Abortive Infection

Abortive Infection: Bacterial Suicide as an Antiviral Immune Strategy

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Abstract

Facing frequent phage challenges, bacteria have evolved numerous mechanisms to resist phage infection. A commonly used phage resistance strategy is abortive infection, in which the infected cell commits suicide before the phage could complete its replication cycle. Abortive infection prevents the phage epidemic from spreading to nearby cells, thus protecting the bacterial colony. The abortive infection strategy is manifested by a plethora of mechanistically diverse defense systems that are abundant in bacterial genomes. In turn, phages have developed equally diverse mechanisms to overcome bacterial abortive infection. This review summarizes the current knowledge on bacterial defense via cell suicide. It describes the principles of abortive infection, details how these principles are implemented in a variety of natural defense systems, and discusses phage counter-defense mechanisms.

INTRODUCTION

Bacteria encode multiple lines of defense against viruses that infect them, termed phages. These include defense systems with well understood mechanism of action, for example restriction-modification and CRISPR-Cas, as well as many recently discovered systems with unknown modes of action (for recent reviews see (1–3)). In general, bacteria are known to resist phage infections by mutating or altering their surface receptors (4), by targeting the phage nucleic acids (3), by producing small molecules that poison phage replication (5), or by committing suicide upon phage infection. The latter mode of protection, termed abortive infection (or Abi for short), is the topic of this review article.

The term “abortive infection” in the context of nonproductive phage replication appeared in the literature already in the 1950s. However, only in the 1980s the term became widely adopted

to describe a mode of bacterial defense in which the infection process is inhibited at a relatively late stage of the infection cycle and the bacterial cells are killed in the process (6). Over the years, many bacterial defense systems that have been discovered were tagged as Abi systems; however, in many cases (especially in systems detected in *Lactococci*), cell suicide or growth arrest was not explicitly demonstrated. In this review, we primarily describe defense by abortive infection in the strict sense, meaning that we mainly discuss defense systems in which cell suicide or growth impairment clearly plays a major role in the mechanism of defense. In some cases, Abi systems are known to have bacteriostatic effects (inflicting metabolic arrest) rather than bactericidal effects (killing the cell); we also describe such systems in the current review, because the lines between metabolic arrest and cell death are sometimes blurry, and because prolonged bacteriostasis can eventually result in cell death (7).

OPERATIONAL PRINCIPLES OF ABORTIVE INFECTION

Abortive infection is not a "defense system" per se; it is rather an immune strategy that is manifested in many different kinds of defense systems encoded by bacteria. The core principle of this strategy is that a bacterial cell, after sensing the infection, commits suicide before the phage can complete its replication cycle. This ensures that no mature phage particles emerge from the infected cell, so that the phage epidemic cannot spread to infect nearby cells, and the colony ultimately survives (**Figure 1**). Abortive infection can be regarded as an "altruistic" trait, as one cell sacrifices itself to benefit the community. However, considering that bacteria frequently live in colonies of isogenic or almost isogenic cells, and since phages are often highly specific and infect just one species or subspecies of bacteria, this altruism is expected to primarily protect only the very closely related kin (8–10).

Because defense systems such as CRISPR-Cas and restriction-modification can mitigate phage infection without killing the infected cells, abortive infection makes biological sense mainly when the first lines of defense have failed and chances of survival of the infected cell are anyway low. Therefore, whereas restriction enzymes target the phage DNA at very early stages of the infection, Abi systems are expected to become activated only when the phage reached the middle or the late stages of its infection cycle (3, 6, 11). In this scenario, abortive infection systems will not lead to cell death if the cell overcame the infection by other means at some

earlier stages; but if the first lines of defense have failed and the phage proceeded into advanced stages of its replication cycle, abortive infection is activated as a defense of last resort.

Every abortive infection system must contain at least two functional modules: one that senses the phage infection, and one that kills the cell or shuts down metabolism once the phage has been sensed. Abi systems can sense intermediates of phage genome replication (12), structural phage proteins that are sensed during their production within the cell (13, 14), or other phage proteins expressed in the cell during infection (15, 16). Abi systems can also sense extensive transcription from phage DNA (17, 18) or phage-mediated shutoff of host gene expression (19); and in some cases, an Abi system is activated only when it senses that a phage has inhibited another, non-Abi defense system (12).

Once phage infection has been sensed, the cell-killing module becomes activated. This module must be tightly suppressed before infection so that it would not impair bacterial growth under normal conditions (1). When activated, the module must function relatively rapidly as it must cause cell death or metabolic arrest before new phage particles mature in the infected cell. Abi systems can lead to cell death by degrading (20) or depolarizing (16, 21, 22) the inner membrane, or by indiscriminately degrading phage and host DNA (23). Other Abi systems, when activated, degrade tRNAs (24), cleave essential proteins in the host translation machinery (25), or indiscriminately degrade phage and host mRNAs (17, 18, 26, 27). In these cases, the host cell enters an immediate growth arrest that can result in eventual cell death but is sometimes reversible (28). It was also suggested that temporary growth arrest can "buy time" for other defense systems, such as restriction enzymes and CRISPR-Cas, to inactivate the phage (1, 29).

The following sections describe individual examples of abortive infection systems in which at least one of the modules (phage-sensing or cell-killing) is understood. We describe "classical" Abi systems that were discovered decades ago, and expand on recently discovered systems, including CBASS and type III CRISPR-Cas systems, that utilize small molecule signaling as a way to transmit the information between the phage-sensing and the cell-killing modules. We also describe a variety of mechanisms by which phages can evade bacterial Abi systems.

DIVERSITY OF ABORTIVE INFECTION SYSTEMS IN BACTERIA

For many years, *Escherichia coli* has served as the most widely used model organism to study phage infection, and so most of the mechanistic understandings of Abi systems were achieved in

E. coli model systems (30). *Lactococcus lactis* has also been extensively studied for its capacity to restrict phage infections, and in fact, more than 20 different defense genes or systems were reported in that bacterium as Abi systems (designated AbiA to AbiZ) (11, 31–33). However, to our knowledge only for a small minority of these (for example AbiZ described in more detail below) the mechanism of phage sensing and/or cell killing is understood.

One of the first abortive infection systems to be deciphered in *E. coli* was **Rex** (3, 34). The Rex system is expressed from a repressed lambda prophage and comprises two genes, *rexA* and *rexB*, both of which are required for the defensive activity (35, 36). The system was shown to restrict plaque formation of several lambdoid phages, as well as particular strains of T4, T7, and T5 phages (37). The RexA protein is thought to sense a protein-DNA complex that may be produced as an intermediate of phage replication or recombination (12). Two copies of RexA then activate one copy of RexB, a membrane-anchored protein containing four transmembrane helices (38). When activated, RexB forms an ion channel in the inner membrane, resulting in a severe loss of membrane potential and a consequent drop in cellular ATP levels; this inhibits bacterial growth and aborts phage infection (**Figure 2*A***). Some studies have suggested that RexB activation results in cell death (38), while other studies proposed that Rex toxicity induces a stationary phase-like state from which some cells can recover after viral infection is mitigated (28). As part of the arms race of bacterial defense and phage counter-attacks, phages have evolved ways to overcome the Rex system. For example, the wild-type T4 phage encodes two proteins (RIIA and RIIB) that mitigate the activity of the Rex system via an unknown mechanism, and hence only mutant T4 strains in which the rII locus is inactivated are blocked by the Rex system (39).

The abortive infection gene **AbiZ**, which protects *L. lactis* against the infection of phage phi31, also induces cell-killing by damaging the cell membrane of the infected cell (16). This membrane-bound protein acts cooperatively with the lysin and holin proteins of the phage that are expressed late in the infection cycle and accelerates cell lysis (**Figure 2*A***). Premature cell lysis causes the infected cells to burst and to release unassembled phage particles that are not capable of infecting further cells (16).

Another Abi system that was described in *E. coli* is **PifA**, which aborts the infection of phage T7 midway through its infection cycle. While the initial steps of T7 infection occur normally, late gene transcription is severely reduced and phage DNA replication is abolished. Activation of

PifA leads to leakage of ATP through loss of membrane integrity and, although the infected cells do not lyse, they die (22). The *pifA* gene, encoded on the F plasmid, is responsible for the abortive infection phenotype (40). This gene is coexpressed on the same operon with *pifC*, a transcriptional repressor that inhibits its own expression as well as the expression of *pifA* (41), a concept that was later referred to as "type IV toxin-antitoxin" and is also manifested by the abortive infection system AbiE (42). The PifA protein was found to be associated with the membrane, although no transmembrane helices can be detected in the protein sequence, and it contains an ATP/GTP binding domain essential for its defensive activity (40). The T7 capsid protein gp10 is the trigger for activation of PifA toxicity (13), but the exact mode by which PifA senses this protein is unknown. Another T7 protein that induces the toxicity of PifA is gp1.2, an inhibitor of deoxynucleotide hydrolysis in the infected cell (13). Accordingly, T7 phages carrying mutations both in *gp1.2* and *gp10* genes escape the PifA-mediated defense (42a).

Several abortive infection systems, including **Lit** and **PrrC,** have been shown to induce cell death by inactivating the host translation machinery. Lit is a protease that is encoded by the defective e14 prophage of *E. coli* K12 (43). Its name, an acronym for late inhibition of T4, stems from its original identification as a protein that inhibits T4 late gene expression (44). The Lit protease is activated when gp23, the major head protein of phage T4, is expressed in the cell (25). This phage head protein binds the translation elongation factor EF-Tu and this complex is identified by Lit (14). Once activated, Lit cleaves EF-Tu between Gly^{59} and Ile^{60} in the conserved nucleotide-binding domain of EF-Tu (44a), thus inhibiting cellular protein translation and arresting bacterial growth (14). Mutations in T4 gp23 that resulted in escape from Lit localized the domain identified by Lit to a short sequence (29 amino acids) denoted Gol (for grow on lit-producing bacteria) (45) that is highly conserved in head proteins of T-even phages (46).

PrrC is a unique abortive infection gene that is activated as a secondary line of defense, only when the first defensive lines have collapsed (**Figure 2*B***). The *prrC* gene was found to be encoded by the clinical *E. coli* strain CT196 (47, 48). Under normal conditions, PrrC binds the type I restriction endonuclease *Eco*prrI and does not interfere with its endonuclease activity (24, 49). Rather, PrrC monitors the normal activity of the restriction enzyme and becomes activated only when the restriction enzyme is tempered with. Specifically, phage T4 encodes a short peptide called Stp, which binds and inhibits the *Eco*prrI restriction enzyme (50). Inhibition of

*Eco*prrI by Stp activates a ribonuclease domain in PrrC, and activated PrrC then cleaves $tRNA^{lys}$ causing protein synthesis to halt (51). The T4 phage encodes a mechanism that enables the re-ligation of $tRNA^{lys}$, and it is thought that this mechanism (manifested by two enzymes: polynucleotide kinase and RNA ligase) has evolved to overcome PrrC defense (52). Therefore, PrrC defends, through abortive infection, only against T4 phages in which either the polynucleotide kinase or the RNA ligase have been inactivated (53).

A recently discovered Abi gene was shown to protect species of *Staphylococcus* against *Siphoviridae* phages via phosphorylation of cellular proteins (15). The kinase gene, **Stk2**, is activated by a phage protein called PacK, which was hypothesized to play a role in phage DNA packaging (**Figure 2*C***). Once activated, Stk2 phosphorylates multiple proteins in the cell, eventually leading to cell death (15).

TOXIN-ANTITOXIN SYSTEMS AND ABORTIVE INFECTION

A toxin-antitoxin (TA) system comprises a pair of genes, usually transcribed from the same operon, one of which is toxic and the second confers immunity to this toxicity (54). TA systems have been divided into six types, according to the nature of the toxin and antitoxin molecules and the mode of immunity regulation. The most common type is type II TA systems, where both the toxin and the antitoxin are proteins, and the antitoxin physically binds to the toxin and prevents its toxic activity (55). According to the paradigm of type II TA systems mode of action, the antitoxin is less stable than the toxin and is more prone to be degraded by cellular proteases. When the cell enters conditions of stress and protein production halts, the antitoxin is rapidly degraded and the stable toxin is no longer suppressed (56).

Type II TA systems are extremely abundant in microbial genomes, with some genomes encoding more than 80 such systems (57). They have been shown to play roles in bacterial responses to various stress conditions (58), confer resistance to antibiotics via persistence (59), take part in biofilm formation regulation (60), and plasmid maintenance (61). TA systems have also been proposed to play a role in phage defense via abortive infection (1, 3), but evidence for such roles are limited to very few well-established examples, and a general role for TA systems in phage defense is still under dispute (62).

RnlAB (also called RNase LS) is a type II TA system encoded by *E. coli* K12, which protects against certain strains of phage T4 via abortive infection (19) (**Figure 2*D***). RnlA is an

endoribonuclease toxin that is inhibited by direct interaction with its cognate antitoxin, RnlB. While the half-life of the RnlA toxin is more than 25 minutes, the half-life of RnlB is much shorter, estimated to be ~2 minutes due to rapid proteolysis by *E. coli* housekeeping proteases (19). Because T4 infection rapidly shuts off host gene expression (63), the unstable antitoxin RnlB gets degraded soon upon phage infection, releasing RnlA to become an active endoribonuclease that indiscriminately degrades phage and host mRNAs, presumably leading to cell death (26, 64). A homologous system called LsoAB encoded on a plasmid of *E. coli* strain O157:H7 shows similar antiphage functionality (26). Interestingly, the wild-type T4 phage encodes a gene called *dmd* that can overcome defense by RnlAB. This gene is expressed early in infection and encodes an antitoxin mimic that binds and neutralizes the RnlA toxin (26). In accordance with this, RnlAB provides resistance only against T4 phage strains that are mutated in *dmd*.

Another example of TA-mediated abortive infection is the **ToxIN** system**,** which was shown to provide phage resistance in various enteric bacteria, including *Erwinia carotovora, Serratia marcescens* and *E. coli* (27). ToxIN (originally identified as AbiQ of *L. lactis* (65)) is a type III TA system where the antitoxin (ToxI) is a noncoding RNA that physically binds the ToxN toxin protein and inhibits its activity (66). In uninfected cells ToxI and ToxN are found as an inactive RNA:protein complex comprised of three ToxI RNAs and three ToxN proteins (66). ToxN is an endoribonuclease, and upon phage infection it becomes activated and presumably cleaves cellular and phage RNA, stopping phage production and causing bacteriostasis or cell death (27). Homologs of *toxN* are found in the genomes of a wide range of Gram-positive and Gram-negative bacteria, with most of the *toxN*-encoding loci located on plasmids, suggesting that these defense systems spread in bacteria via extensive horizontal gene transfer (27). Interestingly, some phages escape ToxIN by expressing a noncoding RNA that mimics the ToxI antitoxin and inhibits ToxN toxicity (67).

CBASS: CYCLIC-OLIGONUCLEOTIDE-BASED ANTIPHAGE SIGNALING SYSTEMS

Recently, a large new family of abortive infection systems, collectively called **CBASS**, has been described (20). In these systems the phage-sensing module is disconnected from the cell-killing module and the proteins that encode these two functions do not physically interact. Rather, when

the phage-sensing protein identifies phage infection, it produces a small secondary messenger molecule comprised of two or three nucleotides covalently linked to form a cyclic molecule (cyclic di-nucleotide or cyclic tri-nucleotide), and this secondary messenger molecule activates the cell-killing "effector" protein that carries out the abortive infection (20) (**Figure 3**). Accordingly, these systems were denoted cyclic-oligonucleotide-based antiphage signaling systems – or CBASS for short (20).

The most extensively studied CBASSs to date are two homologous systems encoded by *Vibrio cholerae* El Tor and *E. coli* TW11681. When cloned into a lab strain of *E. coli* that lacks CBASS these systems protected against a wide variety of phages (20). The key components of the system are a protein called DncV that can produce cyclic GMP-AMP molecules (cGAMP) (68), and CapV, which is a phospholipase activated by cGAMP (69). The cGAMP-producing DncV protein is inactive in uninfected cells, but infection by phage triggers the production of cGAMP that accumulates to micromolar-level concentrations in the cell. The cGAMP then binds and activates the cognate phospholipase protein, which degrades the inner membrane. leading to cell lysis and death (20).

The activity of CBASS was shown to be temporally coordinated so that, on one hand, cell death occurs before the phage can generate mature particles, and, on the other hand, the cell commits to death only after the phage has advanced to late stages in its replicative cycle. When infected by phage P1, cells encoding the *E. coli* TW11681-derived CBASS began generating the secondary messenger cGAMP 30–40 minutes after the initial infection, and culture lysis due to CapV phospholipase activity was observed a few minutes after that (20). Considering that P1-infected CBASS-lacking cultures lyse after 60–70 minutes due to phage-induced lysis, the CBASS system seems to become active only when the phage has temporally completed about half of its replicative cycle. It was shown that cell lysis by CBASS does not release mature infective particles, because a CBASS-encoding culture in which only 20% of bacteria were initially infected does not undergo collapse (20). The phage component sensed by the *E. coli* TW11681 CBASS is currently unknown.

The bacterial CBASS shows structural and functional homology with the cGAS-STING antiviral pathway of animal cells. CBASS systems were identified in more than 10% of sequenced bacterial and archaeal genomes and span a remarkable diversity of oligonucleotide cyclase and effector proteins activities (20, 70). Oligonucleotide cyclase proteins in different

CBASSs can produce a variety of cyclic oligonucleotide signals in addition to cGAMP, including cyclic AMP-UMP, cyclic UMP-UMP, cyclic AMP-AMP-GMP, and others (70). The cell-killing domain in the effector gene also varies, with effectors including, in addition to phospholipases, also endonucleases that can indiscriminately degrade DNA (23, 71), transmembrane domains that are thought to form membrane-spanning ion channels (20, 72), and other domains whose mechanisms of cell killing is unknown. In many cases CBASS operons contain, in addition to the two core genes, also ancillary genes of unknown function that were shown to be necessary for protection against some (but not all) of the phages (20).

The abundance and diversity of CBASS in microbes suggests that transferring the information on phage-sensing via a secondary messenger mediator is a successful strategy. The enzymatic activity of the oligonucleotide cyclase rapidly amplifies the signal when the phage is sensed, so that in principle a single sensor can activate multiple cell-killing effectors. Presumably, this accelerates the process and shortens the time that elapses from phage sensing to the execution of cell-killing, reducing the chances that the phage would complete its replication during that time.

ABORTIVE INFECTION IN CRISPR-CAS DEFENSE

CRISPR-Cas is the adaptive immune system of bacteria and archaea, capable of acquiring short pieces of phage DNA and storing them as "spacers" between repetitive sequences to form an immune memory (73–75). These spacers are subsequently expressed and processed into short RNAs (crRNAs) that become complexed with CRISPR-associated (Cas) proteins, forming a complex that searches for and destroys phage nucleic acids that base pair with the crRNA (73–75).

Type I CRISPR-Cas systems, which are the most abundant CRISPR-Cas systems in nature (76), and type II systems, which include Cas9, both degrade phage DNA and are capable of abolishing the infection without necessitating cell death. However, recent findings show that type III CRISPR-Cas systems can sometimes lead to abortive infection (17, 18). In these systems, the crRNA-Cas complex binds to phage RNA rather than DNA. The phage RNA is recognized during active transcription from the phage genome (77) and then cleaved by the Csm3 (in CRISPR-Cas type IIIA) or Cmr4 (type IIIB) subunit of the crRNA/protein complex. At the same time, another component of the complex (Cas10) cleaves the DNA from which the RNA was

transcribed (78, 79). It was recently shown that recognition and cleavage of phage RNA induces a third enzymatic activity by the PALM domain of Cas10 (17, 18). Once the phage RNA is identified by base-pairing with the crRNA, Cas10 synthesizes a cyclic oligoadenylate (cOA) small-molecule secondary messenger comprised of four or six adenosine monophosphate (AMP) molecules covalently bound to each other in a cyclic configuration (17, 18) (**Figure 4**). The cOA molecules then bind and activate an effector RNase called Csm6 that indiscriminately degrades both phage and host RNAs, presumably leading to cell dormancy and sometimes death (17, 18).

There is a clear biological rational in the mode of defense employed by type III CRISPR-Cas systems. These systems become activated only if the first lines of defense (for example type I or type II CRISPR-Cas, or restriction enzymes) have failed to inactivate the phage by degrading its DNA, because transcription from the phage DNA is a signature for active propagation of the phage infection process. When phage transcription is recognized, the type III CRISPR-Cas system attempts to inactivate it by cleaving both the transcribed RNA and the phage DNA. During that time, it produces a limited amount of the cOA signal. Presumably, a limited amount of cOA molecules produced by a single recognition event will not be sufficient to induce full-fledged toxicity by Csm6; but if multiple phage loci are identified by the type III CRISPR-Cas system, it would mean that the phage genome has already managed to replicate and phage gene expression is abundant. In this case, the chances that the cell would eventually survive are poor, and accumulation of a high concentration of cOA in the cell will maximally activate Csm6 presumably leading to cell death (80). Interestingly, recent findings show that bacteria encode enzymes that can hydrolyze cOA molecules; these enzymes can clear the cell from residual cOAs in case it was able to survive the phage infection, and resuscitate it from Csm6-induced dormancy (81, 82).

Abortive infection was also suggested to be the outcome of type VI CRISPR-Cas systems (83). The effector protein in these systems is Cas13, which uses crRNAs to locate phage RNA. Base-pairing with phage RNA results in cleavage of the RNA, but also induces a nonspecific RNase activity of Cas13 that cleaves both cell and phage mRNAs, resulting in dormancy (83, 84).

SUMMARY AND OUTLOOK

This essay reviews abortive infection systems that have been discovered and studied over a period of more than four decades. As multiple excellent reviews have covered early discoveries on abortive infection systems in depth (3, 32, 84a), we naturally expanded the discussion on the newer discoveries from the past few years. As indicated above, we have not enumerated all known defense systems that have been described as abortive infection systems, but rather focused on in-depth description of systems that comply with the strict definition, i.e., in which cell death or dormancy was clearly attributed to result from the activity of the defense system. We also attempted to focus on Abi systems in which at least part of the mechanism has been elucidated.

While the mechanistic diversity of abortive infection systems has been recognized for several decades, their abundance in nature was not fully appreciated. The recent discovery of CBASS and type III CRISPR-Cas systems as widespread and abundant abortive infection systems suggests that cell suicide is an immunological strategy that may be much more abundant in bacteria and archaea than previously realized. Historically, Abi was studied mainly in *E. coli* and *Lactococci*. With the huge expansion of genomic information available today, studies focusing on defense systems in non-model organisms are likely to reveal many additional systems that rely on suicide of the infected cell for the benefit of colony survival. Indeed, multiple new anti-phage systems found to be encoded by prophages in *Mycobacterium* and *Gordonia* were suggested to function via abortive infection (84b, 84c, 84d). Future studies on these systems, as well as on other defense systems encoded in bacteria, may result in an additional wave of discoveries that would expand the already fascinating diversity of known abortive infection mechanisms.

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FIGURE LEGENDS

Figure 1 Key principles of the abortive infection strategy. A. Infection of a culture that does not encode an abortive infection system. B. Infection of a culture that encodes an abortive infection (Abi) system.

Figure 2 Mechanisms of action of a selected set of phage abortive infection systems. (A) The RexAB and AbiZ systems target bacterial cell membranes. RexA senses a phage protein-DNA complex formed as an intermediate of phage replication, and activates RexB, which forms the ion channel in the membrane. AbiZ recognizes the holins and lysins of the phage and cooperatively acts with them to induce premature cell lysis. (B) PrrC is a protein that monitors the normal activity of the type I restriction enzyme EcoprrI. PrrC in its latent form binds to the EcoprrI protein complex. The T4 phage protein Stp binds EcoprrI and inhibits it, leading to the activation of PrrC. Activated PrrC cleaves $tRNA^{lys}$ causing protein synthesis to halt and phage infection to be terminated. (C) The Stk2 kinase is activated by the PacK phage protein. Activated Stk2 kinase phosphorylates multiple cell proteins eventually leading to cell death and termination of phage infection. (D) RnlAB is a type II toxin-antitoxin system consisting of the endoribonuclease toxin RnlA and its cognate antitoxin RnlB. During T4 phage infection, the host protein synthesis is shut off, and because the antitoxin is unstable it gets rapidly degraded. This releases the RnlA toxin, which indiscriminately cleaves host and phage RNA.

Figure 3 The CBASS abortive infection system. The system is composed of an oligonucleotide cyclase and an effector gene. The oligonucleotide cyclase (in the current figure – cGAS: cyclic GMP-AMP synthase) is activated during phage infection by an unknown phage component. Production of the cyclic oligonucleotide activates a cell-killing effector protein, which (depending on the CBASS type) can be a phospholipase, a nuclease, or a membrane-spanning protein.

Figure 4 The mechanism of abortive infection by type III CRISPR-Cas systems. The crRNA-Cas complex binds to phage RNA during its active transcription from phage DNA. The crRNA-Cas complex cleaves both the phage RNA and the DNA from which it was transcribed. At the same time, it produces cyclic oligoadenylate (cOA) molecules. The cOA molecules bind and activate the nonspecific RNase Csm6, which subsequently indiscriminately degrades phage and host RNA.

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