III CRISPR systems will activate cognate SAVED-containing proteins for antiviral defense, but these mechanisms have so far not been studied in any detail.

Perhaps due to their adoption as tools for genome editing, CRISPR systems are typically thought of as precise, RNA-guided molecular scissors that detect and destroy foreign nucleic acids to provide immunity from MGEs. However, the reality is that many CRISPR systems function using a combination of specific and collateral degradative mechanisms. The champion in this regard is type III CRISPR, which has the option of activating the HD nuclease built into the Cas10 subunit to degrade ssDNA—analogous to Cas12, but which can also generate cyclic nucleotide second messengers. This type of molecular outsourcing provides several advantages, including the ability to amplify a small initial signal (viral RNA) by up to three orders of magnitude and the flexibility to utilize a wide variety of effector nucleases.

Although type III systems have been observed to generate a range of cOA species in vitro, they may be tuned in vivo to generate a particular molecule, such as cA_6 in *Streptococcus* (61), cA_4 in *Sulfolobus* (34), and cA_3 in *Vibrio* (49), determined by the preference of their associated effector enzymes. Notably, although multiple effector genes are commonly observed in type III CRISPR loci, they tend to respond to the same cOA species. Cyclic nucleotide signaling thus presents a challenge to viruses, but also an opportunity. If the signal can be destroyed rapidly enough, viruses can triumph in the race to replicate, so it is unsurprising that viral ring nucleases with superior turnover numbers exist. Indeed, the ability to target and destroy the message rather than recipient protein frees these Acrs from the requirement to maintain recognition of evolving protein structures, effectively broadening their host range as seen for AcrIII-1. By the same token, cells with type III CRISPR systems may experience pressure to swap second messengers and effectors when a specific viral ring nuclease becomes predominant in their environment—a development that may have occurred in *Bacillus* lineages when the *yddf* ring nuclease gene was introduced in a prophage (54).

Type III CRISPR systems are arguably a specialized form of CBASS, and the two certainly overlap in terms of second messengers and effectors (**Figure 5**). However, they should probably be classified separately as there are profound differences, particularly in their viral-sensing and activation mechanism. In this regard, type III CRISPR has a beautifully elegant means to detect viral RNA, whereas classical CBASS cyclases do not have this luxury. While their mechanism of

CBASS activation remains mysterious, this is unlikely to persist for long. CBASS cyclases do have one key advantage over the Cas10 polymerase domain, which is the ability to utilize a broader set of nucleotide triphosphates and to link them with either 2′, 5′ or 3′, 5′ phosphodiester bonds. This remarkable plasticity has been estimated to allow the potential for dozens of different second messengers to be synthesized (70). When this is coupled with the large number of effector proteins available, the diversity is quite mind boggling. Once again, this may reflect the requirement to evolve away from pressures caused by viral counter-defense.

5.2. Anti-CBASS?

It is already apparent that CBASSs are functional against only certain phage lineages, but it is not yet clear whether this is due to differences in the detection of infection or to anti-CBASS proteins—a still-hypothetical entity at the time of writing. Many mechanisms that block and counteract antiviral signaling have been uncovered in eukaryotic viruses. Some viruses shield DNA to prevent detection by cGAS (87), degrade cGAS using proteases (88), prevent cyclic nucleotide synthesis by disabling DNA sensing or promoting dissociation (89–91), and inhibit various stages of downstream signaling, including by proteolytic degradation of STING (92). In a striking parallel with the discovery of cA_4-specific viral ring nucleases, the Kranzusch lab identified a pox virus protein, poxin, that degrades cGAMP to neutralize the cGAS-STING pathway (93). This signposts the likely direction of travel toward a time when there will be as many confirmed anti-CBASS phage protein families as there are Acrs (94). A prime candidate would be a virally encoded cA_3-specific ring nuclease, which could provide broad protection against both CRISPR and CBASS defenses that utilize this signaling molecule.

5.3. Inter-Cell Communication?

Another open question is whether cyclic nucleotides are used to communicate between cells in case of infection. Dissemination of 2′,3′-cGAMP between mammalian cells occurs via gap junctions and confers cell intrinsic immunity to bystander cells (95). Although prokaryotic cells are not connected like mammalian cells, cOA may be disseminated locally to warn neighbors of nearby viral predators. By way of a precedent, the intracellular parasite *Listeria monocytogenes* exports c-di-AMP using a multidrug efflux pump, triggering host innate immunity (96). Remarkably, 2′,3′-cGAMP can be packaged into virions during viral replication and delivered into cells during subsequent rounds of infection, activating innate immunity pathways (97, 98). cOA concentrations are predicted to reach high micromolar levels during infection (35, 61); therefore, cOA could conceivably be loaded into virions during viral replication and delivered to newly infected cells. Accelerated immune activation could be advantageous for type III CRISPR immunity, allowing priming of defenses when a viral infection is imminent and where there exists a means to deactivate the self-destruct button in case of a false alarm, using ring nucleases. It is less clear that CBASS defense involving Abi would benefit from such an approach, as uninfected cells committing suicide unnecessarily might not confer much benefit to the population.

6. CONCLUDING REMARKS

To conclude, the past few years have seen breathtaking advances in our knowledge of cyclic nucleotide-based antiviral defense systems in prokaryotes. These discoveries have built on seminal bioinformatic analyses by the Koonin/Makarova, Aravind, and Sorek groups and have placed cyclic nucleotides center stage as second messengers in antiviral signaling, revealing unexpected parallels between innate immunity systems in bacteria and eukaryotes. Fundamental questions around

CBASS activation on phage infection remain unanswered. In particular, there are few published studies of CBASS in their natural hosts, which will be essential to provide a full understanding of their mechanisms. We can expect many more exciting developments, along with a few surprises, in the years to come for this field.

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LITERATURE CITED

1. Makarova KS, Wolf YI, Alkhnbashi OS, Costa F, Shah SA, et al. 2015. An updated evolutionary classification of CRISPR–Cas systems. *Nat. Rev. Microbiol.* 13:722–36
2. Haft DH, Selengut J, Mongodin EF, Nelson KE. 2005. A guild of 45 CRISPR-associated (Cas) protein families and multiple CRISPR/Cas subtypes exist in prokaryotic genomes. *PLOS Comput. Biol.* 1:e60
3. Makarova KS, Haft DH, Barrangou R, Brouns SJ, Charpentier E, et al. 2011. Evolution and classification of the CRISPR–Cas systems. *Nat. Rev. Microbiol.* 9:467–77
4. Makarova KS, Aravind L, Wolf YI, Koonin EV. 2011. Unification of Cas protein families and a simple scenario for the origin and evolution of CRISPR-Cas systems. *Biol. Direct* 6:38
5. Jackson RN, Wiedenheft B. 2015. A conserved structural chassis for mounting versatile CRISPR RNA-guided immune responses. *Mol. Cell* 58:722–28
6. Jiang F, Doudna JA. 2015. The structural biology of CRISPR-Cas systems. *Curr. Opin. Struct. Biol.* 30:100–11
7. Tamulaitis G, Venclovas C, Siksnys V. 2017. Type III CRISPR-Cas immunity: major differences brushed aside. *Trends Microbiol.* 25:49–61
8. Lin J, Fuglsang A, Kjeldsen AL, Sun K, Bhoobalan-Chitty Y, Peng X. 2020. DNA targeting by subtype I-D CRISPR–Cas shows type I and type III features. *Nucleic Acids Res.* 48:10470–78
9. Manav MC, Van LB, Lin J, Fuglsang A, Peng X, Brodersen DE. 2020. Structural basis for inhibition of an archaeal CRISPR–Cas type I-D large subunit by an anti-CRISPR protein. *Nat. Commun.* 11:5993
10. Hale CR, Zhao P, Olson S, Duff MO, Graveley BR, et al. 2009. RNA-guided RNA cleavage by a CRISPR RNA-Cas protein complex. *Cell* 139:945–56
11. Hale CR, Cocozaki A, Li H, Terns RM, Terns MP. 2014. Target RNA capture and cleavage by the Cmr type III-B CRISPR–Cas effector complex. *Genes Dev.* 28:2432–43
12. Staals RH, Zhu Y, Taylor DW, Kornfeld JE, Sharma K, et al. 2014. RNA targeting by the type III-A CRISPR-Cas Csm complex of *Thermus thermophilus*. *Mol. Cell* 56:518–30
13. Tamulaitis G, Kazlauskiene M, Manakova E, Venclovas C, Nwokeoji AO, et al. 2014. Programmable RNA shredding by the type III-A CRISPR-Cas system of *Streptococcus thermophilus*. *Mol. Cell* 56:506–17
14. Zebec Z, Manica A, Zhang J, White MF, Schleper C. 2014. CRISPR-mediated targeted mRNA degradation in the archaeon *Sulfolobus solfataricus*. *Nucleic Acids Res.* 42:5280–88
15. Samai P, Pyenson N, Jiang W, Goldberg GW, Hatoum-Aslan A, Marraffini LA. 2015. Co-transcriptional DNA and RNA cleavage during type III CRISPR-Cas immunity. *Cell* 161:1164–74
16. Makarova KS, Aravind L, Grishin NV, Rogozin IB, Koonin EV. 2002. A DNA repair system specific for thermophilic Archaea and bacteria predicted by genomic context analysis. *Nucleic Acids Res.* 30:482–96

17. Makarova KS, Grishin NV, Shabalina SA, Wolf YI, Koonin EV. 2006. A putative RNA-interference-based immune system in prokaryotes: computational analysis of the predicted enzymatic machinery, functional analogies with eukaryotic RNAi, and hypothetical mechanisms of action. *Biol. Direct* 1:7
18. Estrella MA, Kuo FT, Bailey S. 2016. RNA-activated DNA cleavage by the type III-B CRISPR-Cas effector complex. *Genes Dev*. 30:460–70
19. Elmore JR, Sheppard NF, Ramia N, Deighan T, Li H, et al. 2016. Bipartite recognition of target RNAs activates DNA cleavage by the Type III-B CRISPR–Cas system. *Genes Dev*. 30:447–59
20. Kazlauskiene M, Tamulaitis G, Kostiuk G, Venclovas C, Siksnys V. 2016. Spatiotemporal control of type III-A CRISPR-Cas immunity: coupling DNA degradation with the target RNA recognition. *Mol. Cell* 62:295–306
21. Han W, Li Y, Deng L, Feng M, Peng W, et al. 2017. A type III-B CRISPR-Cas effector complex mediating massive target DNA destruction. *Nucleic Acids Res*. 45:1983–93
22. Liu TY, Iavarone AT, Doudna JA. 2017. RNA and DNA targeting by a reconstituted *Thermus thermophilus* type III-A CRISPR-Cas system. *PLOS ONE* 12:e0170552
23. Deng L, Garrett RA, Shah SA, Peng X, She Q. 2013. A novel interference mechanism by a type IIIB CRISPR-Cmr module in *Sulfolobus*. *Mol. Microbiol.* 87:1088–99
24. Goldberg GW, Jiang WY, Bikard D, Marraffini LA. 2014. Conditional tolerance of temperate phages via transcription-dependent CRISPR-Cas targeting. *Nature* 514:633–37
25. Liu TY, Liu JJ, Aditham AJ, Nogales E, Doudna JA. 2019. Target preference of type III-A CRISPR-Cas complexes at the transcription bubble. *Nat. Commun.* 10:3001
26. Niewoehner O, Garcia-Doval C, Rostol JT, Berk C, Schwede F, et al. 2017. Type III CRISPR–Cas systems produce cyclic oligoadenylate second messengers. *Nature* 548:543–48
27. Kazlauskiene M, Kostiuk G, Venclovas C, Tamulaitis G, Siksnys V. 2017. A cyclic oligonucleotide signaling pathway in type III CRISPR-Cas systems. *Science* 357:605–9
28. Jiang W, Samai P, Marraffini LA. 2016. Degradation of phage transcripts by CRISPR-associated RNases enables type III CRISPR-Cas immunity. *Cell* 164:710–21
29. Foster K, Kalter J, Woodside W, Terns RM, Terns MP. 2019. The ribonuclease activity of Csm6 is required for anti-plasmid immunity by Type III-A CRISPR-Cas systems. *RNA Biol*. 16:449–60
30. Hatoum-Aslan A, Maniv I, Samai P, Marraffini LA. 2014. Genetic characterization of antiplasmid immunity through a type III-A CRISPR-Cas system. *J. Bacteriol.* 196:310–17
31. Chen JS, Ma E, Harrington LB, Da Costa M, Tian X, et al. 2018. CRISPR-Cas12a target binding unleashes indiscriminate single-stranded DNase activity. *Science* 360:436–39
32. Li SY, Cheng QX, Liu JK, Nie XQ, Zhao GP, Wang J. 2018. CRISPR-Cas12a has both *cis*- and *trans*-cleavage activities on single-stranded DNA. *Cell Res*. 28:491–93
33. East-Seletsky A, O'Connell MR, Knight SC, Burstein D, Cate JH, et al. 2016. Two distinct RNase activities of CRISPR-C2c2 enable guide-RNA processing and RNA detection. *Nature* 538:270–73
34. Rouillon C, Athukoralage JS, Graham S, Gruschow S, White MF. 2018. Control of cyclic oligoadenylate synthesis in a type III CRISPR system. *eLife* 7:e36734
35. Athukoralage JS, Graham S, Rouillon C, Grüschow S, Czekster CM, White MF. 2020. The dynamic interplay of host and viral enzymes in type III CRISPR-mediated cyclic nucleotide signalling. *eLife* 9:e55852
36. Niewoehner O, Jinek M. 2016. Structural basis for the endoribonuclease activity of the type III-A CRISPR-associated protein Csm6. *RNA* 22:318–29
37. Anantharaman V, Makarova KS, Burroughs AM, Koonin EV, Aravind L. 2013. Comprehensive analysis of the HEPN superfamily: identification of novel roles in intra-genomic conflicts, defense, pathogenesis and RNA processing. *Biol. Direct* 8:15
38. Cooper DA, Banerjee S, Chakrabarti A, Garcia-Sastre A, Hesselberth JR, et al. 2015. RNase L targets distinct sites in influenza A virus RNAs. *J. Virol.* 89:2764–76
39. Lintner NG, Frankel KA, Tsutakawa SE, Alsbury DL, Copie V, et al. 2011. The structure of the CRISPR-associated protein Csa3 provides insight into the regulation of the CRISPR/Cas system. *J. Mol. Biol.* 405:939–55
40. Kim YK, Kim YG, Oh BH. 2013. Crystal structure and nucleic acid-binding activity of the CRISPR-associated protein Csx1 of *Pyrococcus furiosus*. *Proteins* 81:261–70

41. Rostol JT, Marraffini LA. 2019. Non-specific degradation of transcripts promotes plasmid clearance during type III-A CRISPR–Cas immunity. *Nat. Microbiol.* 4:656–62
42. Grüschow S, Athukoralage JS, Graham S, Hoogeboom T, White MF. 2019. Cyclic oligoadenylate signalling mediates *Mycobacterium tuberculosis* CRISPR defence. *Nucleic Acids Res.* 47:9259–70
43. Makarova KS, Anantharaman V, Grishin NV, Koonin EV, Aravind L. 2014. CARF and WYL domains: ligand-binding regulators of prokaryotic defense systems. *Front. Genet.* 5:102
44. McMahon SA, Zhu W, Graham S, Rambo R, White MF, Gloster TM. 2020. Structure and mechanism of a Type III CRISPR defence DNA nuclease activated by cyclic oligoadenylate. *Nat. Commun.* 11:500
45. Zhu W, McQuarrie S, Gruschow S, McMahon SA, Graham S, et al. 2021. The CRISPR ancillary effector Can2 is a dual-specificity nuclease potentiating type III CRISPR defence. *Nucleic Acids Res.* 49:2777–89
46. Rostol JT, Xie W, Kuryavyi V, Maguin P, Kao K, et al. 2021. The Card1 nuclease provides defence during type III CRISPR immunity. *Nature* 590:614–29
47. Lau RK, Ye Q, Birkholz EA, Berg KR, Patel L, et al. 2020. Structure and mechanism of a cyclic trinucleotide-activated bacterial endonuclease mediating bacteriophage immunity. *Mol. Cell* 77:723–33
48. Ye Q, Lau RK, Mathews IT, Birkholz EA, Watrous JD, et al. 2020. HORMA domain proteins and a Trip13-like ATPase regulate bacterial cGAS-like enzymes to mediate bacteriophage immunity. *Mol. Cell* 77:709–22.e7
49. Grüschow S, Adamson CS, White MF. 2021. Specificity and sensitivity of an RNA targeting type III CRISPR complex coupled with a NucC endonuclease effector. *Nucleic Acids Res.* 49(22):13122–34
50. Millman A, Melamed S, Amitai G, Sorek R. 2020. Diversity and classification of cyclic-oligonucleotide-based anti-phage signalling systems. *Nat. Microbiol.* 5:1608–15
51. McDonald ND, Regmi A, Morreale DP, Borowski JD, Boyd EF. 2019. CRISPR-Cas systems are present predominantly on mobile genetic elements in *Vibrio* species. *BMC Genom.* 20:105
52. Athukoralage JS, Rouillon C, Graham S, Grüschow S, White MF. 2018. Ring nucleases deactivate Type III CRISPR ribonucleases by degrading cyclic oligoadenylate. *Nature* 562:277–80
53. Molina R, Jensen ALG, Marchena-Hurtado J, López-Méndez B, Stella S, Montoya G. 2021. Structural basis of cyclic oligoadenylate degradation by ancillary Type III CRISPR-Cas ring nucleases. *Nucleic Acids Res.* 49:12577–90
54. Athukoralage JS, McMahon SA, Zhang C, Gruschow S, Graham S, et al. 2020. An anti-CRISPR viral ring nuclease subverts type III CRISPR immunity. *Nature* 577:572–75
55. Samolygo A, Athukoralage JS, Graham S, White MF. 2020. Fuse to defuse: a self-limiting ribonuclease-ring nuclease fusion for type III CRISPR defence. *Nucleic Acids Res.* 48:6149–56
56. Yan X, Guo W, Yuan YA. 2015. Crystal structures of CRISPR-associated Csx3 reveal a manganese-dependent deadenylation exoribonuclease. *RNA Biol.* 12:749–60
57. Athukoralage JS, McQuarrie S, Gruschow S, Graham S, Gloster TM, White MF. 2020. Tetramerisation of the CRISPR ring nuclease Crn3/Csx3 facilitates cyclic oligoadenylate cleavage. *eLife* 9:e57627
58. Makarova KS, Timinskas A, Wolf YI, Gussow AB, Siksnys V, et al. 2020. Evolutionary and functional classification of the CARF domain superfamily, key sensors in prokaryotic antivirus defense. *Nucleic Acids Res.* 48:8828–47
59. Athukoralage JS, Graham S, Gruschow S, Rouillon C, White MF. 2019. A type III CRISPR ancillary ribonuclease degrades its cyclic oligoadenylate activator. *J. Mol. Biol.* 431:2894–99
60. Jia N, Jones R, Yang G, Ouerfelli O, Patel DJ. 2019. CRISPR-Cas III-A Csm6 CARF domain is a ring nuclease triggering stepwise cA_4 cleavage with ApA>p formation terminating RNase activity. *Mol. Cell* 75:944–56.e6
61. Smalakyte D, Kazlauskiene M, Havelund JF, Ruksenaite A, Rimaite A, et al. 2020. Type III-A CRISPR-associated protein Csm6 degrades cyclic hexa-adenylate activator using both CARF and HEPN domains. *Nucleic Acids Res.* 48:9204–17
62. Garcia-Doval C, Schwede F, Berk C, Rostol JT, Niewoehner O, et al. 2020. Activation and self-inactivation mechanisms of the cyclic oligoadenylate-dependent CRISPR ribonuclease Csm6. *Nat. Commun.* 11:1596
63. Athukoralage JS, White MF. 2021. Cyclic oligoadenylate signalling and regulation by ring nucleases during type III CRISPR defence. *RNA* 27:855–67

64. Oke M, Carter LG, Johnson KA, Liu H, McMahon SA, et al. 2010. The Scottish Structural Proteomics Facility: targets, methods and outputs. *J. Struct. Funct. Genom.* 11:167–80
65. Wirth JF, Snyder JC, Hochstein RA, Ortmann AC, Willits DA, et al. 2011. Development of a genetic system for the archaeal virus *Sulfolobus* turreted icosahedral virus (STIV). *Virology* 415:6–11
66. Auchtung JM, Aleksanyan N, Bulku A, Berkmen MB. 2016. Biology of ICE*Bs1*, an integrative and conjugative element in *Bacillus subtilis*. *Plasmid* 86:14–25
67. Diner EJ, Burdette DL, Wilson SC, Monroe KM, Kellenberger CA, et al. 2013. The innate immune DNA sensor cGAS produces a noncanonical cyclic dinucleotide that activates human STING. *Cell Rep.* 3:1355–61
68. 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* 339:786–91
69. Cai X, Chiu YH, Chen ZJ. 2014. The cGAS-cGAMP-STING pathway of cytosolic DNA sensing and signaling. *Mol. Cell* 54:289–96
70. Whiteley AT, Eaglesham JB, de Oliveira Mann CC, Morehouse BR, Lowey B, et al. 2019. Bacterial cGAS-like enzymes synthesize diverse nucleotide signals. *Nature* 567:194–99
71. Cohen D, Melamed S, Millman A, Shulman G, Oppenheimer-Shaanan Y, et al. 2019. Cyclic GMP-AMP signalling protects bacteria against viral infection. *Nature* 574:691–95
72. Davies BW, Bogard RW, Young TS, Mekalanos JJ. 2012. Coordinated regulation of accessory genetic elements produces cyclic di-nucleotides for *V. cholerae* virulence. *Cell* 149:358–70
73. Burroughs AM, Zhang D, Schaffer 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.* 43:10633–54
74. Severin GB, Ramliden MS, Hawver LA, Wang K, Pell ME, et al. 2018. Direct activation of a phospholipase by cyclic GMP-AMP in El Tor *Vibrio cholerae*. *PNAS* 115:E6048–55
75. Lowey B, Whiteley AT, Keszei AFA, Morehouse BR, Mathews IT, et al. 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* 182:38–49.e17
76. 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):5039–51.e5
77. Morehouse BR, Govande AA, Millman A, Keszei AFA, Lowey B, et al. 2020. STING cyclic dinucleotide sensing originated in bacteria. *Nature* 586:429–33
78. Govande AA, Duncan-Lowey B, Eaglesham JB, Whiteley AT, Kranzusch PJ. 2021. Molecular basis of CD-NTase nucleotide selection in CBASS anti-phage defense. *Cell Rep.* 35:109206
79. Burroughs AM, Aravind L. 2020. Identification of uncharacterized components of prokaryotic immune systems and their diverse eukaryotic reformulations. *J. Bacteriol.* 202:e00365-20
80. Lopatina A, Tal N, Sorek R. 2020. Abortive infection: bacterial suicide as an antiviral immune strategy. *Annu. Rev. Virol.* 7:371–84
81. Tal N, Morehouse BR, Millman A, Stokar-Avihail A, Avraham C, et al. 2021. Cyclic CMP and cyclic UMP mediate bacterial immunity against phages. *Cell* 184:5728–39.e16
82. Seifert R. 2017. cCMP and cUMP across the tree of life: from cCMP and cUMP generators to cCMP- and cUMP-regulated cell functions. *Handb. Exp. Pharmacol.* 238:3–23
83. Doron S, Melamed S, Ofir G, Leavitt A, Lopatina A, et al. 2018. Systematic discovery of antiphage defense systems in the microbial pangenome. *Science* 359:eaar4120
84. Ofir G, Herbst E, Baroz M, Cohen D, Millman A, et al. 2021. Antiviral activity of bacterial TIR domains via immune signalling molecules. *Nature* 600:116–20
85. Ka D, Oh H, Park E, Kim JH, Bae E. 2020. Structural and functional evidence of bacterial antiphage protection by Thoeris defense system via NAD^+ degradation. *Nat. Commun.* 11:2816
86. 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.* 12:6381
87. Lahaye X, Satoh T, Gentili M, Cerboni S, Conrad C, et al. 2013. The capsids of HIV-1 and HIV-2 determine immune detection of the viral cDNA by the innate sensor cGAS in dendritic cells. *Immunity* 39:1132–42

88. Aguirre S, Luthra P, Sanchez-Aparicio MT, Maestre AM, Patel J, et al. 2017. Dengue virus NS2B protein targets cGAS for degradation and prevents mitochondrial DNA sensing during infection. *Nat. Microbiol.* 2:17037
89. Wu JJ, Li W, Shao Y, Avey D, Fu B, et al. 2015. Inhibition of cGAS DNA sensing by a herpesvirus virion protein. *Cell Host Microbe* 18:333–44
90. Huang ZF, Zou HM, Liao BW, Zhang HY, Yang Y, et al. 2018. Human cytomegalovirus protein UL31 inhibits DNA sensing of cGAS to mediate immune evasion. *Cell Host Microbe* 24:69–80.e4
91. Biolatti M, Dell'Oste V, Pautasso S, Gugliesi F, von Einem J, et al. 2018. Human cytomegalovirus tegument protein pp65 (pUL83) dampens type I interferon production by inactivating the DNA sensor cGAS without affecting STING. *J. Virol.* 92:e01774-17
92. Aguirre S, Maestre AM, Pagni S, Patel JR, Savage T, et al. 2012. DENV inhibits type I IFN production in infected cells by cleaving human STING. *PLOS Pathog.* 8:e1002934
93. Eaglesham JB, Pan YD, Kupper TS, Kranzusch PJ. 2019. Viral and metazoan poxins are cGAMP-specific nucleases that restrict cGAS-STING signalling. *Nature* 566:259–63
94. Bondy-Denomy J, Davidson AR, Doudna JA, Fineran PC, Maxwell KL, et al. 2018. A unified resource for tracking anti-CRISPR names. *CRISPR J.* 1:304–5
95. Ablasser A, Schmid-Burgk JL, Hemmerling I, Horvath GL, Schmidt T, et al. 2013. Cell intrinsic immunity spreads to bystander cells via the intercellular transfer of cGAMP. *Nature* 503:530–34
96. Woodward JJ, Iavarone AT, Portnoy DA. 2010. c-di-AMP secreted by intracellular *Listeria monocytogenes* activates a host type I interferon response. *Science* 328:1703–5
97. Bridgeman A, Maelfait J, Davenne T, Partridge T, Peng Y, et al. 2015. Viruses transfer the antiviral second messenger cGAMP between cells. *Science* 349:1228–32
98. Gentili M, Kowal J, Tkach M, Satoh T, Lahaye X, et al. 2015. Transmission of innate immune signaling by packaging of cGAMP in viral particles. *Science* 349:1232–36
99. Jumper J, Evans R, Pritzel A, Green T, Figurnov M, et al. 2021. Highly accurate protein structure prediction with AlphaFold. *Nature* 596:583–89
100. Mirdita M, Schütze K, Moriwaki Y, Heo L, Ovchinnikov S, Steinegger M. 2021. ColabFold—making protein folding accessible to all. bioRxiv 2021.08.15.456425. **https://doi.org/10.1101/2021.08.15.456425**

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