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Bacterial defense systems: Mechanisms, homology to eukaryotic immune systems, and applications

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ABSTRACT

Bacteria, as fundamental members of the microbial biosphere, are under constant threat from mobile genetic elements (MGEs), including bacteriophages and plasmids. In response, diverse molecular defense strategies have emerged to detect, neutralize, and eliminate these invaders. This review provides a comprehensive framework for categorizing known bacterial defense systems based on mechanistic principles and modes of action, alongside a synthesis of core operational themes that underlie their functional diversity. Particular attention is given to evolutionary parallels between bacterial immunity and antiviral mechanisms in eukaryotes, with emphasis on conserved molecular modules that reflect a convergent logic of immune defense across biological domains. Biotechnology platforms harnessing bacterial defense systems are also examined, with a focus on their application across practical domains, including genetic enhancement of crops and livestock, development of molecular diagnostics and precision therapies, and optimization of microbial strains for industrial fermentation. By integrating mechanistic architecture, evolutionary relationships, and translational advances into a coherent framework, this review broadens the conceptual understanding of bacterial immunity and lays the groundwork for its expanded application in both research and biotechnological innovation.

Keywords: Phage; Bacteria; Defense system; Antiphage mechanism; Homology

INTRODUCTION

Bacteria in natural environments experience persistent parasitic pressure from mobile genetic elements (MGEs), including bacteriophages and plasmids, whose invasion can compromise cellular function and frequently results in cell death (Mayo-Muñoz et al., 2023). Among these elements, bacteriophages constitute the dominant threat and are estimated to account for 20%–40% of daily bacterial mortality (Fortier & Sekulovic, 2013; Suttle, 2007). Sustained exposure to such intense selective forces has driven the evolution of a wide spectrum of molecular defense strategies that intercept MGEs at distinct stages of infection. Collectively, these mechanisms form the diverse repertoire referred to as bacterial defense systems.

Systematic investigation of bacterial defense emerged in the mid-twentieth century, following the discovery of bacteriophages (d'Herelle, 1917; Twort, 1915) and early interest in phage-based therapies. Subsequent observations revealed that bacterial populations could acquire resistance to phage infection (Davison, 1922; Muckenfuss & Korb, 1928), a phenomenon later shown to extend beyond alterations of cell surface receptors (Henry & Henry, 1946) and instead rely on intracellular inhibitory processes (Echols, 1971; Hunter, 1947; Kaiser, 1957). These findings established the conceptual foundation of bacterial immunity. Over subsequent decades, multiple defensive strategies were elucidated, including restriction-modification (RM) systems (Arber & Dussoix, 1962; Glover et al., 1963; Lederberg, 1957) and abortive infection (Abi) pathways (Lindahl et al., 1970; Parma et al., 1992), revealing that bacterial populations deploy both self-preserving and population-level protective responses. A pivotal advance occurred with the identification of clustered palindromic repeat sequences in the *Escherichia coli* genome (Ishino et al., 1987), later recognized as Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) arrays, which ultimately enabled the development of transformative molecular tools such as CRISPR-CRISPR-associated proteins

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(CRISPR-Cas) 9 (Jinek et al., 2012).

Recent advances in high-throughput sequencing and bioinformatics have transformed the field from isolated characterization of individual systems to systematic discovery at scale. The introduction of the “defense island” concept (Makarova et al., 2011), coupled with genomic neighborhood and “guilt-by-association” strategies, has enabled efficient prediction of previously unrecognized defense loci (Doron et al., 2018; Gao et al., 2020). Experimental validation of many such candidates has confirmed widespread antiphage activity, exemplified by systems such as Hachiman, Shedu, and Gabija (Néron et al., 2023; Tesson et al., 2022, 2024).

This review establishes a conceptual, mechanism-centered framework that organizes known bacterial defense systems according to functional principles and modes of action, broadly distinguishing passive barriers from active effector-driven responses. Building on this framework, this review examines the striking parallels between bacterial defense systems and eukaryotic antiviral immunity from a comparative and evolutionary perspective. Comparative genomic analyses have uncovered homologous effector architectures and conserved molecular modules shared across domains of life (Makarova et al., 2009; Van Den Berg et al., 2024), indicating convergent or deeply rooted evolutionary solutions to viral threat and revealing common logic underlying immune defense. These observations provide new insight into the evolutionary trajectories of antiviral strategies and identify shared molecular foundations with relevance beyond bacterial systems. Beyond theoretical interest, bacterial defense systems have generated substantial translational impact. Defense-derived molecular architectures have been adapted into versatile biotechnological platforms, including programmable genome editing systems and sensitive nucleic acid detection technologies. This review also discusses the application of these technologies across key sectors, encompassing genetic improvement of crops and livestock, development of diagnostic and therapeutic strategies for cancer and infectious disease, and microbial strain engineering for industrial fermentation (Baltz, 2018; Laurent et al., 2024; Qian et al., 2023). By integrating mechanistic diversity, evolutionary context, and applied innovation into a unified framework, this review advances understanding of bacterial defense systems and highlights their expanding relevance across basic biology and biotechnology.

MECHANISM-BASED FRAMEWORK OF DEFENSE SYSTEMS

Bacterial defense systems can be systematically analyzed along three mechanistic axes that define their operation: the trigger axis, which describes how the presence of MGEs is sensed and how this signal is relayed to downstream effectors; the effector axis, which encompasses the molecular strategies used to neutralize the threat; and the outcome axis, which characterizes the physiological consequences for the host following system activation. Within this tripartite framework, defense mechanisms are broadly distinguished as either active or passive, based primarily on their effector function and host impact, spanning the spectrum from targeted neutralization to self-destructive containment.

Active defense systems execute targeted, antagonistic responses against invading MGEs. These systems rely on effectors capable of precise recognition and selective inactivation of foreign genetic material, typically preserving host viability and representing a relatively low-risk defense strategy.

In contrast, passive defense systems adopt non-aggressive, non-antagonistic strategies that prioritize population-level protection over individual survival. These systems do not directly engage MGEs but instead limit their propagation through abortive infection (Abi) (Lopatina et al., 2020), a host self-destructive process that is activated upon detection of phage invasion. In this context, the infected cell initiates programmed death, effectively terminating phage replication and safeguarding the broader population. Another well-characterized passive mechanism is superinfection exclusion (SIE), which likewise acts in a non-antagonistic manner, preventing phage entry through mechanisms such as receptor modification or adsorption inhibition (Taylor et al., 2019b).

While the active-passive dichotomy provides an intuitive conceptual framework, mechanistic boundaries can be blurred. Certain active systems, such as DRT9 (Song et al., 2025), limit host damage as a side effect of defense. However, this damage is tightly regulated and remains sublethal due to intrinsic regulatory mechanisms that generally restrict effector activity. Such cases should not be conflated with passive strategies, as the host-directed injury is neither indiscriminate nor essential to defense. Conversely, some passive systems exhibit seemingly active features. For instance, CmdTAC (Vassallo et al., 2024) displays seemingly active behavior by attacking invading MGEs yet lacks discrimination between self and non-self and fails to restrain effector activity once triggered. The ensuing cell death is not incidental but integral to their defense strategy, with MGE damage occurring as a result of programmed self-destruction rather than from a regulated or targeted attack. A further level of complexity arises in multilayered systems that exhibit stage-dependent switching between active and passive behaviors. A prominent example is the type III CRISPR-Cas system (Mayo-Muñoz et al., 2022), which functions as an active defense under manageable invasion loads by degrading foreign nucleic acids and preserving host viability. However, when the invader burden exceeds the clearance threshold, secondary pathways may be triggered, leading to dormancy or cell death, effectively transitioning the system to a passive protective state.

Beyond the primary classification, additional subgroups can be delineated based on dominant mechanistic features expressed along different conceptual axes—trigger, effector, or outcome. However, the rapid discovery of novel bacterial defense strategies and growing recognition of their molecular complexity make it impossible for a single conceptual axis to fully capture all aspects of a system. As a result, many systems exhibit overlapping characteristics when viewed from alternative mechanistic perspectives. This inherent complexity has prompted recent reviews to adopt diverse classification strategies emphasizing different operational dimensions (Georjon & Bernheim, 2023; Hobbs & Kranzusch, 2024; Laub & Typas, 2024). Therefore, the framework proposed here is not intended as a rigid or definitive taxonomy, but rather as a flexible scaffold for conceptualizing the diversity of bacterial immune strategies. It provides an intuitive, structured lens through which to examine the interplay between sensing, effector deployment, and physiological outcome, offering a coherent foundation for understanding how bacteria deploy multilayered defenses to limit MGE propagation.

Active defense

To prevent damage caused by MGEs, bacteria deploy active defense systems that target and eliminate invading nucleic acids before deleterious effects arise. These mechanisms function as the first line of defense, preserving genomic integrity and enabling targeted elimination of exogenous DNA. Active defense strategies can be classified into five functional subgroups based on their specific modes of action (Figure 1), namely modification-based defense systems, acquired immune systems, chemical defense systems, nucleotide contamination-based systems, and additional systems with unique functional features.

Modification-based defense systems: Nucleic acid modification serves as a widespread molecular signature enabling discrimination between self- and non-self-DNA. These systems typically mark host DNA through covalent modifications, followed by effector-mediated elimination of unmodified, non-self-DNA. The classical example is the RM system, as well as newly discovered variants that share discriminatory nucleic acid modification as their central defensive mechanism.

RM systems (Table 1) are nearly ubiquitous, present in approximately 90% of bacterial genomes (Oliveira et al., 2014), and are composed of DNA methyltransferases (MTases) that label host DNA and restriction endonucleases (REases) that degrade unmodified DNA, thereby achieving self-/non-self-discrimination and defense against invaders. Four major types have been defined based on subunit architecture and enzymatic function (Tock & Dryden, 2005). Type I systems form ATP-dependent oligomeric complexes of REase and MTase that cleave DNA at variable, often distal, distances from the recognition motif. Type II systems encode

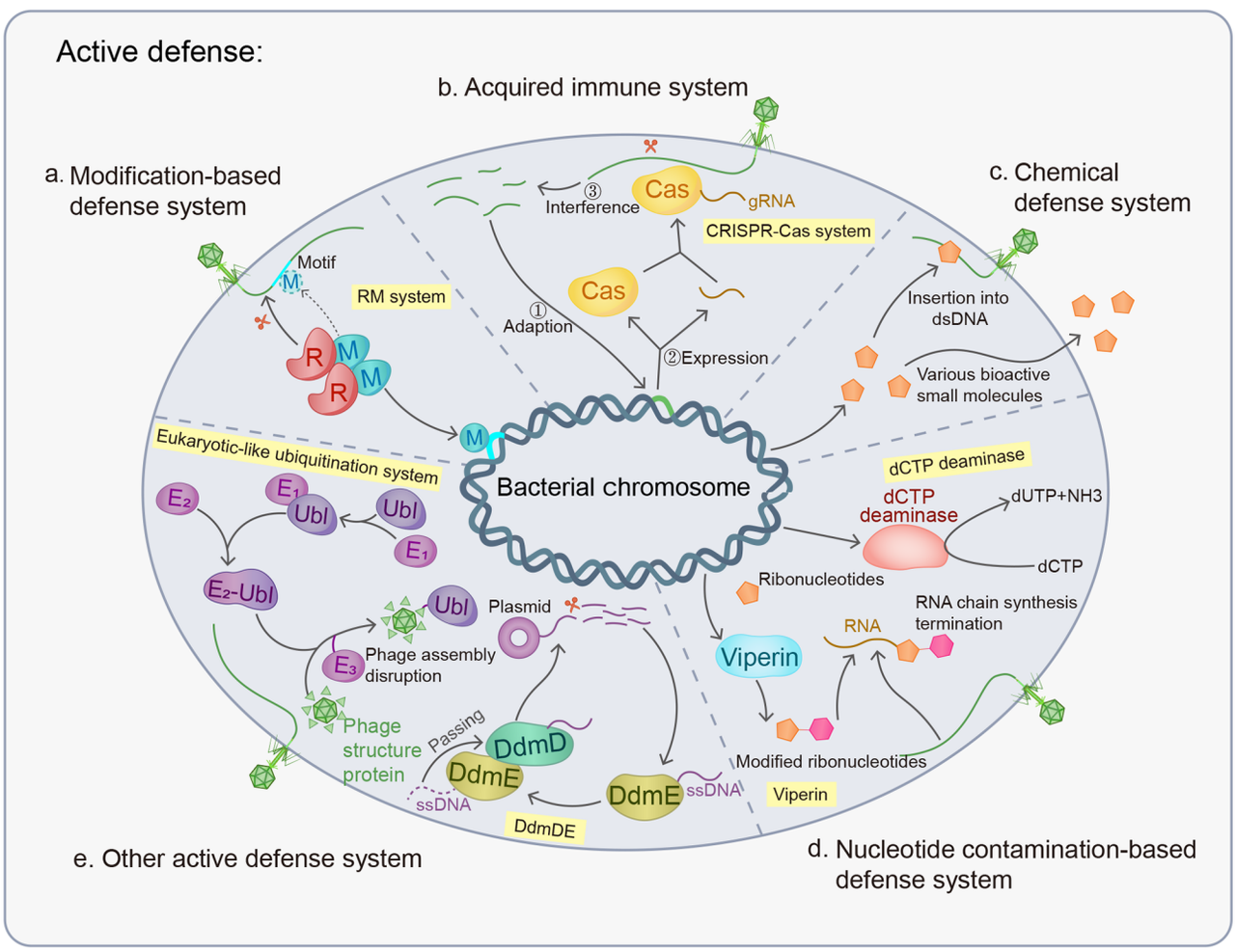

Figure 1 Active defense systems in bacteria

Schematic overview of bacterial active defense systems classified by core molecular mechanisms that directly eliminate mobile genetic elements. a: Modification-based systems distinguish self from non-self by marking host DNA with specific chemical modifications, Unmarked or differently modified foreign DNA is targeted for degradation, as exemplified by restriction-modification (RM) systems (Loenen et al., 2014). Exceptions include systems such as the BacteRiophage EXclusion system, which rely on non-DNA-degrading mechanisms for defense (Goldfarb et al., 2015). b: Acquired immune systems are typified by the Clustered Regularly Interspaced Short Palindromic Repeats-CRISPR-associated proteins (CRISPR-Cas) system, which acquire and memorize foreign sequences, using guide-target base pairing for targeted interference during reinfection (Hille et al., 2018; Koonin & Makarova, 2019; McGinn & Marraffini, 2019). c: Chemical defense systems synthesize diverse bioactive small molecules with antiphage properties. These metabolites are often diffusible, providing protective effects at both the individual and community level (Kronheim et al., 2018). d: Nucleotide contamination-based defense systems inhibit phage propagation by introducing modified or noncanonical nucleotides that disrupt nucleic acid synthesis (Bernheim et al., 2021). e: Other active defense systems comprise mechanistically distinct modules not encompassed by the above categories, frequently involving unconventional enzymatic activities or structural components.

Table 1 Activation and effects of active defense systems

Mechanism-oriented group	System	Activation	Effect	References
Modification-based systems	Type I-III RM	Recognizes specific sites on unmethylated DNA.	Distinguishes host DNA from foreign DNA via site-specific modification; cleaves foreign DNA.	Loenen et al., 2014
	Type IV RM	Recognizes specific sites on methylated DNA.	Distinguishes host DNA from foreign DNA via site-specific modification; cleaves foreign DNA.	Loenen et al., 2014
	Dnd, Ssp	Recognizes specific sites on non-phosphorothioated DNA.	Distinguishes host from foreign DNA via site-specific modification; cleaves foreign DNA.	Wang et al., 2019, 2021a; Xiong et al., 2020; Xu et al., 2010
	DISARM	Recognizes specific sites on unmethylated DNA.	Distinguishes host from foreign DNA via site-specific modification; degrades foreign DNA.	Ofir et al., 2018
	BREX	Recognizes specific sites on unmethylated DNA.	Distinguishes host from foreign DNA via site-specific modification; impedes phage propagation through unknown, non-DNA-degrading mechanisms.	Drobiazko et al., 2025; Goldfarb et al., 2015
Acquired immune systems	Type I, II, IV CRISPR-Cas	Recognizes specific foreign DNA.	Type I, II: Cleaves target DNA; Type IV: Mostly uncharacterized, with some subtypes (e.g., Type IV-A) capable of interfering with transcription or cleaves target DNA.	Baca & Marraffini, 2025; Makarova et al., 2020
	Type III CRISPR-Cas	Recognizes specific foreign RNA.	Cleaves RNA and ssDNA in a target RNA-dependent manner (Abi can also be triggered under certain conditions).	Deng et al., 2013; Lin et al., 2025; Molina et al., 2020; Samai et al., 2015
	Type V CRISPR-Cas	Recognizes specific foreign DNA or RNA.	Cleaves target DNA or RNA with trans-cleavage activity.	Yan et al., 2019
	Type VI, VII CRISPR-Cas	Recognizes specific foreign RNA.	Cleaves target RNA. Type VI also exhibit trans-cleavage activity.	Altae-Tran et al., 2023; Yang et al., 2024; Zetsche et al., 2015
Chemical defense systems	Various bioactive small molecules (e.g., daunorubicin)	N/A	Inserts into dsDNA; inhibits phage protein synthesis, etc.	Hardy et al., 2023; Kronheim et al., 2018
Nucleotide contamination-based defense systems	dCTP deaminase, dGTPases	Unknown	dCTP converts dCTP into deoxyuridine nucleotides; dGTPases degrades dGTP into dG.	Tal et al., 2022
	Viperin	Unknown	Modify ribonucleotides to produce ddhCTP, ddhGTP, and ddhUTP as chain terminators, inhibiting RNA synthesis.	Bernheim et al., 2021
Other active defense systems	Eukaryote-like Ubiquitination System	Senses phage proteins.	E1 activates ubiquitin-like protein; E2 and E3 mediate covalent attachment of ubiquitin-like proteins to phage structure proteins.	Chambers et al., 2024; Hör et al., 2024
	DdmDE	Recognizes foreign DNA via complementary sequences.	DdmD unwinds and cleaves DNA.	Bravo et al., 2024; Jaskólska et al., 2022
	Wadjet	Specifically recognizes conformation of small-sized circular plasmids.	Cleaves small-sized circular plasmids.	Deep et al., 2022; Liu et al., 2022
	Kiwa	Senses interactions between phage tail fiber proteins and cell membrane.	KwaB binds to phage DNA and inhibits late gene expression.	Zhang et al., 2025c

N/A: Not applicable; RM: Restriction-modification; DISARM: Defense island system associated with restriction-modification; BREX: BacteRiophage EXclusion; CRISPR-Cas: Clustered regularly interspaced short palindromic repeats-CRISPR-associated proteins; Abi: Abortive infection; dG: Phosphate-free deoxyguanosine; ddhCTP: 3'-deoxy-3',4'-didehydro-CTP; ddhGTP: 3'-deoxy-3',4'-didehydro-GTP; ddhUTP: 3'-deoxy-3',4'-didehydro-UTP; ssDNA: Single-strand DNA; dsDNA: Double-strand DNA.

separate or fused REase and MTase components that act near or at recognition sites. Type III systems employ a heteromeric ATP-dependent REase-MTase complex that introduces cuts outside the recognition sequence. Type IV systems encode methylation-dependent REases without associated MTases and cleave modified DNA at random distances from the recognition site. These systems are proposed to target phages that evade restriction by acquiring genome modifications characteristic of type I–III defense mechanisms. A recently characterized RM-like system, PcaRCI, identified in *Pectobacterium carotovorum*, confers protection against both phages and plasmids (Birkholz et al., 2022). Beyond methylation-dependent RM systems, bacteria also harbor defense systems that exploit alternative DNA modifications for self-non-self-discrimination, expanding the known diversity of modification-based immunity. For example, the Dnd system introduces phosphorothioate linkages into host DNA via DndABCD proteins and restricts unmodified DNA using DndFGH (Wang et al., 2019; Xu et al., 2010). Similarly, the Ssp system employs SspABCD to phosphorothioate a single DNA strand, coupling with effectors such as SspE or SspFGH to degrade unmodified foreign DNA, with SspABCD serving as a "sensor" (Wang et al., 2021a; Xiong et al., 2020).

Beyond canonical RM systems, several bacterial systems also utilize DNA modifications but operate through more complex mechanisms, which remain poorly characterized. Among these, the Defense Island System Associated with Restriction-Modification (DISARM) (Table 1) is broadly distributed across bacterial and archaeal taxa. It encodes five

components, including a DNA methylase, a helicase, a phospholipase D domain, a DUF1998 domain, and one uncharacterized protein. DISARM does not inhibit phage adsorption but appears to act early during infection by restricting phage DNA. Like RM systems, it recognizes self-DNA via specific methylation marks (e.g., CCWGG motifs in *Bacillus paralicheniformis*), although the precise molecular mechanism is not yet fully understood (Bravo et al., 2022; Ofir et al., 2018). Another prominent DNA modification-linked system is BacteRiophage EXclusion (BREX) (Table 1), which constitutes a large and diverse superfamily encompassing six subtypes. The best-characterized variant, Type I BREX, comprises six genes encoding a S-adenosyl-L-methionine (SAM)-dependent methyltransferase (PglX/BrxX), a putative alkaline phosphatase (PglZ), a putative ATPase (BrxC), a Lon-like protease (BrxL), a DNA-binding protein (BrxA), and a protein of unknown function (BrxB) (Goldfarb et al., 2015). Site-specific DNA methylation is catalyzed by PglX, and functional orthology has been confirmed for BrxX in *E. coli* (Gordeeva et al., 2019). Methylation motifs vary among species, including TAGGAG in *Bacillus cereus* (Goldfarb et al., 2015), and GGTAAG (Gordeeva et al., 2019), GCTAAT (Picton et al., 2021), and CANCATC (Jiang et al., 2024) in *E. coli.* Activation of the BREX system may depend on BrxX-catalyzed host methylation, likely requiring the formation of a multicomponent BrxBCXZ complex (Drobiazko et al., 2025). Functional characterization has revealed additional features distinguishing BREX from classical RM systems. BrxL, a multimeric DNA-binding protein, exhibits ATP-dependent binding to double-stranded DNA (dsDNA), while BrxA acts as a monomeric DNA-binding protein (Beck et al., 2022; Shen et al., 2023). However, unlike classical RM systems, the effects of DNA methylation in the BREX system are more complex and remain poorly understood. Although BREX restricts phage replication at an early stage, it does not degrade DNA, block adsorption, or trigger Abi phenotypes (Goldfarb et al., 2015). In addition to its antiphage activity, BREX also impedes plasmid establishment, as evidenced by reduced transformation efficiency of plasmids carrying BREX recognition sites in BREX-positive *E. coli* strains (Jiang et al., 2024).

Acquired immune systems: The CRISPR-Cas system constitutes the only known form of adaptive immunity identified in prokaryotes. It provides sequence-specific protection against previously encountered MGEs by recognizing and eliminating foreign nucleic acids, thereby restricting horizontal gene transfer and maintaining genomic integrity (Zaremba et al., 2022). The CRISPR locus comprises an array of short direct repeats interspersed with unique spacers derived from foreign genetic material, representing a heritable molecular record of past invaders. Cas genes encode proteins essential for all stages of immune activity (Barrangou et al., 2007). CRISPR-Cas immunity proceeds through three functionally distinct stages: adaptation, expression, and interference. During adaptation, fragments of invading nucleic acids are captured and integrated into the CRISPR array. The expression stage involves transcription of the array into precursor CRISPR RNA (pre-crRNA), which is processed into mature crRNAs that are incorporated into ribonucleoprotein complexes with Cas proteins. In the interference stage, detection of a target sequence adjacent to a protospacer adjacent motif (PAM) triggers cleavage of the foreign DNA or RNA, resulting in neutralization of the invader (Hille et al., 2018; Koonin & Makarova, 2019; McGinn & Marraffini, 2019). Despite considerable structural and functional diversity among Cas proteins, each contributes to one or more stages of immunity. Their roles include acquisition of foreign sequences through nuclease or recombinase activity, processing of pre-crRNAs via endoribonucleases, assembly of effector complexes with crRNA, and cleavage of target nucleic acids by nucleases (Chaudhuri et al., 2022).

CRISPR-Cas systems exhibit extensive mechanistic and structural diversity, encompassing two major classes, seven types (including the recently characterized Type VII), and more than 30 subtypes (Koonin et al., 2017; Makarova et al., 2018, 2020). Class 1 systems, which include Types I, III, and IV, are defined by multi-subunit effector complexes typically organized around Cas5 and Cas7 scaffolds. Type I systems are the most widespread in nature and have been experimentally validated across various subtypes to confer robust phage resistance. Type IV systems, in contrast, are primarily associated with defense against plasmids and other MGEs (Chaudhuri et al., 2022; Özcan et al., 2019; Taylor et al., 2019a; Xu et al., 2023). Unlike Type I systems, which achieve defense via nucleic acid cleavage, Type IV systems were traditionally thought to function through non-nucleolytic mechanisms. For example, Type IV-A3 has been shown to interfere with the expression of exogenous MGEs (Williams et al., 2025; Zhou et al., 2021). However, recent studies have revealed that Type IV-A2 systems possess nuclease activity capable of direct DNA cleavage, broadening the recognized functional diversity of Type IV systems (Makarova et al., 2025). Type III systems are unique within Class 1 due to their ability to target both RNA and DNA. Their effector complexes can cleave RNA with high specificity via the Csm3 or Cmr4 subunits and, simultaneously, trigger single-stranded DNA (ssDNA) cleavage by the HD domain of Cas10 upon recognition of cognate target RNA (Deng et al., 2013; Hale et al., 2009; Lin et al., 2025; Molina et al., 2020; Samai et al., 2015). These two defense mechanisms form the primary active defense axis of Type III systems. Under conditions of extensive MGE challenge, a secondary, passive defense layer may be activated (Mayo-Muñoz et al., 2022), wherein Cas10 catalyzes the synthesis of cyclic oligoadenylates (cOAs) from ATP. These cOAs serve as second messengers that activate auxiliary effectors, ultimately inducing growth arrest or programmed cell death (PCD) to block phage propagation (Kazlauskiene et al., 2017; Lin et al., 2025; Molina et al., 2020; Niewoehner et al., 2017). Through this dual-layered response, Type III systems integrate features of both active immunity and Abi, a functional convergence elaborated further in the passive defense section of this review. In addition, a recently described Type VII system adds further complexity to Class 1, employing a Cas5-Cas7-based complex to mediate RNA-targeted cleavage (Altae-Tran et al., 2023; Yang et al., 2024).

Class 2 systems, including Types II, V, and VI, are distinguished by single-protein effectors. Cas9 (Type II), Cas12 (Type V), and Cas13 (Type VI) each mediate interference through distinct modes of nucleic acid recognition and cleavage (Chaudhuri et al., 2022; Yan et al., 2019; Zetsche et al., 2015). Type II systems, particularly Cas9, have been widely adopted in gene editing and have greatly contributed to advancements in this field (Doudna & Charpentier, 2014). Cas12 enzymes primarily cleave DNA, exhibiting unique characteristics such as dual ribonuclease

activity, distal cleavage relative to the PAM site, and robust trans-cleavage activity (Zhang et al., 2024b). In contrast, Cas13 enzymes exclusively target RNA and also display trans-cleavage activity upon target recognition (Perčulija et al., 2021). Beyond their immune functions, CRISPR-Cas systems have been implicated in shaping bacterial ecology and behavior, including biofilm formation and suppression of swarm motility (Ghosh et al., 2022; Heussler et al., 2015).

Chemical defense systems: In competitive natural environments, bacteria produce a diverse array of bioactive small molecules—classified as specialized or secondary metabolites—that confer ecological advantages and support survival under phage threat (Davies, 2013). These compounds play multifaceted roles in microbial defense and communication and can interfere with phage infection through distinct chemical modes of action. The antiviral potential of such molecules was first observed more than five decades ago, when *Streptomyces* species were shown to secrete compounds capable of inhibiting *E. coli* phages (Morita et al., 1979; Parisi & Soller, 1964). More recently, a high-throughput screening of 4 960 bioactive compounds identified several *Streptomyces*-derived metabolites—including daunorubicin, doxorubicin, epirubicin, and idarubicin (Table 1)—that suppress phage replication by intercalating into dsDNA, thereby preventing transcription and genome replication (Kronheim et al., 2018). In contrast to many protein-based systems that exhibit subtype specificity, these DNA-interacting small molecules act broadly against dsDNA phages and provide early-stage protection during the infection cycle. Moreover, due to their diffusible nature, these compounds may also offer community-level protection, extending effects beyond the producing bacterium to the surrounding microbial community. In addition to DNA intercalators, other chemically distinct metabolites with antiviral activity have been identified. For example, several compounds inhibit phage propagation by disrupting protein biosynthesis, highlighting the mechanistic diversity of small-molecule-mediated defense (Hardy et al., 2023).

Nucleotide contamination-based defense systems: Phages replication demands a continuous and abundant supply of nucleotides to support genome synthesis and transcription. Several bacterial defense systems exploit this metabolic vulnerability by enzymatically disrupting the integrity of the nucleotide pool, creating a hostile intracellular environment that impairs phage propagation. In contrast to RM systems—where nucleotide modification serves primarily as a self-non-self recognition mechanism—these systems use nucleotide alteration or degradation as a direct antiviral strategy. One prominent example is the family of dCTP deaminases (Table 1), which are frequently encoded near known antiphage loci and catalyze the conversion of dCTP into deoxyuridine derivatives during phage infection, thereby perturbing the pool of usable cytidine nucleotides. Similarly, specific dGTPases degrade dGTP into phosphate-free deoxyguanosine (dG), with both enzymes thus disrupting the normal nucleotide pool by converting canonical nucleotides into nonfunctional forms (Tal et al., 2022). Another notable system is mediated by Viperin (Table 1), which modifies ribonucleotides to generate 3'-deoxy-3',4'-didehydro-CTP (ddhCTP), 3'-deoxy-3',4'-didehydro-GTP (ddhGTP), and 3'-deoxy-3',4'-didehydro-UTP (ddhUTP). These aberrant nucleotides act as chain terminators. When incorporated by phage RNA polymerases into nascent viral transcripts, they terminate elongation prematurely, thereby suppressing RNA-dependent replication (Bernheim et al., 2021). Interestingly, although these systems disrupt fundamental metabolic substrates, they have not been shown to cause significant host toxicity under experimental conditions. This contrasts with systems such as RADAR and CmdTAC (Duncan-Lowey et al., 2023; Gao et al., 2023; Vassallo et al., 2024), which also disrupt nucleotide pools but induce Abi phenotypes that compromise host viability. The lack of evident toxicity suggests the existence of intrinsic regulatory features that constrain metabolic disruption to a tolerable level. As such, nucleotide contamination-based systems are provisionally classified as active defense mechanisms, distinct from classical Abi strategies, despite their shared reliance on metabolic interference.

Other active defense systems: Recent research has reported a growing set of bacterial defense mechanisms with distinctive modes of action that fall outside established categories, including both newly identified systems and functionally unconventional strategies.

Nuclease-driven defense systems: Beyond canonical RM systems, a diverse array of nuclease-driven defense systems employ nucleolytic degradation to eliminate invasive nucleic acids. These systems diverge fundamentally from RM-mediated restriction, which relies on methylation-based self-recognition. Instead, these systems display varied and often poorly understood mechanisms of activation and target selection, reflecting distinct evolutionary solutions to phage resistance. Long prokaryotic Argonautes (pAgos), for example, encode programmable endonucleases that bind and cleave complementary sequences guided by short foreign single-stranded RNA (ssRNA) or DNA fragments, although the acquisition pathways for these mechanisms remain unclear (Kuzmenko et al., 2020; Swarts et al., 2017). Their capacity for sequence-specific cleavage has also attracted interest as a platform for programmable genome engineering (Hegge et al., 2018; Huang et al., 2023). A distinct variant of this strategy was identified in *Vibrio cholerae* isolates from the seventh pandemic. This system, termed DdmDE (Table 1), combines an Argonaute-like protein, DdmE, which uses a ssDNA guide to bind target DNA, with an effector nuclease, DdmD. When DdmE binds the target, DdmD is recruited to form a complex, and the target is handed over to DdmD. Subsequently, DdmD transitions from an inactive dimer into an ATP-dependent monomeric nuclease that cleaves plasmid DNA. (Bravo et al., 2024; Jaskólska et al., 2022). Another structurally distinct but functionally related system, Wadjet (Table 1) (also known as JetABCD, MksBEFG, or EptABCD), targets plasmids via recognition of their physical conformation. This system employs structural maintenance of a chromosome (SMC) complex that recognizes and cleaves small circular DNA molecules, enabling shape-based discrimination (Deep et al., 2022; Liu et al., 2022). In addition to these mechanistically defined systems, an expanding cohort of newly discovered nuclease-driven defenses, such as Shedu (Loeff et al., 2025), Gabija (Li et al., 2024a), Septu (Li et al., 2024d), Druantia (Doron et al., 2018; Wang et al., 2023c), and Mokosh (Millman et al., 2022), share endonuclease-based architectures but remain incompletely characterized, representing an expanding frontier of bacterial antiphage immunity.

Membrane-sensing defense systems: The Kiwa system (Table 1) was recently identified as a membrane-activated antiphage mechanism that detects infection via early

interactions at the bacterial cell envelope, representing a previously unrecognized mode of immune surveillance. The system comprises two components, KwaA and KwaB, and is characterized by a temporal separation between sensing and effector activation. KwaA, a transmembrane protein, detects phage infection at the earliest stage—specifically when the phage tail fiber interacts with the bacterial membrane—thereby initiating the response. Following activation, KwaB rapidly binds incoming phage DNA but does not disrupt early or middle-stage transcription. Instead, it selectively inhibits the expression of late-stage phage genes through direct DNA binding (Zhang et al., 2025c).

Bacterial ubiquitination-like systems: Bacteria encode eukaryotic-like ubiquitination systems consisting of E1, E2, and E3 enzymes that catalyze the covalent attachment of ubiquitin or ubiquitin-like proteins (Ubls) (Table 1) to lysine residues on target proteins. During phage infection, this system ubiquitinates phage proteins, disrupting virion assembly and impairing infection progression (Chambers et al., 2024; Hör et al., 2024). The system is evolutionarily homologous to eukaryotic ubiquitination machinery, reflecting a conserved strategy for post-translational regulation.

Reverse transcriptase (RT)-mediated systems: RTs, RNA-dependent DNA polymerases that synthesize DNA using RNA templates, are widely distributed across bacterial genomes in diverse genetic contexts. In several cases, RTs form integral components of antiphage defense systems and participate directly in infection responses. These systems can be broadly classified into retrons and members of the UG/Abi family (Mayo-Muñoz et al., 2025), both of which are frequently linked to Abi phenotypes and therefore often considered part of the passive defense repertoire. Within the UG/Abi clade, RTs with unknown biological functions and unresolved phylogenetic relationships have been designated as originating from Unknown Groups (UGs) (Mestre et al., 2022). A subset of these has been experimentally demonstrated to function in phage defense and is collectively referred to as defense-associated RTs (DRTs) (Gao et al., 2020). These systems exhibit broad mechanistic diversity, ranging from active responses to passive restriction, although most remain poorly characterized at the molecular level. Among these, DRT9 represents the only experimentally studied DRT known to function through an active defense mechanism. This system senses phage infection via intracellular depletion of dATP, which activates reverse transcription of its associated noncoding RNA into poly (A)-rich single-stranded cDNA. The resulting cDNA sequesters the phage-encoded ssDNA binding (SSB) protein, an essential factor for viral genome replication, thereby inhibiting phage propagation. Notably, the cDNA displays higher affinity for phage SSB than for host SSB, resulting in a transient growth arrest rather than irreversible cell death (Song et al., 2025). This controllable cytotoxicity indicates that DRT9 does not operate through self-sacrifice but instead exemplifies an active defense mechanism that restricts phage replication while preserving host viability.

Phage satellite-associated defense: Phage satellites are MGEs that parasitize co-infecting helper phages by hijacking the phage capsid machinery to preferentially package their own genome. This process often disrupts the normal assembly of the helper phage, and although phage satellites are fundamentally MGEs, their role in limiting phage proliferation has led to their classification by some as indirect contributors to bacterial immunity (Fillol-Salom et al., 2018; Hays & Seed, 2020; O'Hara et al., 2017).

Passive defense

Bacteria confronted with invasion by MGEs employ not only active immune responses but also a diverse array of passive defense strategies to curtail infection. These mechanisms, often associated with Abi phenotypes—excluding superinfection exclusion SIE—eliminate the infected host cell through programmed pathways that halt phage replication and prevent viral dissemination. This self-limiting response plays a community-protective role by preserving uninfected neighboring cells (Lopatina et al., 2020). Comprehensive genomic surveys indicate that approximately 72.5% of prokaryotic genomes encode at least one Abi-related module, emphasizing the evolutionary prevalence and ecological importance of passive defense (Rousset & Sorek, 2023). In this review, passive defense systems are categorized into four mechanistic categories (Figure 2): toxin-antitoxin (TA) systems, key molecule depletion-based defense systems, signaling pathway-mediated systems, and other passive defense systems, including a brief discussion of SIE.

Toxin-antitoxin systems: TA systems constitute a major class of Abi-associated mechanisms that are broadly distributed across bacterial and archaeal lineages and play important roles in cellular homeostasis and stress responses (Fineran et al., 2009). These systems typically consist of two genetically linked components, one encoding a toxin that inhibits growth or induces cell death and one encoding an antitoxin that neutralizes toxin activity under non-stress conditions. Coexpression of both components maintains cellular homeostasis by restraining toxic activity during normal growth. However, upon phage infection or other stress conditions, antitoxins are frequently destabilized or degraded, leading to unrestrained toxin activity and consequent growth arrest or PCD, contributing to population-level survival (Page & Peti, 2016). TA systems are currently classified into eight mechanistic types based on the molecular nature and interaction modes of the toxins and antitoxins (Leroux & Laub, 2022).

Type I TA systems comprise protein toxin and antisense RNA antitoxin. In the hok/sok system (Table 2), for example, the antitoxin RNA binds to the toxin mRNA, preventing translation initiation (Pecota & Wood, 1996). During phage infection, antisense RNA is degraded, allowing translation of the hok peptide, which forms pores in the membrane and disrupts membrane integrity. Similar mechanisms are observed in other systems such as fst/RNAII, bsrG/SR4, and tisB/istR-1, where antisense RNAs bind different regions of the toxin mRNA to block translation (Shore et al., 2024). Type II TA systems are defined by direct protein-protein interactions (Zhang et al., 2025a): In the DarTG system (Table 2), DarT functions as an ADP-ribosyltransferase that modifies ssDNA upon phage infection, thereby interfering with viral DNA replication. The antitoxin DarG counteracts this activity either through direct interaction with DarT or by enzymatically reversing the ADP-ribose modifications from DNA (Leroux et al., 2022). The recently characterized phage anti-restriction-induced system (PARIS) (Table 2) represents another Type II example. In uninfected cells, the antitoxin AirA assembles into a hexameric structure that suppresses the nuclease AirB. Upon phage infection, phage-encoded Ocr proteins bind AirA, triggering its conformational rearrangement and releasing active AirB dimers, which cleave lysine tRNAs and suppress

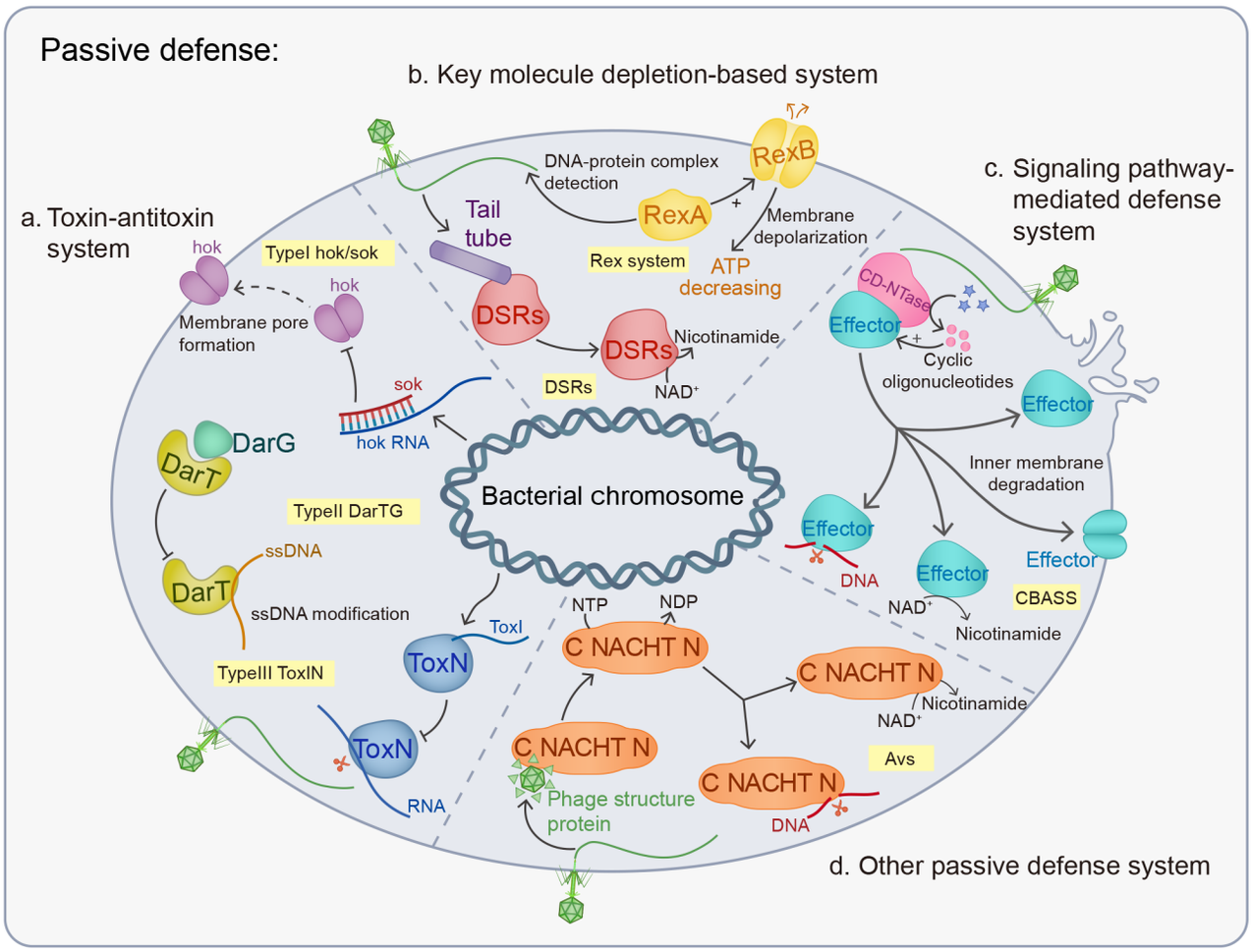

Figure 2 Passive defense systems in bacteria

Schematic representation of bacterial passive defense systems classified by core molecular mechanisms that eliminate phage threats through superinfection exclusion (SIE) or activation of abortive infection (Abi). a: Toxin-antitoxin systems operate via conditional neutralization of toxic proteins by cognate antitoxins. Under normal conditions, antitoxins inhibit toxin activity; disruption of this balance, often during phage infection, results in toxin activation, inducing growth arrest or programmed cell death to prevent phage replication. b: Key molecule depletion-based systems induce cellular suicide by depleting critical intracellular molecules essential for life. Representative targets include ATP (Molineux, 1991; Parma et al., 1992; Snyder, 1995; Snyder & McWilliams, 1989) and NAD^+ (Garb et al., 2022; Huang et al., 2024), whose depletion disrupts metabolism and promotes cell death to block phage spread. c: Signaling pathway-mediated defense systems, typified by the cyclic oligonucleotide-based antiphage signaling system (CBASS), primarily produce second messengers in response to infection, which activate diverse downstream effectors capable of nucleic acid degradation, membrane disruption, NAD^+ depletion, membrane pore formation, ultimately triggering cell suicide (Duncan-Lowey et al., 2021; Fatma et al., 2021; Hogrel et al., 2022; Jenson & Chen, 2024; Rousset & Sorek, 2023; Rousset et al., 2023; Severin et al., 2018; Wang & Zhang, 2023). d: Other passive defense systems include mechanistically distinct or incompletely characterized modules that contribute to Abi- or SIE-based defense but do not fit into the preceding categories.

host translation to limit phage propagation (Deep et al., 2024). Type III TA systems also involve protein toxin and RNA antitoxin, but with a distinct interaction mode. In the ToxIN system (Table 2), the labile antitoxin ToxI binds and neutralizes the endoribonuclease ToxN (Blower et al., 2009). Phage infection halts ToxI production, permitting ToxN accumulation and degradation of phage transcripts, resulting in growth arrest. Types IV–VIII are distinguished by increasingly diverse mechanisms of toxin neutralization (Bonabal & Darfeuille, 2024; Lin et al., 2023). In type IV systems such as CbeA/CbtA and ShosTA, the antitoxin is a protein that stabilizes toxin targets rather than binding the toxin directly (Heller et al., 2017; Pu et al., 2025). DarTG may also fall under this category based on its mode of action noted above (Leroux et al., 2022). In Type V systems such as GhoTS, the antitoxin is an RNase that degrades toxin-encoding transcripts (Wang et al., 2012). Type VI antitoxins function as adaptor proteins that target proteolytic degradation (Aakre et al., 2013). Type VII systems neutralize toxins through covalent modification of the toxin protein (Yao et al., 2020), while Type VIII systems comprise a small RNA toxin and a crRNA-like antitoxin that counteracts its action (Choi et al., 2018). Although most TA systems encode distinct toxin and antitoxin components, the CapRel system (Table 2) encodes a single polypeptide with both activities. Under non-stress conditions, the C-terminal domain inhibits the toxic N-terminal domain. During phage infection, CapRel binds newly synthesized phage capsid proteins, releasing autoinhibition. The active toxin domain then pyrophosphorylates tRNA, halting protein synthesis and impairing phage replication (Zhang et al., 2022).

Several multi-component phage defense systems operate

Table 2 Activation and effects of passive defense systems

Mechanism-oriented group	System	Activation	Effect	References
Toxin-antitoxin systems	hok/sok (Type I)	Unknown (some evidence suggests it may be caused by transcriptional shutoff).	In the absence of sok, toxin hok is translated and forms pores in the membrane.	Kelly et al., 2023; Pecota & Wood, 1996
	DarTG (Type II or IV)	Unknown	In the absence of DarG, DarT acts as an ADP-ribosyltransferase to modify ssDNA.	Leroux et al., 2022
	PARIS (Type II)	Phage Ocr protein binds AirA.	AirB is released and activates nuclease activity, cleaving lysine tRNA to block protein translation.	Deep et al., 2024
	ToxIN (Type III)	Upon phage infection, transcription of antitoxin ToxI is disrupted.	In the absence of ToxI, ToxN is released and degrades mRNA as an RNase.	Blower et al., 2009; Guegler & Laub, 2021
	CapRel	Senses newly synthesized phage capsid proteins.	Upon release from self-inhibition, toxic N-terminus pyrophosphorylates tRNA, blocking protein synthesis.	Zhang et al., 2022
	CmdTAC (tripartite TA system)	CmdC recognizes newly synthesized phage capsid proteins.	CmdA is destabilized and degraded, releasing CmdT, which modifies ssRNA and halts mRNA translation.	Vassallo et al., 2024
	Lamassu	Senses exogenous DNA ends.	LmuABC complex disintegrates and releases LmuA, which has nuclease activity, to cleave DNA.	Li et al., 2025a
Key molecule depletion-based systems	DSRs	Recognizes monomeric phage tail tube.	Deplete intracellular NAD^+ via SIR2 domain activity.	Garb et al., 2022; Huang et al., 2024
	SPARSA, SPARTA	Detects and binds foreign nucleic acid.	Deplete intracellular NAD^+ via SIR2 or TIR domain activity.	Koopal et al., 2022; Kottur et al., 2024; Ryazansky et al., 2018; Zaremba et al., 2022; Zhang et al., 2024a
	Rex	RexA senses DNA-protein complex formed after phage infection.	Activated RexB acts as an ion channel, causing membrane depolarization and ATP depletion.	Molineux, 1991; Parma et al., 1992; Snyder, 1995; Snyder & McWilliams, 1989
Signaling pathway-mediated defense systems	CBASS	At least triggered by folate depletion, phage nucleic acids and function protein; full mechanism mostly remains unknown.	Cyclic oligonucleotides serve as second messengers, triggering Abi via membrane perforation, phospholipase-mediated inner membrane degradation, and DNA cleavage.	Banh et al., 2023; Brenzinger et al., 2024; Richmond-Buccola et al., 2024; Severin et al., 2023; Ye et al., 2020
	Type III CRISPR-Cas	Recognizes specific foreign RNA.	cOA serves as second messenger, activating various downstream effectors to induce Abi.	Deng et al., 2013; Lin et al., 2025; Molina et al., 2020; Samai et al., 2015
	Kongming	Senses phage DNKs.	dITP serves as second messenger, activating effector complex KomBC to deplete cellular NAD^+.	Zeng et al., 2025
	Stk2	Recognizes phage protein Pack.	Phosphorylation cascade may be involved in signal transduction; upon activation, Stk2 phosphorylates multiple downstream proteins involved in cellular processes, leading to Abi.	Depardieu et al., 2016
	Thoeris	Senses phage capsid proteins.	An isomer of cADPR serves as second messengers to activate ThsA, which degrades NAD^+.	Chou et al., 2023; Ka et al., 2020; Roberts et al., 2024; Shi et al., 2024
Other passive defense systems	Avs	Senses conserved phage structures such as large terminase subunit and portal protein.	N-terminal effector domain exerts multiple effects, including DNA degradation and NAD^+ depletion.	Gao et al., 2022
	Gasdermin	Unknown	Activation leads to formation of pores in the membrane.	Johnson et al., 2022
	retron-Ec86	Phage-encoded DNA methylases modify msDNA and disrupt its inhibitory interaction with effector.	NDT-like effector depletes cellular NAD^+.	Bobonis et al., 2022
	DRT2	Unknown	Upon activation, toxic Neo protein is translated repetitively.	Wilkinson et al., 2024
	Hailong	Phage DNA exonucleases degrade short DNA probes.	Upon activation, HalA causes membrane depolarization and arrests cell growth.	Tan et al., 2025
	SIE	N/A	Modifies bacterial surface receptors or blocks phage genome injection.	Taylor et al., 2019b

N/A: Not applicable; ssDNA: Single-strand RNA; ssRNA: Single-strand RNA; PARIS: Phage anti-restriction-induced system; DSRs: Dense-associated sirtuins; SPARTA: Short pAgos in conjunction with the associated TIR; SIR2: Silent information regulator 2; SPARSA: Short pAgos in conjunction with the associated SIR2; TIR: Toll and interleukin-1 receptor; CBASS: Cyclic oligonucleotide-based antiphage signaling system; CRISPR-Cas: Clustered regularly interspaced short palindromic repeats-CRISPR-associated proteins; cOA: Cyclic oligoadenylates; dITP: Deoxyinosine 5'-triphosphate; cADPR: Cyclic ADP-ribose; Avs: Antiviral signal transduction ATPases with numerous domains proteins; NDT: Nucleoside deoxyribosyltransferase; DRT2: Defense-associated reverse transcriptase 2; SIE: Superinfection exclusion.

through mechanisms analogous to TA modules and are thus broadly classified as generalized TA systems. One recently characterized example is the CmdTAC system (Table 2), a tripartite module comprising a toxin (CmdT), an antitoxin (CmdA), and a dedicated chaperone (CmdC) (Vassallo et al., 2022). Under basal conditions, CmdC stabilizes CmdA and enables effective suppression of CmdT toxicity. Upon sensing newly synthesized phage capsid proteins, CmdC dissociates from the complex, resulting in CmdA degradation and CmdT activation. The released CmdT, an ADP-ribosyltransferase, modifies adenines at GA dinucleotides in ssRNA, thereby inhibiting phage translation and triggering Abi (Vassallo et al., 2024). Similarly, the Lamassu system, composed of LmuA, LmuB, and LmuC, achieves coordinated defense through a modular architecture. Under normal conditions, the nuclease LmuA is sequestered by LmuB and LmuC. Upon recognition of bacteriophage or plasmid DNA, the LmuB ATPase domain activates, triggering a conformational rearrangement within the LmuABC complex and releasing LmuA. The liberated LmuA then assembles into an active tetrameric nuclease that degrades DNA and activates Abi (Li et al., 2025a).

Key molecule depletion-based systems: Key molecule depletion-based systems limit phage propagation by inducing suicide through the depletion of critical molecules involved in essential cellular processes, thereby halting infection. For example, nicotinamide adenine dinucleotide (NAD^+), a coenzyme required for a wide array of metabolic and signaling pathways, is indispensable for normal cellular function and plays a central role in immune-mediated cell death in both eukaryotes and prokaryotes. Upon activation, certain bacterial defense modules rapidly deplete NAD^+ pools to induce PCD and restrict phage proliferation. Multiple recently identified Abi systems mediate this effect via NAD^+-consuming enzymes harboring Toll/Interleukin-1 receptor (TIR) or sirtuin catalytic domains (Wang et al., 2023b). For instance, dense-associated sirtuin (DSR) systems (Table 2) encode silent information regulator 2 (SIR2) domain-containing proteins that are activated in response to phage structural components. DSR2 elicits rapid NAD^+ consumption and Abi-associated cell death, while DSR1 triggers transient NAD^+ depletion, allowing partial cellular recovery following restriction of viral replication (Garb et al., 2022; Huang et al., 2024). A subset of short pAgo-associated systems, unlike long pAgo systems above—such as short pAgos in conjunction with the associated SIR2 (SPARSA) and short pAgos in conjunction with the associated TIR (SPARTA) (Table 2)—also utilize TIR or SIR2 effectors to mediate NAD^+ depletion (Koopal et al., 2022; Kottur et al., 2024; Ryazansky et al., 2018; Zaremba et al., 2022; Zhang et al., 2024a). These short variants complement long pAgo systems, which collectively provide defense against diverse MGEs such as phages, plasmids, and transposons (Zander et al., 2017). In addition to NAD^+, some systems act by depleting ATP to achieve a similar defensive outcome. The Rex system (Table 2), one of the earliest described Abi modules, consists of RexA and RexB, where phage-activated RexA triggers RexB-mediated membrane depolarization, subsequently impairing ATP synthesis and inhibiting cell growth (Molineux, 1991; Parma et al., 1992; Snyder, 1995; Snyder & McWilliams, 1989). This section specifically highlights systems in which metabolite depletion functions as the principal effector mechanism. Other systems, such as RT-associated systems, may include similar enzymatic activities but rely on multiple concurrent effectors rather than a single, defining mode of action.

Signaling pathway-mediated defense systems: Signaling pathway-mediated defense systems constitute a mechanistically diverse and dynamically regulated arm of antiphage immunity. A primary example is the cyclic oligonucleotide-based antiphage signaling system (CBASS) (Table 2), which induces PCD in response to phage infection. This system relies on cyclic GMP-AMP synthase (cGAS)/DncV-like nucleotidyltransferases (CD-NTases) to synthesize cyclic oligonucleotides, which detect phage-derived signals and generate cyclic oligonucleotides that initiate Abi through a range of downstream effectors (Cohen et al., 2019; Davies et al., 2012; Severin et al., 2018). Canonical CBASS architectures include at least two core proteins: a CD-NTase that synthesizes cyclic oligonucleotide messengers upon phage sensing, and an effector protein that recognizes these signals to induce cellular suicide. Many systems also encode auxiliary CD-NTase-associated proteins (Caps), which modulate signal propagation, effector activation, or overall pathway dynamics (Millman et al., 2020b). CBASS systems activate diverse effector mechanisms, including NAD^+ depletion, inner membrane disruption, pore formation, ATP depletion, and nucleic acid degradation. However, additional effectors types likely remain unidentified, as other passive defense systems have been shown to induce Abi through an even broader range of mechanisms (Duncan-Lowey et al., 2021; Fatma et al., 2021; Hogrel et al., 2022; Jenson & Chen, 2024; Rousset & Sorek, 2023; Rousset et al., 2023; Severin et al., 2018; Wang & Zhang, 2023).

Millman et al. (2020b) proposed a classification framework for CBASS based on operon architecture, effector function, and the chemical identity of the cyclic oligonucleotide signals synthesized by the associated cyclases. Type I systems comprise a compact two-gene module encoding a CD-NTase and its effector, often a pore-forming membrane protein with transmembrane helices that disrupt cellular integrity upon activation (Burroughs et al., 2015). These modules are broadly distributed among bacterial phyla. Type II systems contains canonical CD-NTase-effector modules and two accessory genes, Cap2 and Cap3, which encode ubiquitin-like domains (Lowey et al., 2020). Cap2 harbors fused E1- and E2-like domains and promotes cyclic oligonucleotide production through a ubiquitination-like mechanism, whereas Cap3 antagonizes this process via its deubiquitinase-like domain, functioning as a negative regulator (Jenson et al., 2023; Ledvina et al., 2023; Yan et al., 2024). The effector of Type II systems frequently contains phospholipase domains that degrade the inner membrane upon activation (Cohen et al., 2019; Severin et al., 2018). Type III systems include accessory genes Cap6 and Cap7, which encode proteins bearing TRIP13/Pch2 and HORMA domains, respectively. These systems produce cyclic tri-adenylate (cAAA) molecules that activate nucleases, leading to degradation of both phage and host DNA. Cap7 promotes CD-NTase activity, while Cap6 inhibits this interaction, paralleling the interplay between TRIP13/Pch2 and HORMA domain proteins in eukaryotic systems (Aravind & Koonin, 1998; Rosenberg & Corbett, 2015; Vader, 2015). Type IV systems encode Cap9 and Cap10, which harbor domains implicated in nucleotide modification and likely mediate DNA cleavage through restriction-modification-like mechanisms, although their precise roles remain unclear (McCarty et al., 2009; Reader et al., 2004). In rare instances, CBASS loci encode gene

fusions combining CD-NTase and effector domains within a single gene product (Millman et al., 2020b). Importantly, within each CBASS type, CD-NTases generate structurally diverse cyclic oligonucleotide signals that are selectively recognized by corresponding cOs-sensing domains in effector proteins. These recognition domains are finely tuned to discriminate among specific signaling molecules, conferring signal-effector specificity across CBASS architectures (Burroughs et al., 2015; Millman et al., 2020b). Despite emerging insights, the mechanisms underlying CBASS activation remain largely unexplored. Known triggers include folate depletion, detection of phage-derived nucleic acids or functional proteins (such as a phage prohead protease), and detection of specific peptides produced during phage infection (Banh et al., 2023; Brenzinger et al., 2024; Richmond-Buccola et al., 2024; Severin et al., 2023; Ye et al., 2020). Furthermore, mutations in phage capsid proteins that enable evasion of CBASS-mediated defense suggest that these structural elements may also play a role in CBASS activation, although this hypothesis remains under investigation (Huiting et al., 2023; Stokar-Avihail et al., 2023).

Several bacterial antiviral systems beyond CBASS likewise employ second-messenger-mediated signaling to coordinate passive defense responses. In Type III CRISPR-Cas systems (Table 2), recognition of target RNA by the Csm complex activates Cas10, which synthesizes cyclic oligoadenylates (cA*n*, *n*=2–6) via its Palm domain (Kazlauskiene et al., 2017). The cA4/cA6 molecules bind to the CRISPR-associated Rossmann Fold (CARF) domain, which functions as a sensor of cyclic oligoadenylates (cOAs) and activates downstream effectors. One such effector, CRISPR-associated adenosine deaminase (CAAD), converts ATP to inosine triphosphate (ITP). ITP is subsequently hydrolyzed to inosine monophosphate (IMP) by Nudix hydrolases, ultimately leading to growth arrest (Athukoralage & White, 2021; Li et al., 2025b). These cyclic molecules also activate Cam1/Csx23, inducing membrane depolarization, or engage Csm6/Csx1/Can1 effectors to mediate nucleotide degradation or hyper-supercoiled DNA cleavage (Lin et al., 2025). Another second-messenger-based system, Thoeris (Table 2), is composed of two core proteins, ThsA and ThsB. Following phage infection, viral capsid proteins trigger the TIR domain of ThsB to produce a cyclic ADP-ribose (cADPR) isomer, which binds and activates ThsA. ThsA contains a sirtuin-like domain and a SLOG domain and functions as a potent NAD^+ hydrolase, inducing NAD^+ depletion and subsequent cell death (Chou et al., 2023; Ka et al., 2020; Roberts et al., 2024; Shi et al., 2024). Thoeris systems are characterized into Types I and II based on the signaling molecules produced and the domain architecture of ThsA. In Type I, ThsA features an N-terminal SIR2 domain and a C-terminal SLOG domain that recognizes 1′′-3′-gcADPR. In Type II, ThsA contains an N-terminal transmembrane region and a Macro domain at the C-terminus, which binds ADPR derivatives instead of the SLOG domain (Sabonis et al., 2025). A recently identified type III variant contains a distinct SLOG domain that belongs to the SMF/DprA superfamily, differing from the STALD (SIR2/TIR-associated SLOG domains)-type SLOGs in types I and II (Van Den Berg et al., 2024). Similarly, the Pycsar system, another nucleotide-based defense module, uses specialized cyclase enzymes to produce monocyclic pyrimidine molecules, such as cUMP and cCMP, as signaling molecules (Tal et al., 2021). In addition, the Kongming system (Table 2) uses phage-encoded deoxynucleotide monophosphate kinases (DNKs) to synthesize deoxyinosine 5'-triphosphate (dITP), which activates the KomBC effector complex to deplete cellular NAD^+ (Zeng et al., 2025). Beyond second-messenger signaling, phosphorylation cascades, commonly found in eukaryotic systems, have also been implicated in bacterial phage defense. In *Staphylococcus* species, the eukaryotic-like kinase Stk2 (Table 2) confers resistance through phosphorylation-dependent signaling. Upon interaction with the phage protein Pack, Stk2 undergoes phosphorylation and activates Stk1, triggering a phosphorylation cascade that modifies multiple cellular proteins, ultimately leading to phage-induced cell death (Depardieu et al., 2016).

Other passive defense systems: This category encompasses antiviral strategies that do not conform to previously described mechanistic categories or for which molecular mechanisms remain incompletely characterized.

Autonomous sensor-effector systems with eukaryotic-like modules: In addition to the eukaryotic-like signaling architectures exemplified by systems such as CBASS, recent studies have identified a distinct class of defense modules that incorporate eukaryotic-like domains but operate independently of canonical signaling cascades. These autonomous systems directly couple pathogen detection with effector activation within a single molecular complex, bypassing the need for second messengers. For instance, antiviral STAND proteins (Avs) (Table 2), which belong to the Signal Transduction ATPases with Numerous Domains (STAND) superfamily, play key roles in phage defense and exhibit a tripartite architecture comprising a central NTPase domain, an extended C-terminal sensor domain, and an N-terminal effector domain. Upon detection of conserved viral structural proteins, Avs undergo oligomerization, leading to activation of their effector domains (e.g., DNases, SIRs) to induce cell death or other defense responses (Gao et al., 2022). Another well-characterized system is the Gasdermin (GSDM) family (Table 2), which is conserved across prokaryotes and metazoans. Upon phage infection, bacterial GSDMs (bGSDMs) are relieved from autoinhibition and form large pores in the membrane, thereby compromising membrane integrity and leading to cell death (Johnson et al., 2022).

RT-mediated systems: RT-mediated passive defense systems have been documented in both retron-associated modules and members of the UG/Abi family. In retron-based defense systems, core components include an RT, a non-coding RNA (ncRNA) that is reverse transcribed into multicopy ssDNA (msDNA), and one or more dedicated effector proteins. As RTs and msDNA alone generally lack intrinsic antiviral activity, protection is conferred by associated effectors, which, in most characterized cases, function as toxins. Mechanistically, many retron-associated systems parallel TA architectures. Under basal conditions, reverse transcription generates msDNA that suppresses effector activity, whereas phage infection disrupts this neutralization and triggers Abi. For example, retron-Ec86 (Table 2) consists of a ncRNA template, an RT, and an NDT (Nucleoside deoxyribosyltransferase)-like effector. These components assemble into a supramolecular Ec86-effector filament, where effector dimers are confined within a cage-like architecture formed by RT-msDNA complexes. During phage infection, phage-encoded Dcm-mediated methylation of msDNA destabilizes this architecture, releasing the effector. The activated effector then depletes intracellular NAD^+, inducing

Abi and blocking phage replication (Bobonis et al., 2022). Similar retron-based systems, including retron-Eco1, retron-Sen2, retron-Eco2, and retron-Ec78, differ in effector identity but retain conserved tripartite organization and activation resembling TA modules (Carabias et al., 2024; George et al., 2025; Wang et al., 2024c, 2025e). Recent study on the retron-Ec83-Septu system revealed a notable deviation from this paradigm. Although activation follows the canonical retron-associated framework, the effector in this system exhibits no detectable host toxicity, in contrast to retron-Ec78-Septu, in which effector activity is intrinsically cytotoxic in the absence of msDNA. These findings suggest mechanistic differences among retron-associated defenses that remain incompletely understood (George et al., 2025; Wang et al., 2025b). In addition, certain retron systems exhibit a unique association with the integrity of host immune complexes. For example, retron-Ec48 monitors the integrity of the host RecBCD complex and becomes activated when RecBCD function is damaged or functionally perturbed, triggering effector-mediated Abi (Millman et al., 2020a). Within the UG/Abi family, several RTs historically associated with Abi defense, such as AbiK, AbiA, and AbiP2, represent early examples of RT-associated defense, although their precise molecular mechanisms remain unresolved (Bouchard & Moineau, 2004; Domingues et al., 2008; Emond et al., 1997; Gapińska et al., 2024). Among defense-associated RTs, aside from DRT9 discussed in the active defense context, DRT2 and DRT10 are the most extensively characterized. The DRT2 system (Table 2) responds to phage infection by synthesizing repeated DNA molecules via a Type II RT. These DNA products are transcribed into repetitive RNA and translated into toxic Neo proteins, whose accumulation induces bacterial growth arrest and halts phage propagation (Wilkinson et al., 2024). DRT10 contains an RT, a SLATT protein, and a ncRNA template, and functions in a manner similar to eukaryotic telomerase by repeatedly copying the RNA template to generate regular tandem-repeat cDNA. The resulting cDNA is proposed to function as a regulatory signal that modulates SLATT activity, ultimately leading to phage infection failure (Tang et al., 2025). Despite the abundance of RTs encoded in retrons and UG/Abi systems, only a small fraction have been functionally linked to immune defense, leaving a vast knowledge gap yet to be explored (Mestre et al., 2022).

Decoy-triggered defense systems: The Hailong system (Table 2) exemplifies a distinct antiphage strategy wherein bacterial cells proactively synthesize molecular decoys to detect phage enzymatic activity. This system comprises an NTase (HalB) and a membrane ion channel protein (HalA). HalB generates short DNA fragments that function as decoy substrates for viral DNA exonucleases required for phage genome processing. Upon degradation of these decoys by viral DNA enzymes, HalA is activated, disrupting membrane potential and suppressing host cell growth to block phage propagation. This mechanism represents a unique trap-based defense mode in which viral enzymatic activity directly triggers immune activation (Tan et al., 2025).

SIE: Prophages integrated into bacterial genomes can confer resistance against homologous or similar phages via SIE (Taylor et al., 2019b) (Table 2). This strategy typically functions by modifying surface receptors or blocking phage DNA injection (Getz & Maxwell, 2024), thereby preventing entry without inducing Abi. Although categorized as a passive defense due to the absence of active targeting or elimination of invading MGEs, SIE can influence population-level dynamics by promoting preferential infection of related phages, which arguably violates a central principle of canonical passive defense, namely the self-sacrifice of infected cells for the benefit of the bacterial population. This effect is illustrated by the recently proposed anti-Kronos model, in which prophage-mediated protection at the single-cell level facilitates broader infection of the bacterial population (Taylor et al., 2025).

Other Abi systems with undefined mechanisms: Despite the growing understanding of bacterial defense systems, many Abi-associated systems remain unknown or poorly characterized. A number of recently discovered or bioinformatically predicted systems have been shown to exhibit typical Abi phenotypes, yet their molecular mechanisms remain largely unresolved. Notable examples include the Hachiman system (Cui et al., 2025; Tuck et al., 2024), Zorya system (Costa et al., 2024; Doron et al., 2018; Hu et al., 2025), pdpSau (Kuntová et al., 2023), and Hna protein (Sather et al., 2023). These systems underscore both the evolutionary ingenuity of bacterial immunity and the considerable gaps that persist in defining how many Abi pathways operate at the molecular level.

EVOLUTIONARY HOMOLOGY BETWEEN BACTERIAL AND EUKARYOTIC IMMUNITY

Bacteria and eukaryotes are continually exposed to viral threats, and both have evolved complex immune systems that exhibit striking mechanistic parallels. This is primarily due to deep evolutionary conservation of molecular components that trace back to ancient antiviral architectures (Wein & Sorek, 2022). Conserved elements include the cGAS-stimulator of interferon genes (STING) axis (Kranzusch et al., 2014), effector proteins such as Viperin (Bernheim et al., 2021), GSDM family members (Johnson et al., 2022), programmable nucleases like pAgo (Swarts et al., 2014b), and canonical signaling domains such as TIR and nucleotide-binding oligomerization domain-like receptors (NLR) (Chou et al., 2023; Ofir et al., 2021), which span both bacterial and eukaryotic immune systems.

A critical distinction must be made between mechanistic analogy and true evolutionary homology. Numerous prokaryotic antiviral strategies mirror the logic of eukaryotic immune responses, not through shared ancestry but as a consequence of convergent evolution driven by similar selective pressures. This phenomenon is exemplified by systems such as CRISPR-Cas, which archives molecular signatures of prior infections to guide sequence-specific defense, a strategy functionally analogous to the antibody-based memory in eukaryotic adaptive immunity. Similarly, Abi, whereby infected bacterial cells undergo programmed self-elimination to halt phage dissemination, mirrors the immunological function of PCD in eukaryotes for maintaining internal homeostasis.

Such cases illustrate that similar defensive strategies can arise across domains of life even in the absence of structural homology, representing functional convergence shaped by comparable evolutionary pressures. However, beyond such functional convergence, a subset of immune modules exhibits clear sequence or structural homology, reflecting ancestral continuity rather than mere adaptation to shared challenges. This review focuses on those conserved elements, examining their architectural features, mechanistic roles, and

evolutionary trajectories to elucidate the deep-rooted connections between bacterial and eukaryotic immune strategies and their broader relevance to biomedical science.

Conserved defense modules

Despite extensive evolutionary divergence, organisms across all domains of life have encountered comparable selective pressures from viral invasion and have retained conserved immune modules across vast phylogenetic distances. This conservation highlights deep, shared principles underlying antiviral defense in bacteria and eukaryotes. The following section examines these homologous immune components and their domain-specific adaptations (Table 3).

CD-NTase: Cytosolic DNA serves as a potent danger signal in eukaryotic cells, typically indicative of viral infection. The cGAS protein detects this foreign DNA and catalyzes the synthesis of 2',3'-cGAMP, which activates the receptor protein STING, ultimately inducing the production of interferons—a hallmark of the antiviral cGAS-STING pathway (Hopfner & Hornung, 2020; Sun et al., 2013). In prokaryotes, a functionally analogous cGAMP synthase, DncV, was originally characterized in *Vibrio cholerae*, where it contributes to pathogenesis (Davies et al., 2012). Although cGAS and DncV differ in sequence identity and catalytic direction, their three-dimensional structures and active site configurations are highly conserved, indicating deep evolutionary homology (Kranzusch et al., 2014; Jenson & Chen, 2024). DncV belongs to the CD-NTase family, whose members are broadly distributed across bacterial taxa and play a critical role in CBASS (Lowey et al., 2020). In CBASS, CD-NTases synthesize a variety of cyclic dinucleotide (CDN) and trinucleotide compounds. These molecules activate downstream effectors to initiate robust cell-intrinsic responses such as Abi, thereby halting phage propagation (Whiteley et al., 2019). The bacterial CBASS architecture mirrors the cGAS-STING pathway in eukaryotes both in mode of action and system components (Slavik & Kranzusch, 2023). CBASS activation is tightly regulated in bacteria to prevent autoimmunity. In Type III systems, regulation is achieved through HORMA domain-containing proteins and Trip 13-like AAA+ ATPases. The former activates CD-NTase after recognizing phage infection, while the latter drives the structural remodeling of HORMA domain-containing proteins under phage-free conditions, thereby inhibiting CBASS overactivation and avoiding bacterial damage (Ye et al., 2020). HORMA domains and Trip 13-like AAA+ ATPases are also found in eukaryotes, where they contribute to signal regulation in various pathways (Gu et al., 2022). Furthermore, STING itself is a key component of CBASS. Bacterial STING domains drive oligomerization of downstream TIR effector domains and rapid NAD^+ cleavage upon sensing cyclic dinucleotides; notably, 84% of bacterial STING sequences are encoded within CBASS immune cassettes (Morehouse et al., 2020). Although the bacterial and eukaryotic cGAS-STING pathways are not identical, their core components and functions are conserved, suggesting the presence of a shared antiviral signaling mechanism based on nucleotide second messengers across both prokaryotes and eukaryotes (Slavik & Kranzusch, 2023).

NLR: Abi represents a central bacterial defense strategy in which infected cells undergo self-termination to prevent viral spread. A mechanistically analogous process in mammals is pyroptosis, a proinflammatory form of PCD mediated by NLR signaling. NLRs consist of a nucleotide-binding domain (NBD), leucine-rich repeat (LRR) motifs, and variable C- and N-terminal domains, and operate as multifunctional sensors in innate immunity, regulating inflammation, interferon production, cell fate, autophagy, mitosis, and metabolic homeostasis (Chou et al., 2023). Recent studies have identified a gene encoding a NACHT domain—a signature of metazoan NLRs—adjacent to CBASS loci in *Klebsiella pneumoniae* and found that certain bacterial proteins containing NACHT modules can confer phage resistance, suggesting an evolutionarily conserved structural and functional architecture shared with eukaryotic NLRs (Kibby et al., 2023). Similarly, the bacterial Avs protein, a member of the STAND superfamily, functions as an NLR analog by recognizing phage structural proteins through its C-terminal domain, triggering oligomerization and activating N-terminal

Table 3 Domain and function of homologous modules

Defense system	Conservative defense module	Domain function	Immunity module in eukaryotes	References
CBASS	cGAMP synthase	Senses phages and produce circular oligonucleotides.	cGAS-STING	Lowey et al., 2020; Slavik & Kranzusch, 2023; Sun et al., 2013
CBASS, Avs	NACHT	Hydrolyzes NTPs and activates effector domain through conformational changes.	NLRs	Chou et al., 2023; Gao et al., 2022; Kibby et al., 2023
bGSDM	GSDM	Assembles into large pores in the membrane.	eGSDM	Ding et al., 2016; Feng et al., 2018; Johnson et al., 2022
pViperin	SAM	Catalyzes modified ribonucleotide production.	eViperin	Bernheim et al., 2021; Gizzi et al., 2018
pAgo	Short pAgo: MID, PIWI domain Long pAgo: MID, PIWI, PAZ, N domain	Recognizes and degrades DNA or RNA (Long pAgo); trigger Abi (Short pAgo).	eAgo	Hutvagner & Simard, 2008; Makarova et al., 2009; Ryazansky et al., 2018
BilABCD	E1, E2, DUB, UBI	Ubiquitinates phage proteins nonspecifically.	ISG15	Bhoj & Chen, 2009; Chambers et al., 2024; Freitas et al., 2020
Thoeris	TIR domain protein	Produces cyclic ADP-ribose isomers via NADase activity.	SARM1	Figley & Diantonio, 2020; Ofir et al., 2021; O'Neill & Bowie, 2007
dGTPase	dGTPase	Breaks down dGTP into deoxyguanosine without phosphate.	SAMHD1	Tal et al., 2022

cGAS: Cyclic GMP-AMP (cGAMP) synthase; STING: Stimulator of interferon genes; NLRs: Nucleotide-binding oligomerization domain-like receptors; GSDM: Gasdermin; eGSDM: Eukaryotic Gasdermin; SAM: S-adenosylmethionine; Ago: Argonaute; ISG15: Interferon-stimulated gene 15; TIR: Toll/interleukin-1 receptor; SARM1: Sterile alpha and TIR motif-containing 1; SAMHD1: Sterile alpha motif and HD-domain-containing protein 1.

effector domains—such as DNA endonucleases—to halt infection (Gao et al., 2022). These findings reinforce the conserved immune logic underlying prokaryotic and eukaryotic antiviral defense.

GSDM: GSDMs, known for their key role in pyroptosis in mammalian innate immunity, also have homologs in bacteria. In animal systems, GSDMs contain an N-terminal pore-forming domain that is released following caspase-mediated proteolysis of an inhibitory C-terminal region. The liberated N-terminal domain oligomerizes within cellular membranes to form large pores, typically 10–16 nm in diameter, resulting in membrane rupture and release of intracellular contents (Ding et al., 2016; Feng et al., 2018; Shi et al., 2017). bGSDMs exhibit strong structural homology with the mammalian pore-forming N-terminal domain. In response to phage infection, cleavage by caspase-like or other activating proteases removes the inhibitory C-terminal domain of bGSDMs, allowing the N-terminal domain to assemble into membrane pores. This pore formation induces cell death and restricts phage replication, thereby providing population-level protection (Johnson et al., 2022).

Viperin: Viperin belongs to the radical S-adenosylmethionine (SAM) enzyme superfamily and is up-regulated by interferon-induced protein that suppresses replication of RNA and DNA viruses. In human cells, Viperin catalyzes the SAM-dependent conversion of cytidine CTP into ddhCTP, a nucleotide analog that acts as a chain terminator for viral RNA polymerases, thereby blocking viral replication (Gizzi et al., 2018). Prokaryotic homologs of Viperin have been shown to produce a suite of modified ribonucleotides, including ddhCTP, ddhGTP, and ddhUTP, which inhibit transcription during T7 phage infection. These findings reveal a conserved antiviral strategy based on nucleotide modification (Bernheim et al., 2021) and support the view that key components of innate immune defense originated in prokaryotes and were retained or co-opted during eukaryotic evolution.

Ago: Ago proteins are conserved across all three domains of life—Bacteria, Archaea, and Eukarya—where they mediate nucleic acid-guided defense functions. In eukaryotes, Ago proteins participate in RNA interference (RNAi), a post-transcriptional gene silencing mechanism in which RNase III-like enzymes (e.g., Dicer) cleave long dsRNA into short interfering RNAs (siRNAs). These siRNAs possess phosphorylated 5' termini and two-nucleotide 3' overhangs and are processed into guide-passenger strand duplexes. Eukaryotic Ago (eAgo) selectively retains the guide strand, which directs the cleavage or repression of complementary target mRNA, thereby inhibiting gene expression (Höck & Meister, 2008; Hutvagner & Simard, 2008). Despite functional divergence, the four major domains—MID (guide binding), PIWI (RNase H-like catalytic site), PAZ (guide protection), and N-terminal domain (duplex unwinding)—exhibit substantial structural conservation in both eAgos and pAgos (Swarts et al., 2014b). Interestingly, in contrast to their eukaryotic counterparts, pAgos often contain additional domains and are co-located with genes encoding helicases, nucleases, or DNA-binding proteins, suggesting broader targeting complexity. pAgos lacking PAZ and N-terminal domains are termed “short pAgos”, while those containing all four canonical domains are termed “long pAgos”. Most pAgos preferentially bind DNA rather than RNA as guides (Makarova et al., 2009; Ryazansky et al., 2018). As antiviral effectors, Agos in both bacteria and eukaryotes act against invasive nucleic acids, including viral genomes and transposons. Recent cryo-EM analyses have elucidated the activation mechanism of a short pAgo-based system termed short prokaryotic Argonaute and DNase/RNase-APAZ (SPARDA), which coordinates target recognition and collateral DNA degradation. This system comprises NbaAgo (a catalytically inactive short pAgo), DREN (a DNA/RNA effector nuclease), and APAZ domain-containing effectors. Upon co-loading of guide RNA and target RNA, these components undergo conformational rearrangement and filamentous assembly into an active configuration that facilitates nucleic acid cleavage (Wang et al., 2025c). The oligomerization dynamics of SPARDA effectors closely resemble the polymeric activation of metazoan STING complexes (Ergun et al., 2019; Hou et al., 2023), reflecting convergent evolution of supramolecular assemblies in antiviral immunity.

Ubiquitination-associated protein: Ubiquitin-mediated signaling plays a vital role in regulating immune responses and maintaining cellular integrity in eukaryotic systems. This post-translational modification typically involves the covalent attachment of ubiquitin or ubiquitin-like proteins (Ubls) through an orchestrated cascade of E1 activating enzymes, E2 conjugating enzymes, E3 ligases, as well as deubiquitinases (DUBs) that reverse the modification. In the context of innate immunity, ubiquitination regulates numerous signaling pathways, including those initiated by pattern recognition receptors (PRRs), where polyubiquitin chains act as scaffolds for the assembly of immune signaling complexes (Bhoj & Chen, 2009; Jiang & Chen, 2012). Among interferon-induced Ubls, interferon-stimulated gene 15 (ISG15) exerts antiviral effects through both conjugated and unconjugated forms. ISG15 enhances host immune responses through non-covalent interactions and cytokine-like activity. Upon conjugation to target proteins—a process termed ISGylation—ISG15 perturbs viral replication by covalently modifying viral components, disrupting oligomeric assembly, altering structural architecture, or impairing essential protein functions (Perng & Lenschow, 2018). Ubiquitination-like antiviral strategies have also been identified in bacteria. The recently described BilABCD (bacterial ISG15-like gene ABCD) operon encodes a complete conjugation pathway composed of a Ubl modifier (BilA), an E1-like enzyme (BilD), an E2-like enzyme (BilB), and a deubiquitinase (BilC). Upon phage infection, this system conjugates the Ubl to phage-encoded proteins, thereby disrupting virion assembly and preventing the production of infectious progeny (Chambers et al., 2024). The BilABCD system has been proposed as a functional counterpart of the eukaryotic ISG15 pathway, as both systems deploy a diubiquitin-like Ubl together with a dedicated enzymatic cascade to restrict viral replication. Although the ISG15 pathway likely emerged after eukaryogenesis and lacks direct descent from bacterial or archaeal ancestors, the strong mechanistic convergence between BilABCD and ISG15 strongly suggests that ubiquitin-mediated protein conjugation represents a robust and versatile antiviral strategy across both prokaryotic and eukaryotic domains (Chambers et al., 2024; Freitas et al., 2020).

TIR: TIR domains constitute conserved signaling modules that orchestrate innate immune responses across all domains of life. In eukaryotes, TIR domains mediate intracellular signal transduction downstream of TLRs, initiating cascades through five adaptor families—MyD88, TIRAP, TRIF, TRAM, and SARM—which couple downstream kinases to activate

transcriptional programs driving inflammatory and interferon responses (O'Neill & Bowie, 2007). Prokaryotes also utilize TIR-dependent signaling pathways. For instance, in the previously discussed Thoeris system, the TIR domain in ThsB catalyzes the synthesis of cyclic ADP-ribose isomers, which serve as messengers to activate ThsA, leading to NAD^+ depletion (Ofir et al., 2021). Notably, TIR domains also possess enzymatic activity. For example, the adaptor protein SARM1 (sterile alpha and TIR motif-containing 1) functions as an NADase, hydrolyzing NAD^+ into NAM, ADPR, and cADPR to mediate axonal degeneration following cellular injury or stress (Essuman et al., 2017; Figley & Diantonio, 2020). Similar NAD-cleaving functions have been described in plant NLR immune receptors, where TIR-mediated NAD^+ hydrolysis triggers hypersensitive responses and cell death upon pathogen detection (Wan et al., 2019). TIR domains play a central role in bacterial antiviral defense through their intrinsic NADase activity, which mediates NAD^+ depletion and consequently blocks phage propagation. One representative example is the Pycsar system, in which a low-abundance effector containing a TIR domain is activated by the signaling molecule cUMP to rapidly degrade intracellular NAD^+, leading to Abi (Patel et al., 2022). Similarly, bacterial STING proteins harboring TIR domains undergo oligomerization upon binding cyclic dinucleotides, thereby activating their NADase activity (Morehouse et al., 2020). Collectively, these systems highlight a conserved antiviral paradigm in which TIR-mediated NAD^+ depletion suppresses phage replication. Consistent with this model, large-scale genetic screens in *Escherichia coli* have uncovered multiple TIR-domain proteins with robust antiphage activity, reinforcing the prevalence of TIR-based defense in bacteria and revealing deep evolutionary parallels with plant and animal immune networks (Wang et al., 2024b).

Nucleotide-degrading enzymes: Nucleotide-degrading enzymes represent a key antiviral strategy in bacteria. For example, dCTP deaminase converts dCTP into deoxyuracil nucleotides, while dGTPase hydrolyzes dGTP into deoxyguanosine, eliminating the phosphate group. Both enzymes selectively degrade intracellular dNTP pools, thereby impairing phage DNA replication. dGTPase shares distant homology with the human antiviral factor SAMHD1 (Tal et al., 2022), a multifunctional restriction factor that hydrolyzes dNTPs to restrict retroviral replication, including HIV-1 (Lahouassa et al., 2012). Emerging evidence indicates that SAMHD1-mediated restriction extends beyond dNTP depletion to include interference with DNA replication, suppression of gene expression, disruption of viral particle assembly, and inhibition of lipid metabolism (Cheung et al., 2025). This suggests that there may be unidentified SAMHD1-like proteins in bacteria with similar or variant functions, which may play a yet-to-be-revealed role in defending against MGEs.

Homologous links between bacterial defense systems and eukaryotic immunity: origins and implications

The discovery of structurally homologous and functionally convergent antiviral systems in bacteria and eukaryotes indicates that core components of innate immunity either originated prior to domain divergence or emerged through repeated molecular innovation in response to shared viral threats. Although bacterial and eukaryotic immune architectures exhibit substantial divergence, evolutionary pressures imposed by viral predation have likely driven the emergence of conserved molecular strategies, including nucleic acid sensing, cyclic nucleotide signaling, and programmed cell death. For instance, the cyclic dinucleotides synthesized by prokaryotic CBASS modules mirror the role of second messengers in eukaryotic innate immune cascades, underscoring deep functional parallels.

Archaeal lineages, particularly the Asgard superphylum, serve as a crucial phylogenetic bridge in reconstructing the evolutionary trajectory of antiviral defense. Recent genomic analyses of Asgard archaeans—considered the closest relatives of eukaryotic ancestors—have identified a broad repertoire of antiviral defense modules with homologs in both bacterial and eukaryotic systems, including Agos, Viperin, and CBASS operons. Phylogenetic reconstructions suggest that archaeal Ago (arAgo) proteins are the likely ancestors of eAgo proteins, as evidenced by conserved PIWI and MID domains. pAgos likewise appear to have originated from archaeal lineages (Leão et al., 2024). Similarly, NLR and Viperin proteins display striking sequence and structural conservation between archaeal and eukaryotic representatives, suggesting shared evolutionary origin. Collectively, these findings support the hypothesis that early eukaryotes—including the last eukaryotic common ancestor (LECA)—inherited and integrated antiviral immunity from various bacteria and archaea, particularly Asgard archaea, thereby facilitating the emergence of modern-day eukaryotes. These observations offer valuable insights into virus-host coevolution, the origins of the eukaryotic immune system, and the development of novel antiviral strategies (Leão et al., 2024).

Homology between immune components across domains of life provides a powerful basis for predicting and identifying novel antiviral systems. Recent investigations in *Pseudomonas aeruginosa* uncovered multiple proteins with structural similarity to eukaryotic antiviral factors, enabling the accurate prediction and experimental confirmation of antiphage activity. These components include two key proteins, NucS and SfsA, both containing unique NACHT domains structurally analogous to those found in eukaryotic inflammasomes, and both implicated in phage resistance. Another component, 6A-MBL, incorporates ubiquitin-related elements, suggesting that bacteria may employ ubiquitin-like strategies to counteract viral infection, mirroring mechanisms used in eukaryotic immunity (Van Den Berg et al., 2024). Complementary studies have extended this comparative framework to eukaryotic systems. Using phylogeny-guided approaches, Cury et al. (2024) identified homologs of bacterial defense components Mokosh, Lamassu, and Elos in eukaryotes, namely anti-transposon piRNA pathway proteins, phosphopeptide-binding domain 1 (FHAD1)/chymotrypsin-C (CTRC), and GTPases of immunity-associated proteins (GIMAPs), all exhibiting antiviral activity. Moreover, Bonhomme et al. (2025) investigated eukaryotic homologs of the bacterial SIR2 protein, a known antiphage factor, and discovered a novel protein family, SIRims. Functional studies revealed that the SIRim homolog SIRal participates in human innate immune responses. These findings underscore the growing utility of cross-domain homology in guiding the discovery of uncharacterized immune systems. Advances in computational biology have further accelerated this process. Deep learning models trained on single-cell transcriptomic data have demonstrated the capacity to identify conserved regulatory features across evolutionary boundaries (Zhang et al., 2024c), while machine learning tools trained on bacterial

defense homologs have outperformed conventional guilt-by-association approaches in predicting novel immune elements (Deweirdt et al., 2025). Building on these advances, systematic integration of bacterial and eukaryotic immune datasets could facilitate the construction of cross-domain training frameworks that operate bidirectionally. Such approaches would enhance the discovery of novel bacterial defense mechanisms while also uncovering previously unrecognized components of eukaryotic immunity, offering a unified view of immune evolution across domains.

These findings indicate not only a functional convergence between bacterial and eukaryotic antiviral responses but also the possibility that certain mechanisms originated in ancient cellular lineages and were either conserved or convergently evolved in diverse taxa (Abavisani et al., 2024). Some anti-defense proteins may therefore interfere with both bacterial and eukaryotic immunity by targeting conserved domains. Supporting this view, recent evidence shows that certain phage-encoded proteins can bind TIR domains and cGAS-like proteins in plants and animals, thereby inhibiting host immune signaling through active-site blockade. This cross-domain interference raises the potential for developing phage-derived immunomodulators to treat autoimmune or hyperinflammatory disorders (Yirmiya et al., 2025). In parallel, several homologous modules between bacterial defense systems and eukaryotic immunity retain similar antiviral roles, while others appear to have been repurposed for distinct physiological functions. For example, the recently characterized DRT10 system shows evolutionary connections to the eukaryotic telomerase repeat addition mechanism (Tang et al., 2025). Likewise, homologs of Pycsar-associated enzymes capable of synthesizing or degrading cyclic pyrimidine nucleotides (cCMP and cUMP) have been identified in eukaryotic cells, although the biological significance of these pathways remains unclear (Hobbs & Kranzusch, 2024). These observations suggest that immune modules shaped by viral conflict in bacteria may have diversified in eukaryotes to fulfill broader functional roles beyond host defense.

APPLICATIONS

The distinctive functions of bacterial defense systems have enabled their wide-ranging application across various scientific and industrial sectors. This section first presents the biotechnological innovations derived from these systems, followed by an overview of their implementation in fermentation engineering, agriculture, animal husbandry, and medical diagnostics (Figure 3). By synthesizing current applications across these fields, the analysis highlights critical attributes that may define defense systems with high translational potential.

Application of antiphage defense systems in biotechnology

Bacterial antiphage defense systems constitute a versatile and rapidly expanding toolkit for biotechnology. Prominent examples currently applied for translational purposes include CRISPR-Cas, pAgo, RM systems, and TA systems. These systems operate through diverse molecular mechanisms and have been adapted for gene editing, plasmid stabilization, inducible gene regulation, and nucleic acid detection.

RM systems, among the earliest characterized prokaryotic immune strategies, have proven foundational in molecular biotechnology. REases can recognize and cleave specific DNA sequences and are widely used in recombinant DNA technology, genome mapping, and DNA sequence analysis (Cheng et al., 2018; Guppy et al., 2018; Matsumura, 2015). More recently, REases have gained attention in the field of precision diagnostics. Notably, aberrant DNA methylation patterns are increasingly recognized as early biomarkers of various cancers (Werner et al., 2017). Methylation-sensitive restriction enzymes can distinguish between methylated and unmethylated DNA targets, enabling site-specific epigenetic profiling with high sensitivity (Wang et al., 2021b). This approach offers substantial clinical utility for early cancer detection and longitudinal monitoring, highlighting the translational potential of RM-derived enzymes well beyond their traditional roles in basic research.

The Type II CRISPR-Cas system, exemplified by CRISPR-Cas9, functions as a programmable defense platform that enables precise targeting and cleavage of dsDNA. Specificity is directed by a single-guide RNA (sgRNA), which guides Cas9 to complementary genomic sequences adjacent to a protospacer-adjacent motif (PAM). Target cleavage is executed by the RuvC and HNH nuclease domains, each incising one DNA strand. This modular recognition mechanism forms the foundation of CRISPR-Cas9-based genome editing. Rational engineering of the sgRNA spacer enables targeted intervention at virtually any PAM-compatible locus, facilitating a wide array of genetic manipulations (Jiang et al., 2013; Jinek et al., 2012; Li et al., 2015). Subsequent advances have produced refined Cas9 variants tailored for improved specificity and functional versatility. For example, nickase Cas9 (nCas9), created by introducing a point mutation in one of its nuclease domains, generates a single-strand break and reduces genotoxicity, enabling high-fidelity genome modification (Vu et al., 2024). Building upon this, base editors (BEs) were subsequently developed by coupling nCas9 to DNA deaminases, allowing targeted single-base conversions without double-strand cleavage. However, despite their utility, deaminase-based systems raise concerns regarding off-target editing and context-dependent deamination (Grünewald et al., 2019; Jin et al., 2019; Komor et al., 2016; Zhou et al., 2019). To address these limitations, prime editing (PE) was developed by fusing nCas9 to a reverse transcriptase, enabling precise substitutions, insertions, and deletions using a PE guide RNA (Kantor et al., 2020). Although PE circumvents deaminase-associated risks, its efficiency remains highly variable across loci. In response, increasing attention has turned toward the development of deaminase-free strategies. Glycosylase-based base editors (GBEs), which fuse cytosine- or thymine-DNA glycosylases to nCas9 (Ye et al., 2024), offer improved specificity and reduced molecular complexity while supporting single-nucleotide resolution editing (Tong et al., 2023, 2024). Deactivated Cas9 (dCas9), a catalytically inactive variant lacking both RuvC and HNH nuclease activities, binds specific DNA sequences without inducing cleavage. This DNA-binding capability enables transcriptional repression by obstructing RNA polymerase recruitment or elongation, forming the basis of CRISPR interference (CRISPRi) (Qi et al., 2013). Moreover, dCas9 can also be fused with regulatory elements for locus-specific epigenetic modulation, used in live-cell imaging to visualize chromatin dynamics, or employed in nucleic acid detection platforms where dCas9/single-guide RNA (sgRNA) complexes act as highly specific recognition modules enabling sensitive pathogen identification (Butterfield et al., 2025; Duan

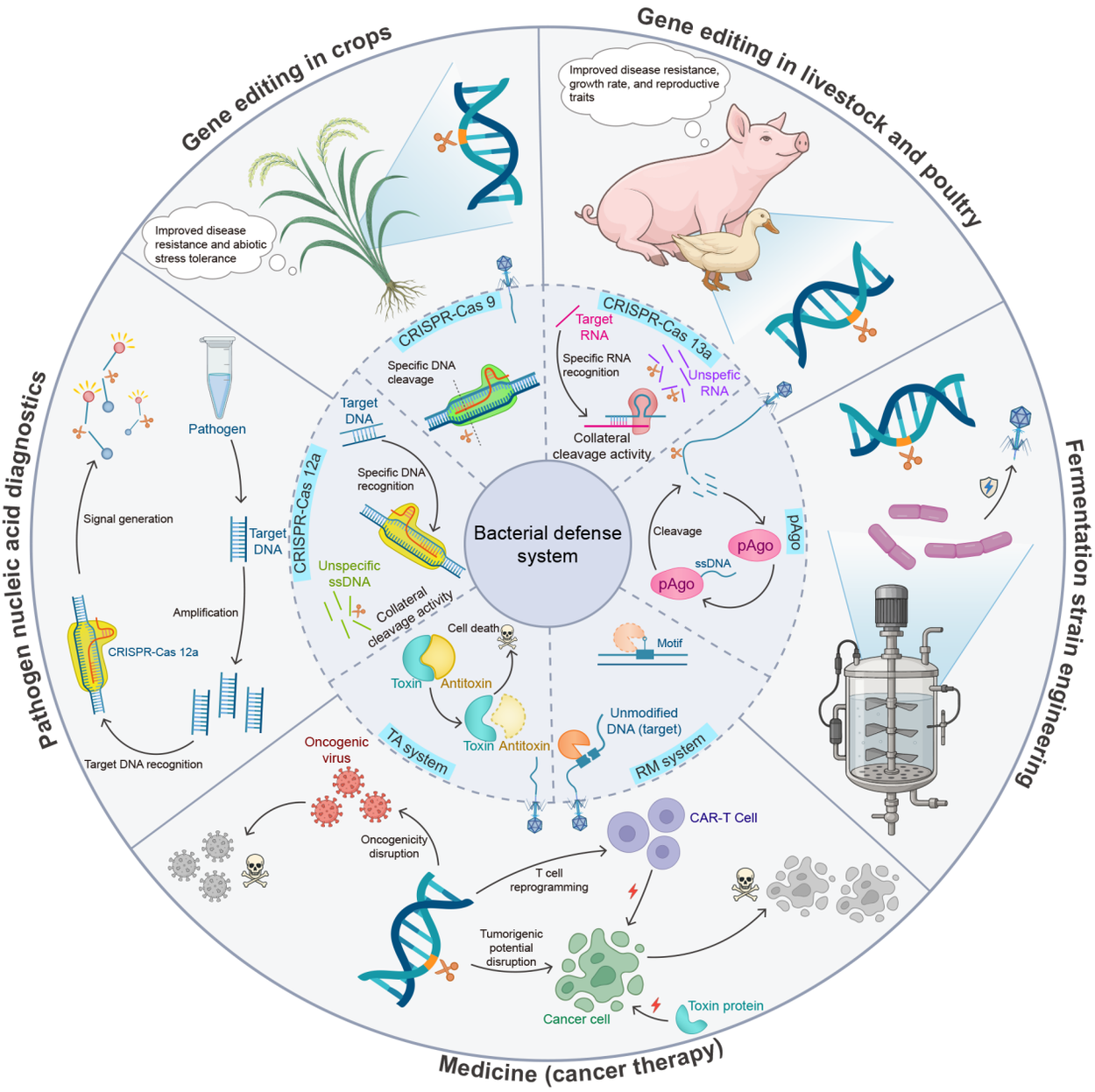

Figure 3 Application of defense systems

Schematic summary of key bacterial defense systems that have given rise to derivative biotechnologies, including Clustered Regularly Interspaced Short Palindromic Repeats-CRISPR-associated proteins (CRISPR-Cas) 9, CRISPR-Cas12, CRISPR-Cas13, prokaryotic Argonautes (pAgo), restriction-modification (RM) systems, and toxin-antitoxin (TA) systems. It also outlines their diverse applications across agriculture and animal husbandry, diagnostics and therapeutics, and fermentation engineering. CRISPR-Cas9 and pAgo platforms are primarily utilized for genome editing, enabling trait improvement in crops and livestock (Raza et al., 2022), targeted gene therapies in oncology (Shojaei Baghini et al., 2022), immune cell engineering (Pan et al., 2022), and microbial strain optimization (Dong et al., 2023; Zou et al., 2022). CRISPR-Cas12 and CRISPR-Cas13 have emerged as powerful tools for nucleic acid detection, particularly for pathogen identification in clinical diagnostics (Chen et al., 2018). The TA system enables targeted cell elimination, including cancer cells (Qiu et al., 2022), by modulating the balance between toxin and antitoxin components. Although RM systems are seldom directly applied as standalone tools, their restriction endonucleases remain fundamental components in gene cloning and molecular biology workflows (Cheng et al., 2018; Guppy et al., 2018; Matsumura, 2015).

et al., 2018; Li et al., 2024c; Wang et al., 2025a). dCas9 has additionally been incorporated into CRISPR-Cas3 platforms for large-fragment genome deletions, overcoming limitations of standard Cas9 excision (Li et al., 2024b). Other engineered Cas9 variants, including hyper-accurate Cas9 and HeFSpCas9, have been developed to enhance editing precision and minimize off-target effects (Chen et al., 2017; Kulcsár et al., 2017). Beyond Cas9 and its variants, a diverse array of CRISPR-Cas systems have been adapted for DNA and RNA manipulation. For instance, Cas12a and the Cascad-Cas3 complex have been utilized for genome editing and nucleic acid detection—Cas12a exhibits collateral cleavage activity, while Cas3 enables large-scale DNA degradation (Csörgő et al., 2020; Liu et al., 2024; Ma et al., 2022). In contrast, the Cas13 family specifically targets RNA. Among them, Cas13d (also known as CasRx) and Cas13X/Y enable efficient RNA editing (Zhu et al., 2024). Notably, Cas13a, with its strong collateral cleavage activity, has been widely adopted

in high-sensitivity diagnostic platforms such as SHERLOCK (Kellner et al., 2019).

The pAgo system shares functional similarities with CRISPR-Cas, enabling sequence-specific cleavage of target DNA. However, unlike CRISPR-Cas systems or classical restriction enzymes, pAgo proteins are not constrained by PAM sequences or fixed recognition sites, allowing more flexible and programmable targeting for genome editing, nucleic acid detection, and synthetic restriction applications (Enghiad & Zhao, 2017; Sun et al., 2023; Xiong et al., 2023). Recent advances include the development of PNP and PNFP editing platforms, which leverage peptide nucleic acid (PNA)-assisted strand invasion and FokI endonuclease fusions to achieve precise dsDNA cleavage under physiological conditions (Marsic et al., 2023; Wang et al., 2024a). Moreover, given that early pAgos (e.g., TtAgo, MpAgo) generally require elevated temperatures (>55°C), limiting their broader utility, there is increasing interest in identifying pAgos that function at lower temperatures (Kaya et al., 2016; Swarts et al., 2014a). Accordingly, efforts have shifted toward characterizing mesophilic pAgos, including ChAgo, PrAgo, and NgAgo, which retain nuclease activity at moderate temperatures (Lee et al., 2021; Peng et al., 2025; Xu et al., 2024).

TA systems, representative of phage-triggered Abi mechanisms, have emerged as programmable components for synthetic biology and biosafety engineering (Lin et al., 2023; Martin et al., 2025). TA-based selective killing modules, composed of a stable toxin and a labile antitoxin, offer programmable control and host versatility, enabling diverse applications such as plasmid maintenance, controlled protein expression, and targeted cancer cell elimination (Chemla et al., 2025; Qiu et al., 2022; Sevillano et al., 2017; Suzuki et al., 2005). For instance, co-expression of an antitoxin with a target protein enables selective survival of only those cells that successfully produce the desired recombinant product, while non-expressing cells are eliminated by toxin activity (Nehlsen et al., 2010). This conditional survival mechanism allows TA systems to act as intracellular filters that couple gene expression to cell viability.

Application of antiphage defense systems in crop and livestock production

Accelerating global food demand and intensifying climate change require transformative solutions to enhance both agricultural yield and stress tolerance (Tyagi et al., 2020; Zhu et al., 2020). Traditional genome editing tools such as zinc finger nucleases (ZFNs), although promising, have been hampered by the technical complexity of protein engineering and prohibitively long development timelines, limiting their scalability in plant and animal systems in agricultural applications (Wright et al., 2005). In contrast, CRISPR-Cas platforms have revolutionized the landscape of agricultural biotechnology by offering a highly programmable, efficient, and target-specific editing approach applicable across diverse taxa (Jabbar et al., 2021; Zhu et al., 2020).

In animal production, CRISPR-based genome interventions have been applied to improve productivity, disease resistance, and nutritional traits (Raza et al., 2022). In cattle, nCas9-enabled insertion of the *NRAMP1* gene generated lines resistant to tuberculosis, with significantly reduced off-target effects compared to conventional Cas9 (Gao et al., 2017). In pigs, CRISPR-Cas9-mediated knockout of viral entry receptors *CD163* and *pAPN* produced resistance to porcine reproductive and respiratory syndrome (PRRS) and transmissible gastroenteritis virus (TGEV) (Xu et al., 2020). In chickens, Challagulla et al. (2021) constructed a Tol2 transposon CRISPR-Cas9 system targeting the early infected-cell polypeptide-4 (ICP4) of Marek's disease virus (MDV), a key factor in MDV replication. They successfully obtained transgenic chickens that stably expressed Cas9 and ICP4-gRNAs (gICP4). This system can significantly inhibit the replication of MDV in chickens. CRISPR technologies have also been used to delete allergenic beta-lactoglobulin (BLG) from dairy animals and replace it with genes encoding beneficial proteins, enabling development of hypoallergenic and nutritionally enriched milk (Koloskova et al., 2021). In sheep, precise introduction of the *FecB* allele into *BMPRIB* using CRISPR-Cas9 produced a high-fecundity lineage with a 62% increase in lambing rate, without compromising wool quality (Zhang et al., 2025b).

CRISPR-Cas systems have driven extensive innovation in crop genome engineering. Cytosine base editing platforms, including those incorporating high-efficiency cytidine deaminases derived from killer whales (Zhu et al., 2020), have enabled targeted edits to improve agronomic traits such as yield, stress tolerance, and nutritional quality. In rice, targeted modification of the *OsLOGL5* gene using CRISPR-Cas9 significantly enhanced grain yield under drought and nitrogen-limited conditions without affecting plant architecture (Wang et al., 2020). In soybean, disruption of *GmFAD2-1A* and *GmFAD2-1B*—genes involved in polyunsaturated fatty acid synthesis—resulted in lines with elevated oleic acid levels and improved monounsaturated fatty acid profiles (Fu et al., 2022). In tomato, CRISPR-mediated inactivation of *SlGAD2* lowered gamma-aminobutyric acid (GABA) accumulation and conferred increased resistance to bacterial wilt disease caused by *Ralstonia solanacearum* (Zhao et al., 2025).

Application of antiphage defense systems in nucleic acid diagnostics and medicine

Defense system-derived biotechnologies offer remarkable versatility and responsiveness for advancing diagnostics and therapeutic interventions in human medicine. Among these, CRISPR-Cas has become the most widely adopted bacterial immune system for molecular diagnostics, driven by trans-cleavage (also known as collateral cleavage) activity. Unlike their canonical sequence-specific nuclease function, certain Cas effectors cleave non-targeted single-stranded nucleic acids upon activation by a cognate target sequence. This trans-cleavage property, when combined with quenched fluorescent probes, enables highly sensitive and specific signal generation—fluorescence is emitted upon probe cleavage, signaling target detection (Hillary & Ceasar, 2023). Cas12 and Cas13 represent the principal systems harnessed for these applications. Cas12 effectors recognize dsDNA targets and catalyze trans-cleavage of ssDNA, forming the basis of the DNA Endonuclease-Targeted CRISPR Trans Reporter (DETECTR) platform (Chen et al., 2018). Building on this, recent advances have yielded dual-enzymatic Cas12a systems and paper-based microfluidic devices integrated with Cas12a (Guan et al., 2025; Tong et al., 2025). In contrast, Cas13 targets RNA and mediates trans-cleavage of ssRNA, enabling detection strategies such as SHERLOCK. As Cas13 activation requires RNA targets, target DNA must first undergo transcription to RNA, with ssRNA used as the reporter (Ackerman et al., 2020; Kellner et al., 2019). Cas13a-based detection platforms have also been developed for one-pot multiplex assays and lateral-flow immunochromatographic formats suitable for rapid pathogen detection (Shang et al.,

2024; Wang et al., 2025d). Compared to conventional methods, CRISPR-based diagnostics offer greater speed, simplicity, and sensitivity, with expanding utility in oncology, infectious disease surveillance, and environmental biosensing (Qian et al., 2023; Wang et al., 2023a). Beyond CRISPR systems, non-CRISPR prokaryotic defense mechanisms are gaining interest for diagnostic innovation. For example, strategies based on programmable pAgo proteins and SspE-derived cleavage activities are under active development (Shuai et al., 2023; Sun et al., 2023), broadening the molecular toolkit available for precision diagnostics.

In therapeutic applications, CRISPR-based technologies have emerged as the dominant bacterial defense-derived platforms, with broad utility across pathogen clearance, cancer treatment, immune enhancement, and genetic disorder correction (Laurent et al., 2024; Peng et al., 2024; Qian et al., 2023; Shojaei Baghini et al., 2022; Zeng et al., 2024). For infectious disease, CRISPR-based systems enable precise targeting of bacterial antibiotic resistance genes, virulence factors, and associated MGEs (e.g., plasmids), offering a powerful strategy to combat multidrug-resistant pathogens (Folliero et al., 2022; Li et al., 2023; Qian et al., 2023; Yao et al., 2022). In antiviral settings, cleavage of essential viral genomic regions or disruption of host-virus interaction sites can facilitate effective viral suppression or elimination (Dong et al., 2015; Lin et al., 2021; Zeng et al., 2024). In oncology, CRISPR-based technologies support the selective inactivation of genes that drive tumor proliferation, migration, and angiogenesis. A growing catalog of validated gene targets has already entered clinical evaluation, underscoring the translational momentum of CRISPR-directed cancer therapeutics (Shojaei Baghini et al., 2022). Concurrently, genome engineering strategies are being deployed to reprogram immune cells—including CAR-NK cells, CAR-macrophages, and CAR-T cells, offering a highly specific strategy for cancer cell elimination (Pan et al., 2022; Peng et al., 2024). In the context of genetic disease, *in vivo* and *ex vivo* delivery of CRISPR constructs is being applied to correct pathogenic mutations in conditions such as β-thalassemia and hemophilia (Laurent et al., 2024; Shojaei Baghini et al., 2022), advancing toward durable and potentially curative interventions.

Beyond CRISPR-Cas platforms, TA systems have also been explored for therapeutic potential, primarily relying on the cytotoxic activity of the toxin component. A major challenge lies in achieving selective cytotoxicity toward target cells while sparing healthy tissue (Boss & Kędzierska, 2023). For example, modified derivatives of the PepA1 toxin have been engineered to retain antibacterial activity while eliminating toxicity to human cells (Solecki et al., 2015). In cancer therapy, TA systems have been embedded into conditionally activated gene circuits for tumor-selective activation. For instance, constructs such as mazF/mazE have been applied to induce apoptosis in malignant cells with minimal off-target effects (Qiu et al., 2022; Shapira et al., 2017).

Application of antiphage defense systems in fermentation engineering

Defense systems not only serve as engineering tools but also provide a molecular arsenal for conferring phage resistance. Phage contamination remains a persistent obstacle across a range of fermentation processes (Baltz, 2018), often causing reduced yield or complete process failure. Documented examples include acetone-butanol fermentation by *Clostridium madisonii*, 1,3-propanediol fermentation by *Klebsiella pneumoniae*, L-amino acid fermentation by *Brevibacterium flavum*, and lactic acid fermentations in yogurt and cheese manufacturing (Jones et al., 2000; Koptides et al., 1992; Murphy et al., 2013; Shen et al., 2016). Consequently, the industry has implemented various physical and operational countermeasures, including strain rotation, airflow management, process optimization, and equipment sanitation (Los, 2012; Wünsche, 1989). Beyond these conventional approaches, advances in understanding natural bacterial defense systems have enabled the development of genetically engineered fermentation strains, offering an effective and targeted strategy for phage control (Baltz, 2018). For example, expression of CRISPR-based plasmids encoding phage-specific guide RNAs can protect host strains from lytic infection while maintaining fermentation performance. In this context, CRISPR-Cas functions as an exogenous defense mechanism rather than a genome editing tool (Dong et al., 2023; Halter & Zahn, 2018; Jakutyte-Giraitiene & Gasiunas, 2016). Defense-oriented strain engineering may also involve multiplexed strategies. In one example, simultaneous introduction of the SspABCD–SspE system and receptor modification via gene editing conferred broad-spectrum resistance against diverse phages (Zou et al., 2022). Alternatively, as many bacterial strains naturally encode CRISPR arrays, synthetic plasmids bearing phage-derived protospacers can be used to stimulate targeted spacer acquisition, effectively arming the strain against specific viral threats (Hynes et al., 2016).

CONCLUSION

Despite considerable mechanistic diversity, bacterial antiviral defense systems follow a unifying logic centered on detection of foreign elements, activation of intracellular responses, and deployment of effectors to eliminate the invader or induce abortive strategies to protect the broader population. This tripartite architecture echoes the logic of eukaryotic immune pathways, and many bacterial defense components exhibit clear structural and evolutionary homology to components of animal immunity. Such parallels highlight a conserved molecular framework that informs both fundamental understanding of immune strategies and cross-domain discovery of novel mechanisms between bacterial and eukaryotic systems (Bonhomme et al., 2025; Van Den Berg et al., 2024). Building on this foundation, compact and mechanistically tractable bacterial systems have emerged as powerful tools for biotechnology, offering advantages of precision, scalability, and low genetic burden, with broad applications across medicine, agriculture, animal husbandry, and fermentation industries.

Nonetheless, key challenges remain. As the catalog of bacterial defense systems continues to expand, their biological functions and ecological roles remain poorly understood—especially in natural or complex microbial communities, where interactions with MGEs and their anti-defense strategies shape intricate co-evolutionary dynamics. From a comparative perspective, further investigation is required to clarify the structural and functional homology between bacterial defense components and elements of the animal immune system. To date, most homologous systems identified in bacteria act through passive defense mechanisms, limiting phage replication without direct neutralization. This pattern raises the possibility that additional

homologous systems, particularly those employing active strategies against MGEs, have yet to be discovered. Identifying such systems would further illuminate the evolutionary links between bacterial defenses and eukaryotic immune pathways. In parallel, translational applications of defense-derived tools face substantial challenges, particularly in achieving targeted and controllable delivery across diverse hosts and environments. These limitations are compounded by technical barriers such as off-target effects, host toxicity, restricted target range, and incomplete mechanistic understanding. Overcoming these obstacles will require the development of delivery platforms that ensure precision, safety, and tunable activity, alongside rational system engineering strategies. Future efforts should therefore emphasize modular design, context-specific regulation of expression, and synergistic integration of multiple defense systems to enable broader, safer, and more effective deployment in clinical, agricultural, and industrial settings.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

Conceptualization: J.H.L.; Writing–Original draft preparation: G.Z.L., Z.Q.L., and C.Y.L.; Writing–Review & Editing: J.H.L., G.Z.L., J.Y., and Z.L.X.; Visualization: G.Z.L., Z.Q.L., and C.Y.L. All authors read and approved the final version of the manuscript.

REFERENCES

Aakre CD, Phung TN, Huang D, et al. 2013. A bacterial toxin inhibits DNA replication elongation through a direct interaction with the β sliding clamp. *Molecular Cell*, **52**(5): 617–628.

Abavisani M, Faraji S, Ansari B, et al. 2024. Exploring the evolutionary links: innate immunity in bacteria and eukaryotes. *Process Biochemistry*, **147**: 240–256.

Ackerman CM, Myhrvold C, Thakku SG, et al. 2020. Massively multiplexed nucleic acid detection with Cas13. *Nature*, **582**(7811): 277–282.

Altae-Tran H, Kannan S, Suberski AJ, et al. 2023. Uncovering the functional diversity of rare CRISPR-Cas systems with deep terascale clustering. *Science*, **382**(6673): eadi1910.

Aravind L, Koonin EV. 1998. The HORMA domain: a common structural denominator in mitotic checkpoints, chromosome synapsis and DNA repair. *Trends in Biochemical Sciences*, **23**(8): 284–286.

Arber W, Dussoix D. 1962. Host specificity of DNA produced by *Escherichia coli*: I. Host controlled modification of bacteriophage λ. *Journal of Molecular Biology*, **5**(1): 18–36.

Athukoralage JS, White MF. 2021. Cyclic oligoadenylate signaling and regulation by ring nucleases during type III CRISPR defense. *RNA*, **27**(8): 855–867.

Baca CF, Marraffini LA. 2025. Nucleic acid recognition during prokaryotic immunity. *Molecular Cell*, **85**(2): 309–322.

Baltz RH. 2018. Bacteriophage-resistant industrial fermentation strains: from the cradle to CRISPR/Cas9. *Journal of Industrial Microbiology and Biotechnology*, **45**(11): 1003–1006.

Banh DV, Roberts CG, Morales-Amador A, et al. 2023. Bacterial cGAS senses a viral RNA to initiate immunity. *Nature*, **623**(7989): 1001–1008.

Barrangou R, Fremaux C, Deveau H, et al. 2007. CRISPR provides acquired resistance against viruses in prokaryotes. *Science*, **315**(5819): 1709–1712.

Beck IN, Picton DM, Blower TR. 2022. Crystal structure of the BREX phage defence protein BrxA. *Current Research in Structural Biology*, **4**: 211–219.

Bernheim A, Millman A, Ofir G, et al. 2021. Prokaryotic viperins produce diverse antiviral molecules. *Nature*, **589**(7840): 120–124.

Bhoj VG, Chen ZJ. 2009. Ubiquitylation in innate and adaptive immunity. *Nature*, **458**(7237): 430–437.

Birkholz N, Jackson SA, Fagerlund RD, et al. 2022. A mobile restriction-modification system provides phage defence and resolves an epigenetic conflict with an antagonistic endonuclease. *Nucleic Acids Research*, **50**(6): 3348–3361.

Blower TR, Fineran PC, Johnson MJ, et al. 2009. Mutagenesis and functional characterization of the RNA and protein components of the *toxIN* abortive infection and toxin-antitoxin locus of *Erwinia*. *Journal of Bacteriology*, **191**(19): 6029–6039.

Bobonis J, Mitosch K, Mateus A, et al. 2022. Bacterial retrons encode phage-defending tripartite toxin–antitoxin systems. *Nature*, **609**(7925): 144–150.

Bonabal S, Darfeuille F. 2024. Preventing toxicity in toxin-antitoxin systems: an overview of regulatory mechanisms. *Biochimie*, **217**: 95–105.

Bonhomme D, Vaysset H, Ednacot EMQ, et al. 2025. A human homolog of SIR2 antiphage proteins mediates immunity via the Toll-like receptor pathway. *Science*, **389**(6758): eadr8536.

Boss L, Kędzierska B. 2023. Bacterial toxin-antitoxin systems' cross-interactions—implications for practical use in medicine and biotechnology. *Toxins*, **15**(6): 380.

Bouchard JD, Moineau S. 2004. Lactococcal phage genes involved in sensitivity to AbiK and their relation to single-strand annealing proteins. *Journal of Bacteriology*, **186**(11): 3649–3652.

Bravo JPK, Aparicio-Maldonado C, Nobrega FL, et al. 2022. Structural basis for broad anti-phage immunity by DISARM. *Nature Communications*, **13**(1): 2987.

Bravo JPK, Ramos DA, Fregoso Ocampo R, et al. 2024. Plasmid targeting and destruction by the DdmDE bacterial defence system. *Nature*, **630**(8018): 961–967.

Brenzinger S, Airoldi M, Ogunleye AJ, et al. 2024. The *Vibrio cholerae* CBASS phage defence system modulates resistance and killing by antifolate antibiotics. *Nature Microbiology*, **9**(1): 251–262.

Burroughs AM, Zhang DP, Schäffer DE, et al. 2015. Comparative genomic analyses reveal a vast, novel network of nucleotide-centric systems in biological conflicts, immunity and signaling. *Nucleic Acids Research*, **43**(22): 10633–10654.

Butterfield GL, Rohm D, Roberts A, et al. 2025. Characterization of diverse Cas9 orthologs for genome and epigenome editing. *Proceedings of the National Academy of Sciences of the United States of America*, **122**(11): e2417674122.

Carabias A, Camara-Wilpert S, Mestre MR, et al. 2024. Retron-Eco1 assembles NAD^+-hydrolyzing filaments that provide immunity against bacteriophages. *Molecular Cell*, **84**(11): 2185–2202. e12.

Challagulla A, Jenkins KA, O'Neil TE, et al. 2021. In vivo inhibition of Marek's disease virus in transgenic chickens expressing Cas9 and gRNA against ICP4. *Microorganisms*, **9**(1): 164.

Chambers LR, Ye QZ, Cai JX, et al. 2024. A eukaryotic-like ubiquitination system in bacterial antiviral defence. *Nature*, **631**(8022): 843–849.

Chaudhuri A, Halder K, Datta A. 2022. Classification of CRISPR/Cas system and its application in tomato breeding. *Theoretical and Applied Genetics*, **135**(2): 367–387.

Chemla Y, Sweeney CJ, Wozniak CA, et al. 2025. Design and regulation of engineered bacteria for environmental release. *Nature Microbiology*, **10**(2): 281–300.

Chen JS, Dagdas YS, Kleinstiver BP, et al. 2017. Enhanced proofreading governs CRISPR-Cas9 targeting accuracy. *Nature*, **550**(7676): 407–410.

Chen JS, Ma EB, Harrington LB, et al. 2018. CRISPR-Cas12a target binding unleashes indiscriminate single-stranded DNase activity. *Science*, **360**(6387): 436–439.

Cheng YH, Liaw JJ, Kuo CN. 2018. REHUNT: a reliable and open source package for restriction enzyme hunting. *BMC Bioinformatics*, **19**(1): 178.

Cheung PHH, Yang H, Wu L. 2025. dNTP depletion and beyond: the multifaceted nature of SAMHD1-mediated viral restriction. *Journal of Virology*, **99**(5): e00302–25.

Choi JS, Kim W, Suk S, et al. 2018. The small RNA, SdsR, acts as a novel type of toxin in *Escherichia coli*. *RNA Biology*, **15**(10): 1319–1335.

Chou WC, Jha S, Linhoff MW, et al. 2023. The NLR gene family: from discovery to present day. *Nature Reviews Immunology*, **23**(10): 635–654.

Cohen D, Melamed S, Millman A, et al. 2019. Cyclic GMP-AMP signalling protects bacteria against viral infection. *Nature*, **574**(7780): 691–695.

Costa AR, Van Den Berg DF, Esser JQ, et al. 2024. Accumulation of defense systems in phage-resistant strains of *Pseudomonas aeruginosa*. *Science Advances*, **10**(8): eadj0341.

Csörgő B, León LM, Chau-Ly IJ, et al. 2020. A compact Cascade-Cas3 system for targeted genome engineering. *Nature Methods*, **17**(12): 1183–1190.

Cui YQ, Dai ZK, Ouyang YF, et al. 2025. Bacterial Hachiman complex executes DNA cleavage for antiphage defense. *Nature Communications*, **16**(1): 2604.

Cury J, Haudiquet M, Hernandez Trejo V, et al. 2024. Conservation of antiviral systems across domains of life reveals immune genes in humans. *Cell Host & Microbe*, **32**(9): 1594–1607. e5.

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

Davies J. 2013. Specialized microbial metabolites: functions and origins. *The Journal of Antibiotics*, **66**(7): 361–364.

Davison WC. 1922. The bacteriolysant therapy of bacillary dysentery in children: therapeutic application of bacteriolysants; d'Herelle's phenomenon. *American Journal of Diseases of Children*, **23**(6): 531–534.

Deep A, Gu YJ, Gao YQ, et al. 2022. The SMC-family Wadjet complex protects bacteria from plasmid transformation by recognition and cleavage of closed-circular DNA. *Molecular Cell*, **82**(21): 4145–4159. e7.

Deep A, Liang QS, Enustun E, et al. 2024. Architecture and activation mechanism of the bacterial PARIS defence system. *Nature*, **634**(8033): 432–439.

Deng L, Garrett RA, Shah SA, et al. 2013. A novel interference mechanism by a type IIIB CRISPR-Cmr module in *Sulfolobus*. *Molecular Microbiology*, **87**(5): 1088–1099.

Depardieu F, Didier JP, Bernheim A, et al. 2016. A eukaryotic-like serine/threonine kinase protects staphylococci against phages. *Cell Host & Microbe*, **20**(4): 471–481.

Deweirdt PC, Mahoney EM, Laub MT. 2025. DefensePredictor: a machine learning model to discover novel prokaryotic immune systems. *bioRxiv*, doi: https://doi.org/10.1101/2025.01.08.631726

d'Herelle F. 1917. Sur un microbe invisible antagoniste des bacilles dysentériques. *Comptes Rendus de l'Académie des Sciences*, **165**: 373–375.

Ding JJ, Wang K, Liu W, et al. 2016. Pore-forming activity and structural autoinhibition of the gasdermin family. *Nature*, **535**(7610): 111–116.

Domingues S, McGovern S, Plochocka D, et al. 2008. The lactococcal abortive infection protein AbiP is membrane-anchored and binds nucleic acids. *Virology*, **373**(1): 14–24.

Dong CS, Qu L, Wang HY, et al. 2015. Targeting hepatitis B virus cccDNA by CRISPR/Cas9 nuclease efficiently inhibits viral replication. *Antiviral Research*, **118**: 110–117.

Dong YQ, Huang YF, Fan HH, et al. 2023. Characterization, complete genome sequencing, and CRISPR/Cas9 system-based decontamination of a novel *Escherichia coli* phage TR1 from fermentation substrates. *Frontiers in Microbiology*, **14**: 1230775.

Doron S, Melamed S, Ofir G, et al. 2018. Systematic discovery of antiphage defense systems in the microbial pangenome. *Science*, **359**(6379): eaar4120.

Doudna JA, Charpentier E. 2014. The new frontier of genome engineering with CRISPR-Cas9. *Science*, **346**(6213): 1258096.

Drobiazko A, Adams MC, Skutel M, et al. 2025. Molecular basis of foreign DNA recognition by BREX anti-phage immunity system. *Nature Communications*, **16**(1): 1825.

Duan JZ, Lu GQ, Hong Y, et al. 2018. Live imaging and tracking of genome regions in CRISPR/dCas9 knock-in mice. *Genome Biology*, **19**(1): 192.

Duncan-Lowey B, McNamara-Bordewick NK, Tal N, et al. 2021. Effector-mediated membrane disruption controls cell death in CBASS antiphage defense. *Molecular Cell*, **81**(24): 5039–5051. e5.

Duncan-Lowey B, Tal N, Johnson AG, et al. 2023. Cryo-EM structure of the RADAR supramolecular anti-phage defense complex. *Cell*, **186**(5): 987–998. e15.

Echols H. 1971. Lysogeny: viral repression and site-specific recombination. *Annual Review of Biochemistry*, **40**(1): 827–854.

Emond E, Holler BJ, Boucher I, et al. 1997. Phenotypic and genetic characterization of the bacteriophage abortive infection mechanism AbiK from *Lactococcus lactis*. *Applied and Environmental Microbiology*, **63**(4): 1274–1283.

Enghiad B, Zhao HM. 2017. Programmable DNA-guided artificial restriction enzymes. *ACS Synthetic Biology*, **6**(5): 752–757.

Ergun SL, Fernandez D, Weiss TM, et al. 2019. STING polymer structure reveals mechanisms for activation, hyperactivation, and inhibition. *Cell*, **178**(2): 290–301. e10.

Essuman K, Summers DW, Sasaki Y, et al. 2017. The SARM1 Toll/interleukin-1 receptor domain possesses intrinsic NAD^+ cleavage activity that promotes pathological axonal degeneration. *Neuron*, **93**(6): 1334–1343. e5.

Fatma S, Chakravarti A, Zeng XK, et al. 2021. Molecular mechanisms of the CdnG-Cap5 antiphage defense system employing 3', 2'-cGAMP as the second messenger. *Nature Communications*, **12**(1): 6381.

Feng SY, Fox D, Man SM. 2018. Mechanisms of Gasdermin family members in inflammasome signaling and cell death. *Journal of Molecular Biology*, **430**(18): 3068–3080.

Figley MD, Diantonio A. 2020. The SARM1 axon degeneration pathway: control of the NAD^+ metabolome regulates axon survival in health and disease. *Current Opinion in Neurobiology*, **63**: 59–66.

Fillol-Salom A, Martínez-Rubio R, Abdulrahman RF, et al. 2018. Phage-inducible chromosomal islands are ubiquitous within the bacterial universe. *The ISME Journal*, **12**(9): 2114–2128.

Fineran PC, Blower TR, Foulds IJ, et al. 2009. The phage abortive infection system, ToxIN, functions as a protein-RNA toxin-antitoxin pair. *Proceedings of the National Academy of Sciences of the United States of America*, **106**(3): 894–899.

Folliero V, Lama S, Franci G, et al. 2022. Casein-derived peptides from the dairy product kashk exhibit wound healing properties and antibacterial activity against *Staphylococcus aureus*: structural and functional characterization. *Food Research International*, **153**: 110949.

Fortier LC, Sekulovic O. 2013. Importance of prophages to evolution and virulence of bacterial pathogens. *Virulence*, **4**(5): 354–365.

Freitas BT, Scholte FEM, Bergeron É, et al. 2020. How ISG15 combats viral infection. *Virus Research*, **286**: 198036.

Fu MX, Chen L, Cai YP, et al. 2022. CRISPR/Cas9-mediated mutagenesis of *GmFAD2–1A* and/or *GmFAD2–1B* to create high-oleic-acid soybean. *Agronomy*, **12**(12): 3218.

Gao LA, Wilkinson ME, Strecker J, et al. 2022. Prokaryotic innate immunity through pattern recognition of conserved viral proteins. *Science*, **377**(6607): eabm4096.

Gao LY, Altae-Tran H, Böhning F, et al. 2020. Diverse enzymatic activities mediate antiviral immunity in prokaryotes. *Science*, **369**(6507): 1077–1084.

Gao YN, Luo X, Li PP, et al. 2023. Molecular basis of RADAR anti-phage supramolecular assemblies. *Cell*, **186**(5): 999–1012. e20.

Gao YP, Wu HB, Wang YS, et al. 2017. Single Cas9 nickase induced generation of *NRAMP1* knockin cattle with reduced off-target effects. *Genome Biology*, **18**(1): 13.

Gapińska M, Zajko W, Skowronek K, et al. 2024. Structure-functional characterization of *Lactococcus* AbiA phage defense system. *Nucleic Acids Research*, **52**(8): 4723–4738.

Garb J, Lopatina A, Bernheim A, et al. 2022. Multiple phage resistance systems inhibit infection via SIR2-dependent NAD^+ depletion. *Nature Microbiology*, **7**(11): 1849–1856.

George JT, Burman N, Wilkinson RA, et al. 2025. Structural basis of antiphage defence by an ATPase-associated reverse transcriptase. *Nature Communications*, **16**(1): 8459.

Georjon H, Bernheim A. 2023. The highly diverse antiphage defence systems of bacteria. *Nature Reviews Microbiology*, **21**(10): 686–700.

Getz LJ, Maxwell KL. 2024. Diverse antiphage defenses are widespread among prophages and mobile genetic elements. *Annual Review of Virology*, **11**(1): 343–362.

Ghosh S, Lahiri D, Nag M, et al. 2022. Precision targeting of food biofilm-forming genes by microbial scissors: CRISPR–Cas as an effective modulator. *Frontiers in Microbiology*, **13**: 964848.

Gizzi AS, Grove TL, Arnold JJ, et al. 2018. A naturally occurring antiviral ribonucleotide encoded by the human genome. *Nature*, **558**(7711): 610–614.

Glover SW, Schell J, Symonds N, et al. 1963. The control of host-induced modification by phage P1. *Genetical Research*, **4**(3): 480–482.

Goldfarb T, Sberro H, Weinstock E, et al. 2015. BREX is a novel phage resistance system widespread in microbial genomes. *The EMBO Journal*, **34**(2): 169–183.

Gordeeva J, Morozova N, Sierro N, et al. 2019. BREX system of *Escherichia coli* distinguishes self from non-self by methylation of a specific DNA site. *Nucleic Acids Research*, **47**(1): 253–265.

Grünewald J, Zhou RH, Garcia SP, et al. 2019. Transcriptome-wide off-target RNA editing induced by CRISPR-guided DNA base editors. *Nature*, **569**(7756): 433–437.

Gu YJ, Desai A, Corbett KD. 2022. Evolutionary dynamics and molecular mechanisms of HORMA domain protein signaling. *Annual Review of Biochemistry*, **91**(1): 541–569.

Guan X, Yang R, Zhang JY, et al. 2025. Programmable multiplexed nucleic acid detection by harnessing specificity defect of CRISPR‐Cas12a. *Advanced Science*, **12**(4): 2411021.

Guegler CK, Laub MT. 2021. Shutoff of host transcription triggers a toxin-antitoxin system to cleave phage RNA and abort infection. *Molecular Cell*, **81**(11): 2361–2373. e9.

Guppy JL, Jones DB, Jerry DR, et al. 2018. The state of "*Omics*" research for farmed penaeids: advances in research and impediments to industry utilization. *Frontiers in Genetics*, **9**: 282.

Hale CR, Zhao P, Olson S, et al. 2009. RNA-guided RNA cleavage by a CRISPR RNA-Cas protein complex. *Cell*, **139**(5): 945–956.

Halter MC, Zahn JA. 2018. Characterization of a novel lytic bacteriophage from an industrial *Escherichia coli* fermentation process and elimination of virulence using a heterologous CRISPR-Cas9 system. *Journal of Industrial Microbiology & Biotechnology*, **45**(3): 153–163.

Hardy A, Kever L, Frunzke J. 2023. Antiphage small molecules produced by bacteria–beyond protein-mediated defenses. *Trends in Microbiology*, **31**(1): 92–106.

Hays SG, Seed KD. 2020. Dominant *Vibrio cholerae* phage exhibits lysis inhibition sensitive to disruption by a defensive phage satellite. *eLife*, **9**: e53200.

Hegge JW, Swarts DC, van der Oost J. 2018. Prokaryotic Argonaute proteins: novel genome-editing tools?. *Nature Reviews Microbiology*, **16**(1): 5–11.

Heller DM, Tavag M, Hochschild A. 2017. CbtA toxin of *Escherichia coli* inhibits cell division and cell elongation via direct and independent interactions with FtsZ and MreB. *PLoS Genetics*, **13**(9): e1007007.

Henry JE, Henry RJ. 1946. Studies on the relationship between bacteriophage and bacterial host cell: I. Adsorption of phage by resistant variants of *Staphylococcus*. *Journal of Bacteriology*, **52**(4): 481–486.

Heussler GE, Cady KC, Koeppen K, et al. 2015. Clustered regularly interspaced short palindromic repeat-dependent, biofilm-specific death of *Pseudomonas aeruginosa* mediated by increased expression of phage-related genes. *mBio*, **6**(3): e00129–15.

Hillary VE, Ceasar SA. 2023. A review on the mechanism and applications of CRISPR/Cas9/Cas12/Cas13/Cas14 proteins utilized for genome engineering. *Molecular Biotechnology*, **65**(3): 311–325.

Hille F, Richter H, Wong SP, et al. 2018. The biology of CRISPR-Cas: backward and forward. *Cell*, **172**(6): 1239–1259.

Hobbs SJ, Kranzusch PJ. 2024. Nucleotide immune signaling in CBASS, Pycsar, Thoeris, and CRISPR antiphage defense. *Annual Review of Microbiology*, **78**(1): 255–276.

Höck J, Meister G. 2008. The Argonaute protein family. *Genome Biology*, **9**(2): 210.

Hogrel G, Guild A, Graham S, et al. 2022. Cyclic nucleotide-induced helical structure activates a TIR immune effector. *Nature*, **608**(7924): 808–812.

Hopfner KP, Hornung V. 2020. Molecular mechanisms and cellular functions of cGAS-STING signalling. *Nature Reviews Molecular Cell Biology*, **21**(9): 501–521.

Hör J, Wolf SG, Sorek R. 2024. Bacteria conjugate ubiquitin-like proteins to interfere with phage assembly. *Nature*, **631**(8022): 850–856.

Hou MH, Wang YC, Yang CS, et al. 2023. Structural insights into the regulation, ligand recognition, and oligomerization of bacterial STING. *Nature Communications*, **14**(1): 8519.

Hu HD, Popp PF, Hughes TCD, et al. 2025. Structure and mechanism of the Zorya anti-phage defence system. *Nature*, **639**(8056): 1093–1101.

Huang JF, Zhu KL, Gao YN, et al. 2024. Molecular basis of bacterial DSR2 anti-phage defense and viral immune evasion. *Nature Communications*, **15**(1): 3954.

Huang S, Wang KH, Mayo SL. 2023. Genome manipulation by guide-directed Argonaute cleavage. *Nucleic Acids Research*, **51**(8): 4078–4085.

Huiting E, Cao XL, Ren J, et al. 2023. Bacteriophages inhibit and evade cGAS-like immune function in bacteria. *Cell*, **186**(4): 864–876. e21.

Hunter GJE. 1947. Phage-resistant and phage-carrying strains of lactic streptococci. *Journal of Hygiene*, **45**(3): 307–312.

Hutvagner G, Simard MJ. 2008. Argonaute proteins: key players in RNA silencing. *Nature Reviews Molecular Cell Biology*, **9**(1): 22–32.

Hynes AP, Labrie SJ, Moineau S. 2016. Programming native CRISPR arrays for the generation of targeted immunity. *mBio*, **7**(3): e00202–16.

Ishino Y, Shinagawa H, Makino K, et al. 1987. Nucleotide sequence of the *iap* gene, responsible for alkaline phosphatase isozyme conversion in *Escherichia coli*, and identification of the gene product. *Journal of Bacteriology*, **169**(12): 5429–5433.

Jabbar A, Zulfiqar F, Mahnoor M, et al. 2021. Advances and perspectives in the application of CRISPR-Cas9 in livestock. *Molecular Biotechnology*, **63**(9): 757–767.

Jakutyte-Giraitiene L, Gasiunas G. 2016. Design of a CRISPR-Cas system to increase resistance of *Bacillus subtilis* to bacteriophage SPP1. *Journal of Industrial Microbiology and Biotechnology*, **43**(8): 1183–1188.

Jaskólska M, Adams DW, Blokesch M. 2022. Two defence systems eliminate plasmids from seventh pandemic *Vibrio cholerae*. *Nature*,

604(7905): 323–329.
Jenson JM, Chen ZJ. 2024. cGAS goes viral: a conserved immune defense system from bacteria to humans. *Molecular Cell*, **84**(1): 120–130.
Jenson JM, Li T, Du FH, et al. 2023. Ubiquitin-like conjugation by bacterial cGAS enhances anti-phage defence. *Nature*, **616**(7956): 326–331.
Jiang WY, Bikard D, Cox D, et al. 2013. RNA-guided editing of bacterial genomes using CRISPR-Cas systems. *Nature Biotechnology*, **31**(3): 233–239.
Jiang XM, Chen ZJ. 2012. The role of ubiquitylation in immune defence and pathogen evasion. *Nature Reviews Immunology*, **12**(1): 35–48.
Jiang XY, Li D, Sun ZW, et al. 2024. Type I BREX system defends against antibiotic-resistant plasmids in *Escherichia coli*. *Antimicrobial Agents and Chemotherapy*, **68**(3): e01128–23.
Jin S, Zong Y, Gao Q, et al. 2019. Cytosine, but not adenine, base editors induce genome-wide off-target mutations in rice. *Science*, **364**(6437): 292–295.
Jinek M, Chylinski K, Fonfara I, et al. 2012. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science*, **337**(6096): 816–821.
Johnson AG, Wein T, Mayer ML, et al. 2022. Bacterial gasdermins reveal an ancient mechanism of cell death. *Science*, **375**(6577): 221–225.
Jones DT, Shirley M, Wu XY, et al. 2000. Bacteriophage infections in the industrial acetone butanol (AB) fermentation process. *Journal of Molecular Microbiology and Biotechnology*, **2**(1): 21–26.
Ka D, Oh H, Park E, et al. 2020. Structural and functional evidence of bacterial antiphage protection by Thoeris defense system via NAD^+ degradation. *Nature Communications*, **11**(1): 2816.
Kaiser AD. 1957. Mutations in a temperate bacteriophage affecting its ability to lysogenize *Escherichia coli*. *Virology*, **3**(1): 42–61.
Kantor A, McClements ME, MacLaren RE. 2020. CRISPR-Cas9 DNA base-editing and prime-editing. *International Journal of Molecular Sciences*, **21**(17): 6240.
Kaya E, Doxzen KW, Knoll KR, et al. 2016. A bacterial Argonaute with noncanonical guide RNA specificity. *Proceedings of the National Academy of Sciences of the United States of America*, **113**(15): 4057–4062.
Kazlauskiene M, Kostiuk G, Venclovas Č, et al. 2017. A cyclic oligonucleotide signaling pathway in type III CRISPR-Cas systems. *Science*, **357**(6351): 605–609.
Kellner MJ, Koob JG, Gootenberg JS, et al. 2019. SHERLOCK: nucleic acid detection with CRISPR nucleases. *Nature Protocols*, **14**(10): 2986–3012.
Kelly A, Arrowsmith TJ, Went SC, et al. 2023. Toxin–antitoxin systems as mediators of phage defence and the implications for abortive infection. *Current Opinion in Microbiology*, **73**: 102293.
Kibby EM, Conte AN, Burroughs AM, et al. 2023. Bacterial NLR-related proteins protect against phage. *Cell*, **186**(11): 2410–2424. e18.
Koloskova EM, Ezerskiy VA, Ostrenko KS. 2021. Modifications of the beta-lactoglobulin gene in bovine and goats for correction of milk composition using CRISPR/Cas9 technology. *Journal of Livestock Science*, **12**: 8–16.
Komor AC, Kim YB, Packer MS, et al. 2016. Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. *Nature*, **533**(7603): 420–424.
Koonin EV, Makarova KS. 2019. Origins and evolution of CRISPR-Cas systems. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **374**(1772): 20180087.
Koonin EV, Makarova KS, Zhang F. 2017. Diversity, classification and evolution of CRISPR-Cas systems. *Current Opinion in Microbiology*, **37**: 67–78.
Koopal B, Potocnik A, Mutte SK, et al. 2022. Short prokaryotic Argonaute systems trigger cell death upon detection of invading DNA. *Cell*, **185**(9): 1471–1486. e19.
Koptides M, Barák I, Šlšová M, et al. 1992. Characterization of bacteriophage BFK20 from *Brevibacterium flavum*. *Microbiology*, **138**(7): 1387–1391.
Kottur J, Malik R, Aggarwal AK. 2024. Nucleic acid mediated activation of a short prokaryotic Argonaute immune system. *Nature Communications*, **15**(1): 4852.
Kranzusch PJ, Lee ASY, Wilson SC, et al. 2014. Structure-guided reprogramming of human cGAS dinucleotide linkage specificity. *Cell*, **158**(5): 1011–1021.
Kronheim S, Daniel-Ivad M, Duan Z, et al. 2018. A chemical defence against phage infection. *Nature*, **564**(7735): 283–286.
Kulcsár PI, Tálas A, Huszár K, et al. 2017. Crossing enhanced and high fidelity SpCas9 nucleases to optimize specificity and cleavage. *Genome Biology*, **18**(1): 190.
Kuntová L, Mašlaňová I, Obořilová R, et al. 2023. *Staphylococcus aureus* prophage-encoded protein causes abortive infection and provides population immunity against kayviruses. *mBio*, **14**(2): e02490–22.
Kuzmenko A, Oguienko A, Esyunina D, et al. 2020. DNA targeting and interference by a bacterial Argonaute nuclease. *Nature*, **587**(7835): 632–637.
Lahouassa H, Daddacha W, Hofmann H, et al. 2012. SAMHD1 restricts the replication of human immunodeficiency virus type 1 by depleting the intracellular pool of deoxynucleoside triphosphates. *Nature Immunology*, **13**(3): 223–228.
Laub MT, Typas A. 2024. Principles of bacterial innate immunity against viruses. *Current Opinion in Immunology*, **89**: 102445.
Laurent M, Geoffroy M, Pavani G, et al. 2024. CRISPR-based gene therapies: from preclinical to clinical treatments. *Cells*, **13**(10): 800.
Leão P, Little ME, Appler KE, et al. 2024. Asgard archaea defense systems and their roles in the origin of eukaryotic immunity. *Nature Communications*, **15**(1): 6386.
Lederberg S. 1957. Suppression of the multiplication of heterologous bacteriophages in lysogenic bacteria. *Virology*, **3**(3): 496–513.
Ledvina HE, Ye QZ, Gu YJ, et al. 2023. An E1-E2 fusion protein primes antiviral immune signalling in bacteria. *Nature*, **616**(7956): 319–325.
Lee KZ, Mechikoff MA, Kikla A, et al. 2021. NgAgo possesses guided DNA nicking activity. *Nucleic Acids Research*, **49**(17): 9926–9937.
Leroux M, Laub MT. 2022. Toxin-antitoxin systems as phage defense elements. *Annual Review of Microbiology*, **76**(1): 21–43.
Leroux M, Srikant S, Teodoro GIC, et al. 2022. The DarTG toxin-antitoxin system provides phage defence by ADP-ribosylating viral DNA. *Nature Microbiology*, **7**(7): 1028–1040.
Li J, Cheng R, Wang ZM, et al. 2024a. Structures and activation mechanism of the Gabija anti-phage system. *Nature*, **629**(8011): 467–473.
Li JZ, Zhao D, Zhang T, et al. 2024b. Precise large-fragment deletions in mammalian cells and mice generated by dCas9-controlled CRISPR/Cas3. *Science Advances*, **10**(11): eadk8052.
Li TY, Li SQ, Kang Y, et al. 2024c. Harnessing the evolving CRISPR/Cas9 for precision oncology. *Journal of Translational Medicine*, **22**(1): 749.
Li X, Bao N, Yan Z, et al. 2023. Degradation of antibiotic resistance genes by VADER with CRISPR-Cas immunity. *Applied and Environmental Microbiology*, **89**(4): e00053–23.
Li Y, Adams DW, Liu HW, et al. 2025a. Structure and activation mechanism of a Lamassu phage and plasmid defense system. *Nature Structural & Molecular Biology*, **32**(12): 2503–2516.
Li YF, Lin ZQ, Huang C, et al. 2015. Metabolic engineering of *Escherichia coli* using CRISPR-Cas9 meditated genome editing. *Metabolic Engineering*, **31**: 13–21.
Li YT, Li ZX, Yan PR, et al. 2025b. Antiviral signaling of a type III CRISPR-associated deaminase. *Science*, **387**(6736): eadr0393.
Li YY, Shen ZF, Zhang MY, et al. 2024d. PtuA and PtuB assemble into an inflammasome-like oligomer for anti-phage defense. *Nature Structural &*

Molecular Biology, **31**(3): 413–423.

Lin HF, Li G, Peng XW, et al. 2021. The use of CRISPR/Cas9 as a tool to study human infectious viruses. *Frontiers in Cellular and Infection Microbiology*, **11**: 590989.

Lin JZ, Bhoobalan-Chitty Y, Peng X. 2025. Cad1 turns ATP into phage poison. *Cell Host & Microbe*, **33**(1): 8–10.

Lin JZ, Guo YX, Yao JY, et al. 2023. Applications of toxin-antitoxin systems in synthetic biology. *Engineering Microbiology*, **3**(2): 100069.

Lindahl G, Sironi G, Bialy H, et al. 1970. Bacteriophage lambda; abortive infection of bacteria lysogenic for phage P2. *Proceedings of the National Academy of Sciences of the United States of America*, **66**(3): 587–594.

Liu HW, Roisné-Hamelin F, Beckert B, et al. 2022. DNA-measuring Wadjet SMC ATPases restrict smaller circular plasmids by DNA cleavage. *Molecular Cell*, **82**(24): 4727–4740. e6.

Liu SY, Liu HL, Wang X, et al. 2024. The immune system of prokaryotes: potential applications and implications for gene editing. *Biotechnology Journal*, **19**(2): 2300352.

Loeff L, Walter A, Rosalen GT, et al. 2025. DNA end sensing and cleavage by the Shedu anti-phage defense system. *Cell*, **188**(3): 721–733. e17.

Loenen WAM, Dryden DTF, Raleigh EA, et al. 2014. Highlights of the DNA cutters: a short history of the restriction enzymes. *Nucleic Acids Research*, **42**(1): 3–19.

Lopatina A, Tal N, Sorek R. 2020. Abortive infection: bacterial suicide as an antiviral immune strategy. *Annual Review of Virology*, **7**(1): 371–384.

Los M. 2012. Minimization and prevention of phage infections in bioprocesses. *Methods in Molecular Biology*, **834**: 305–315.

Lowey B, Whiteley AT, Keszei AFA, 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**(1): 38–49. e17.

Ma EB, Chen K, Shi HL, et al. 2022. Improved genome editing by an engineered CRISPR-Cas12a. *Nucleic Acids Research*, **50**(22): 12689–12701.

Makarova KS, Shmakov SA, Wolf YI, et al. 2025. An updated evolutionary classification of CRISPR–Cas systems including rare variants. *Nature Microbiology*, **10**(12): 3346–3361.

Makarova KS, Wolf YI, Iranzo J, et al. 2020. Evolutionary classification of CRISPR-Cas systems: a burst of class 2 and derived variants. *Nature Reviews Microbiology*, **18**(2): 67–83.

Makarova KS, Wolf YI, Koonin EV. 2018. Classification and nomenclature of CRISPR-Cas systems: where from here?. *The CRISPR Journal*, **1**(5): 325–336.

Makarova KS, Wolf YI, Snir S, et al. 2011. Defense islands in bacterial and archaeal genomes and prediction of novel defense systems. *Journal of Bacteriology*, **193**(21): 6039–6056.

Makarova KS, Wolf YI, van der Oost J, et al. 2009. Prokaryotic homologs of Argonaute proteins are predicted to function as key components of a novel system of defense against mobile genetic elements. *Biology Direct*, **4**(1): 29.

Marsic T, Gundra SR, Wang QC, et al. 2023. Programmable site-specific DNA double-strand breaks via PNA-assisted prokaryotic Argonautes. *Nucleic Acids Research*, **51**(17): 9491–9506.

Martin C, Panosetti M, Tesini E, et al. 2025. Synthetic RNA ligands as activators of type I toxin-antitoxin systems: a novel antimicrobial strategy targeting *Helicobacter pylori*. *Chemical Science*, **16**(29): 13249–13255.

Matsumura I. 2015. Why Johnny can't clone: common pitfalls and not so common solutions. *BioTechniques*, **59**(3): IV–XIII.

Mayo-Muñoz D, Li HJ, Mestre MR, et al. 2025. The role of noncoding RNAs in bacterial immunity. *Trends in Microbiology*, **33**(2): 208–222.

Mayo-Muñoz D, Pinilla-Redondo R, Birkholz N, et al. 2023. A host of armor: prokaryotic immune strategies against mobile genetic elements. *Cell Reports*, **42**(7): 112672.

Mayo-Muñoz D, Smith LM, Garcia-Doval C, et al. 2022. Type III CRISPR-Cas provides resistance against nucleus-forming jumbo phages via abortive infection. *Molecular Cell*, **82**(23): 4471–4486. e9.

McCarty RM, Somogyi Á, Lin GX, et al. 2009. The deazapurine biosynthetic pathway revealed: in vitro enzymatic synthesis of preQ$_0$ from guanosine 5'-triphosphate in four steps. *Biochemistry*, **48**(18): 3847–3852.

McGinn J, Marraffini LA. 2019. Molecular mechanisms of CRISPR-Cas spacer acquisition. *Nature Reviews Microbiology*, **17**(1): 7–12.

Mestre MR, Gao LA, Shah SA, et al. 2022. UG/Abi: a highly diverse family of prokaryotic reverse transcriptases associated with defense functions. *Nucleic Acids Research*, **50**(11): 6084–6101.

Millman A, Bernheim A, Stokar-Avihail A, et al. 2020a. Bacterial retrons function in anti-phage defense. *Cell*, **183**(6): 1551–1561. e12.

Millman A, Melamed S, Amitai G, et al. 2020b. Diversity and classification of cyclic-oligonucleotide-based anti-phage signalling systems. *Nature Microbiology*, **5**(12): 1608–1615.

Millman A, Melamed S, Leavitt A, et al. 2022. An expanded arsenal of immune systems that protect bacteria from phages. *Cell Host & Microbe*, **30**(11): 1556–1569. e5.

Molina R, Sofos N, Montoya G. 2020. Structural basis of CRISPR-Cas type III prokaryotic defence systems. *Current Opinion in Structural Biology*, **65**: 119–129.

Molineux IJ. 1991. Host-parasite interactions: recent developments in the genetics of abortive phage infections. *The New Biologist*, **3**(3): 230–236.

Morehouse BR, Govande AA, Millman A, et al. 2020. STING cyclic dinucleotide sensing originated in bacteria. *Nature*, **586**(7829): 429–433.

Morita J, Tanaka A, Komano T, et al. 1979. Inactivation of phage ϕX174 by anthracycline antibiotics, aclacinomycin A, doxorubicin and daunorubicin. *Agricultural and Biological Chemistry*, **43**(12): 2629–2631.

Muckenfuss RS, Korb C. 1928. Studies on the bacteriophage of d'Hérelle: X. toxin production by normal and by phage-resistant Shiga dysentery bacilli. *Journal of Experimental Medicine*, **48**(2): 277–283.

Murphy J, Royer B, Mahony J, et al. 2013. Biodiversity of lactococcal bacteriophages isolated from 3 Gouda-type cheese-producing plants. *Journal of Dairy Science*, **96**(8): 4945–4957.

Nehlsen K, Herrmann S, Zauers J, et al. 2010. Toxin-antitoxin based transgene expression in mammalian cells. *Nucleic Acids Research*, **38**(5): e32.

Néron B, Denise R, Coluzzi C, et al. 2023. MacSyFinder v2: improved modelling and search engine to identify molecular systems in genomes. *Peer Community Journal*, **3**: e28.

Niewoehner O, Garcia-Doval C, Rostøl JT, et al. 2017. Type III CRISPR-Cas systems produce cyclic oligoadenylate second messengers. *Nature*, **548**(7669): 543–548.

Ofir G, Herbst E, Baroz M, et al. 2021. Antiviral activity of bacterial TIR domains via immune signalling molecules. *Nature*, **600**(7887): 116–120.

Ofir G, Melamed S, Sberro H, et al. 2018. DISARM is a widespread bacterial defence system with broad anti-phage activities. *Nature Microbiology*, **3**(1): 90–98.

O'Hara BJ, Barth ZK, Mckitterick AC, et al. 2017. A highly specific phage defense system is a conserved feature of the *Vibrio cholerae* mobilome. *PLoS Genetics*, **13**(6): e1006838.

Oliveira PH, Touchon M, Rocha EPC. 2014. The interplay of restriction-modification systems with mobile genetic elements and their prokaryotic hosts. *Nucleic Acids Research*, **42**(16): 10618–10631.

O'Neill LAJ, Bowie AG. 2007. The family of five: TIR-domain-containing adaptors in Toll-like receptor signalling. *Nature Reviews Immunology*, **7**(5): 353–364.

Özcan A, Pausch P, Linden A, et al. 2019. Type IV CRISPR RNA processing and effector complex formation in *Aromatoleum aromaticum*. *Nature Microbiology*, **4**(1): 89–96.

Page R, Peti W. 2016. Toxin-antitoxin systems in bacterial growth arrest and persistence. *Nature Chemical Biology*, **12**(4): 208–214.

Pan K, Farrukh H, Chittepu VCSR, et al. 2022. CAR race to cancer immunotherapy: from CAR T, CAR NK to CAR macrophage therapy. *Journal of Experimental & Clinical Cancer Research*, **41**(1): 119.

Parisi B, Soller A. 1964. Studies on the antiphage activity of daunomycin. *Giornale di Microbiologia*, **12**(3): 183–194.

Parma DH, Snyder M, Sobolevski S, et al. 1992. The Rex system of bacteriophage λ: tolerance and altruistic cell death. *Genes & Development*, **6**(3): 497–510.

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

Pecota DC, Wood TK. 1996. Exclusion of T4 phage by the hok/sok killer locus from plasmid R1. *Journal of Bacteriology*, **178**(7): 2044–2050.

Peng L, Sferruzza G, Yang LJ, et al. 2024. CAR-T and CAR-NK as cellular cancer immunotherapy for solid tumors. *Cellular & Molecular Immunology*, **21**(10): 1089–1108.

Peng YH, Zhang Y, Liu Y, et al. 2025. Characterization of Argonaute nuclease from mesophilic bacterium *Chroococcidiopsis*. *International Journal of Molecular Sciences*, **26**(3): 1085.

Perčulija V, Lin JY, Zhang B, et al. 2021. Functional features and current applications of the RNA‐targeting type VI CRISPR‐Cas systems. *Advanced Science*, **8**(13): 2004685.

Perng YC, Lenschow DJ. 2018. ISG15 in antiviral immunity and beyond. *Nature Reviews Microbiology*, **16**(7): 423–439.

Picton DM, Luyten YA, Morgan RD, et al. 2021. The phage defence island of a multidrug resistant plasmid uses both BREX and type IV restriction for complementary protection from viruses. *Nucleic Acids Research*, **49**(19): 11257–11273.

Pu H, Chen YX, Zhao XJ, et al. 2025. A toxin-antitoxin system provides phage defense via DNA damage and repair. *Nature Communications*, **16**(1): 3141.

Qi LS, Larson MH, Gilbert LA, et al. 2013. Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression. *Cell*, **152**(5): 1173–1183.

Qian YZ, Zhou DP, Li M, et al. 2023. Application of CRISPR-Cas system in the diagnosis and therapy of ESKAPE infections. *Frontiers in Cellular and Infection Microbiology*, **13**: 1223696.

Qiu J, Zhai YM, Wei M, et al. 2022. Toxin-antitoxin systems: classification, biological roles, and applications. *Microbiological Research*, **264**: 127159.

Raza SHA, Hassanin AA, Pant SD, et al. 2022. Potentials, prospects and applications of genome editing technologies in livestock production. *Saudi journal of Biological Sciences*, **29**(4): 1928–1935.

Reader JS, Metzgar D, Schimmel P, et al. 2004. Identification of four genes necessary for biosynthesis of the modified nucleoside queuosine. *Journal of Biological Chemistry*, **279**(8): 6280–6285.

Richmond-Buccola D, Hobbs SJ, Garcia JM, et al. 2024. A large-scale type I CBASS antiphage screen identifies the phage prohead protease as a key determinant of immune activation and evasion. *Cell Host & Microbe*, **32**(7): 1074–1088. e5.

Roberts CG, Fishman CB, Banh DV, et al. 2024. A bacterial TIR-based immune system senses viral capsids to initiate defense. *BioRxiv*, doi: https://doi.org/10.1101/2024.07.29.605636

Rosenberg SC, Corbett KD. 2015. The multifaceted roles of the HORMA domain in cellular signaling. *Journal of Cell Biology*, **211**(4): 745–755.

Rousset F, Sorek R. 2023. The evolutionary success of regulated cell death in bacterial immunity. *Current Opinion in Microbiology*, **74**: 102312.

Rousset F, Yirmiya E, Nesher S, et al. 2023. A conserved family of immune effectors cleaves cellular ATP upon viral infection. *Cell*, **186**(17): 3619–3631. e13.

Ryazansky S, Kulbachinskiy A, Aravin AA. 2018. The expanded universe of prokaryotic Argonaute proteins. *mBio*, **9**(6): e01935–18.

Sabonis D, Avraham C, Chang R B, et al. 2025. TIR domains produce histidine-ADPR as an immune signal in bacteria. *Nature*, **642**(8067): 467–473.

Samai P, Pyenson N, Jiang WY, et al. 2015. Co-transcriptional DNA and RNA cleavage during type III CRISPR-Cas immunity. *Cell*, **161**(5): 1164–1174.

Sather LM, Zamani M, Muhammed Z, et al. 2023. A broadly distributed predicted helicase/nuclease confers phage resistance via abortive infection. *Cell Host & Microbe*, **31**(3): 343–355. e5.

Severin GB, Ramliden MS, Ford KC, et al. 2023. Activation of a *Vibrio cholerae* CBASS anti-phage system by quorum sensing and folate depletion. *mBio*, **14**(5): e00875–23.

Severin GB, Ramliden MS, Hawver LA, et al. 2018. Direct activation of a phospholipase by cyclic GMP-AMP in El Tor *Vibrio cholerae*. *Proceedings of the National Academy of Sciences of the United States of America*, **115**(26): E6048–E6055.

Sevillano L, Díaz M, Santamaría RI. 2017. Development of an antibiotic marker-free platform for heterologous protein production in *Streptomyces*. *Microbial Cell Factories*, **16**(1): 164.

Shang YT, Xing GW, Lin JX, et al. 2024. Multiplex bacteria detection using one-pot CRISPR/Cas13a-based droplet microfluidics. *Biosensors and Bioelectronics*, **243**: 115771.

Shapira S, Shapira A, Kazanov D, et al. 2017. Selective eradication of cancer cells by delivery of adenovirus-based toxins. *Oncotarget*, **8**(24): 38581–38591.

Shen BW, Doyle LA, Werther R, et al. 2023. Structure, substrate binding and activity of a unique AAA+ protein: the BrxL phage restriction factor. *Nucleic Acids Research*, **51**(8): 3513–3528.

Shen JT, Zhou JJ, Fu HX, et al. 2016. A *Klebsiella pneumoniae* bacteriophage and its effect on 1, 3-propanediol fermentation. *Process Biochemistry*, **51**(10): 1323–1330.

Shi JJ, Gao WQ, Shao F. 2017. Pyroptosis: gasdermin-mediated programmed necrotic cell death. *Trends in Biochemical Sciences*, **42**(4): 245–254.

Shi Y, Masic V, Mosaiab T, et al. 2024. Structural characterization of macro domain-containing Thoeris antiphage defense systems. *Science Advances*, **10**(26): eadn3310.

Shojaei Baghini S, Gardanova ZR, Abadi SAH, et al. 2022. CRISPR/Cas9 application in cancer therapy: a pioneering genome editing tool. *Cellular & Molecular Biology Letters*, **27**(1): 35.

Shore SFH, Leinberger FH, Fozo EM, et al. 2024. Type I toxin-antitoxin systems in bacteria: from regulation to biological functions. *EcoSal Plus*, **12**(1): eesp–0025-2022.

Shuai YT, Ju Y, Li YH, et al. 2023. A rapid nucleic acid detection platform based on phosphorothioate-DNA and sulfur binding domain. *Synthetic and Systems Biotechnology*, **8**(2): 213–219.

Slavik KM, Kranzusch PJ. 2023. CBASS to cGAS-STING: the origins and mechanisms of nucleotide second messenger immune signaling. *Annual Review of Virology*, **10**(1): 423–453.

Snyder L. 1995. Phage-exclusion enzymes: a bonanza of biochemical and cell biology reagents?. *Molecular Microbiology*, **15**(3): 415–420.

Snyder L, McWilliams K. 1989. The *rex* genes of bacteriophage lambda can inhibit cell function without phage superinfection. *Gene*, **81**(1): 17–24.

Solecki O, Mosbah A, Floc'h MB, et al. 2015. Converting a *Staphylococcus aureus* toxin into effective cyclic pseudopeptide antibiotics. *Chemistry & Biology*, **22**(3): 329–335.

Song XY, Xia YS, Zhang JT, et al. 2025. Bacterial reverse transcriptase synthesizes long poly(A)-rich cDNA for antiphage defense. *Science*, **388**(6753): eads4639.

Stokar-Avihail A, Fedorenko T, Hör J, et al. 2023. Discovery of phage

determinants that confer sensitivity to bacterial immune systems. *Cell*, **186**(9): 1863–1876. e16.

Sun K, Liu Y, Zhao W, et al. 2023. Prokaryotic Argonaute proteins: a new frontier in point-of-care viral diagnostics. *International Journal of Molecular Sciences*, **24**(19): 14987.

Sun LJ, Wu JX, Du FH, et al. 2013. Cyclic GMP-AMP synthase is a cytosolic DNA sensor that activates the type I interferon pathway. *Science*, **339**(6121): 786–791.

Suttle CA. 2007. Marine viruses—major players in the global ecosystem. *Nature Reviews Microbiology*, **5**(10): 801–812.

Suzuki M, Zhang JJ, Liu MH, et al. 2005. Single protein production in living cells facilitated by an mRNA interferase. *Molecular Cell*, **18**(2): 253–261.

Swarts DC, Jore MM, Westra ER, et al. 2014a. DNA-guided DNA interference by a prokaryotic Argonaute. *Nature*, **507**(7491): 258–261.

Swarts DC, Makarova K, Wang YL, et al. 2014b. The evolutionary journey of Argonaute proteins. *Nature Structural & Molecular Biology*, **21**(9): 743–753.

Swarts DC, Szczepaniak M, Sheng G, et al. 2017. Autonomous generation and loading of DNA guides by bacterial Argonaute. *Molecular Cell*, **65**(6): 985–998. e6.

Tal N, Millman A, Stokar-Avihail A, et al. 2022. Bacteria deplete deoxynucleotides to defend against bacteriophage infection. *Nature Microbiology*, **7**(8): 1200–1209.

Tal N, Morehouse BR, Millman A, et al. 2021. Cyclic CMP and cyclic UMP mediate bacterial immunity against phages. *Cell*, **184**(23): 5728–5739. e16.

Tan JMJ, Melamed S, Cofsky JC, et al. 2025. A DNA-gated molecular guard controls bacterial Hailong anti-phage defence. *Nature*, **643**(8072): 794–800.

Tang S, Ramirez JL, Mestre MR, et al. 2025. Antiviral reverse transcriptases reveal the evolutionary origin of telomerase. *bioRxiv*, doi: https://doi.org/10.1101/2025.10.16.682844.

Taylor HN, Warner EE, Armbrust MJ, et al. 2019a. Structural basis of Type IV CRISPR RNA biogenesis by a Cas6 endoribonuclease. *RNA Biology*, **16**(10): 1438–1447.

Taylor VL, Fitzpatrick AD, Islam Z, et al. 2019b. The diverse impacts of phage morons on bacterial fitness and virulence. *Advances in Virus Research*, **103**: 1–31.

Taylor VL, Patel PH, Shah M, et al. 2025. Prophages block cell surface receptors to preserve their viral progeny. *Nature*, **644**(8078): 1049–1057.

Tesson F, Hervé A, Mordret E, et al. 2022. Systematic and quantitative view of the antiviral arsenal of prokaryotes. *Nature Communications*, **13**(1): 2561.

Tesson F, Planel R, Egorov AA, et al. 2024. A comprehensive resource for exploring antiphage defense: DefenseFinder webservice, Wiki and databases. *Peer Community Journal*, **4**: e91.

Tock MR, Dryden DTF. 2005. The biology of restriction and anti-restriction. *Current Opinion in Microbiology*, **8**(4): 466–472.

Tong GD, Nath P, Hiruta Y, et al. 2025. Amplification-free CRISPR/Cas based dual-enzymatic colorimetric nucleic acid biosensing device. *Lab on a Chip*, **25**(4): 536–545.

Tong HW, Liu NN, Wei YH, et al. 2023. Programmable deaminase-free base editors for G-to-Y conversion by engineered glycosylase. *National Science Review*, **10**(8): nwad143.

Tong HW, Wang HQ, Wang XC, et al. 2024. Development of deaminase-free T-to-S base editor and C-to-G base editor by engineered human uracil DNA glycosylase. *Nature Communications*, **15**(1): 4897.

Tuck OT, Adler BA, Armbruster EG, et al. 2024. Genome integrity sensing by the broad-spectrum Hachiman antiphage defense complex. *Cell*, **187**(24): 6914–6928. e20.

Twort FW. 1915. An investigation on the nature of ultra-microscopic viruses. *The Lancet*, **186**(4814): 1241–1243.

Tyagi S, Kumar R, Das A, et al. 2020. CRISPR-Cas9 system: a genome-editing tool with endless possibilities. *Journal of Biotechnology*, **319**: 36–53.

Vader G. 2015. Pch2^{TRIP13}: controlling cell division through regulation of HORMA domains. *Chromosoma*, **124**(3): 333–339.

Van Den Berg DF, Costa AR, Esser JQ, et al. 2024. Bacterial homologs of innate eukaryotic antiviral defenses with anti-phage activity highlight shared evolutionary roots of viral defenses. *Cell Host & Microbe*, **32**(8): 1427–1443. e8.

Vassallo CN, Doering CR, Laub MT. 2024. Anti-viral defence by an mRNA ADP-ribosyltransferase that blocks translation. *Nature*, **636**(8041): 190–197.

Vassallo CN, Doering CR, Littlehale ML, et al. 2022. A functional selection reveals previously undetected anti-phage defence systems in the *E. coli* pangenome. *Nature Microbiology*, **7**(10): 1568–1579.

Vu TV, Nguyen NT, Kim J, et al. 2024. Prime editing: mechanism insight and recent applications in plants. *Plant Biotechnology Journal*, **22**(1): 19–36.

Wan L, Essuman K, Anderson RG, et al. 2019. TIR domains of plant immune receptors are NAD^+-cleaving enzymes that promote cell death. *Science*, **365**(6455): 799–803.

Wang BY, Yu ZY, Zhang ZH, et al. 2025a. A dCas9/sgRNA complex-mediated competitive assay for accurate and sensitive *Pseudomonas aeruginosa* analysis. *Analytical Methods*, **17**(13): 2791–2798.

Wang C, Rish AD, Armbruster EG, et al. 2025b. Disassembly activates Retron-Septu for antiphage defense. *Science*, **389**(6762): eadv3344.

Wang CG, Wang GK, Gao Y, et al. 2020. A cytokinin-activation enzyme-like gene improves grain yield under various field conditions in rice. *Plant Molecular Biology*, **102**(4-5): 373–388.

Wang F, Xu HJ, Zhang CD, et al. 2025c. Target DNA-induced filament formation and nuclease activation of SPARDA complex. *Cell Research*, **35**(7): 510–519.

Wang L, Zhang LL. 2023. The arms race between bacteria CBASS and bacteriophages. *Frontiers in Immunology*, **14**: 1224341.

Wang LR, Jiang SS, Deng ZX, et al. 2019. DNA phosphorothioate modification—a new multi-functional epigenetic system in bacteria. *FEMS Microbiology Reviews*, **43**(2): 109–122.

Wang M, Ji Q, Liu P, et al. 2023b. NAD^+ depletion and defense in bacteria. *Trends in Microbiology*, **31**(5): 435–438.

Wang MX, Chen MH, Wu X, et al. 2023a. CRISPR applications in cancer diagnosis and treatment. *Cellular & Molecular Biology Letters*, **28**(1): 73.

Wang QC, Rao GS, Marsic T, et al. 2024a. Fusion of FokI and catalytically inactive prokaryotic Argonautes enables site-specific programmable DNA cleavage. *Journal of Biological Chemistry*, **300**(9): 107720.

Wang SS, Kuang SR, Song HG, et al. 2024b. The role of TIR domain-containing proteins in bacterial defense against phages. *Nature Communications*, **15**(1): 7384.

Wang SS, Sun EC, Liu YP, et al. 2023c. Landscape of new nuclease-containing antiphage systems in *Escherichia coli* and the counterdefense roles of bacteriophage T4 genome modifications. *Journal of Virology*, **97**(6): e00599–23.

Wang SW, Wan MP, Huang RL, et al. 2021a. SspABCD-SspFGH constitutes a new type of DNA phosphorothioate-based bacterial defense system. *mBio*, **12**(2): e00613–21.

Wang T, Jiang WQ, Huang ZQ, et al. 2025d. Multiplex detection of respiratory RNA viruses without amplification based on CRISPR-Cas13a immunochromatographic test strips. *Virology Journal*, **22**(1): 192.

Wang XF, Zhou SY, Chu CX, et al. 2021b. Dual methylation-sensitive restriction endonucleases coupling with an RPA-assisted CRISPR/Cas13a system (DESCS) for highly sensitive analysis of DNA methylation and its application for point-of-care detection. *ACS Sensors*, **6**(6): 2419–2428.

Wang XX, Lord DM, Cheng HY, et al. 2012. A new type V toxin-antitoxin system where mRNA for toxin GhoT is cleaved by antitoxin GhoS. *Nature Chemical Biology*, **8**(10): 855–861.

Wang YJ, Wang C, Guan ZY, et al. 2024c. DNA methylation activates retron Ec86 filaments for antiphage defense. *Cell Reports*, **43**(10): 114857.
Wang YJ, Wang C, Yin YQ, et al. 2025e. Structural basis of the RNA-mediated Retron-Eco2 oligomerization. *Cell Discovery*, **11**(1): 73.
Wein T, Sorek R. 2022. Bacterial origins of human cell-autonomous innate immune mechanisms. *Nature Reviews Immunology*, **22**(10): 629–638.
Werner RJ, Kelly AD, Issa JPJ. 2017. Epigenetics and precision oncology. *The Cancer Journal*, **23**(5): 262–269.
Whiteley AT, Eaglesham JB, de Oliveira Mann CC, et al. 2019. Bacterial cGAS-like enzymes synthesize diverse nucleotide signals. *Nature*, **567**(7747): 194–199.
Wilkinson ME, Li D, Gao A, et al. 2024. Phage-triggered reverse transcription assembles a toxic repetitive gene from a noncoding RNA. *Science*, **386**(6717): eadq3977.
Williams AA, Redman O, Domgaard H, et al. 2025. Determining the biochemical function of type IV CRISPR ribonucleoprotein complexes and accessory proteins. *Methods in Enzymology*, **712**: 79–114.
Wright DA, Townsend JA, Winfrey RJ Jr, et al. 2005. High-frequency homologous recombination in plants mediated by zinc-finger nucleases. *The Plant Journal*, **44**(4): 693–705.
Wünsche L. 1989. Importance of bacteriophages in fermentation processes. *Acta Biotechnologica*, **9**(5): 395–419.
Xiong XC, Lu ZW, Ma LX, et al. 2023. Applications of programmable endonucleases in sequence- and ligation-independent seamless DNA assembly. *Biomolecules*, **13**(7): 1022.
Xiong XL, Wu G, Wei Y, et al. 2020. SspABCD–SspE is a phosphorothioation-sensing bacterial defence system with broad anti-phage activities. *Nature Microbiology*, **5**(7): 917–928.
Xu K, Zhou YR, Mu YL, et al. 2020. *CD163* and *pAPN* double-knockout pigs are resistant to PRRSV and TGEV and exhibit decreased susceptibility to PDCoV while maintaining normal production performance. *eLife*, **9**: e57132.
Xu TG, Yao F, Zhou XF, et al. 2010. A novel host-specific restriction system associated with DNA backbone S-modification in *Salmonella*. *Nucleic Acids Research*, **38**(20): 7133–7141.
Xu XY, Yang H, Dong HR, et al. 2024. Characterization of argonaute nucleases from mesophilic bacteria *Pseudobutyrivibrio ruminis*. *Bioresources and Bioprocessing*, **11**(1): 94.
Xu ZL, Chen SZ, Wu WY, et al. 2023. Type I CRISPR-Cas-mediated microbial gene editing and regulation. *AIMS Microbiology*, **9**(4): 780–800.
Yan WX, Hunnewell P, Alfonse LE, et al. 2019. Functionally diverse type V CRISPR-Cas systems. *Science*, **363**(6422): 88–91.
Yan Y, Xiao J, Huang FT, et al. 2024. Phage defence system CBASS is regulated by a prokaryotic E2 enzyme that imitates the ubiquitin pathway. *Nature Microbiology*, **9**(6): 1566–1578.
Yang J, Li XZC, He QQ, et al. 2024. Structural basis for the activity of the type VII CRISPR-Cas system. *Nature*, **633**(8029): 465–472.
Yao JY, Zhen XK, Tang KH, et al. 2020. Novel polyadenylylation-dependent neutralization mechanism of the HEPN/MNT toxin/antitoxin system. *Nucleic Acids Research*, **48**(19): 11054–11067.
Yao SG, Wei DW, Tang N, et al. 2022. Efficient suppression of natural plasmid-borne gene expression in carbapenem-resistant *Klebsiella pneumoniae* using a compact CRISPR interference system. *Antimicrobial Agents and Chemotherapy*, **66**(11): e00890–22.
Ye LJ, Zhao DD, Li J, et al. 2024. Glycosylase-based base editors for efficient T-to-G and C-to-G editing in mammalian cells. *Nature Biotechnology*, **42**(10): 1538–1547.
Ye QZ, Lau RK, Mathews IT, et al. 2020. HORMA domain proteins and a Trip13-like ATPase regulate bacterial cGAS-like enzymes to mediate bacteriophage immunity. *Molecular Cell*, **77**(4): 709–722. e7.
Yirmiya E, Hobbs SJ, Leavitt A, et al. 2025. Structure-guided discovery of viral proteins that inhibit host immunity. *Cell*, **188**(6): 1681–1692. e17.
Zander A, Willkomm S, Ofer S, et al. 2017. Guide-independent DNA cleavage by archaeal Argonaute from *Methanocaldococcus jannaschii*. *Nature Microbiology*, **2**(6): 17034.
Zaremba M, Dakineviciene D, Golovinas E, et al. 2022. Short prokaryotic Argonautes provide defence against incoming mobile genetic elements through NAD^+ depletion. *Nature Microbiology*, **7**(11): 1857–1869.
Zeng WJ, Zheng LW, Li YK, et al. 2024. Engineered extracellular vesicles for delivering functional Cas9/gRNA to eliminate hepatitis B virus cccDNA and integration. *Emerging Microbes & Infections*, **13**(1): 2284286.
Zeng ZF, Hu ZY, Zhao RL, et al. 2025. Base-modified nucleotides mediate immune signaling in bacteria. *Science*, **388**(6745): eads6055.
Zetsche B, Gootenberg JS, Abudayyeh OO, et al. 2015. Cpf1 is a single RNA-guided endonuclease of a class 2 CRISPR-Cas system. *Cell*, **163**(3): 759–771.
Zhang H, Tao S, Chen HM, et al. 2025a. Type II toxin-antitoxin systems in *Escherichia coli*. *Infection and Drug Resistance*, **18**: 1083–1096.
Zhang JT, Wei XY, Cui N, et al. 2024a. Target ssDNA activates the NADase activity of prokaryotic SPARTA immune system. *Nature Chemical Biology*, **20**(4): 503–511.
Zhang RX, Chai N, Liu TL, et al. 2024b. The type V effectors for CRISPR/Cas-mediated genome engineering in plants. *Biotechnology Advances*, **74**: 108382.
Zhang SY, Fang X, Chang MY, et al. 2024c. Cross-species single-cell analysis reveals divergence and conservation of peripheral blood mononuclear cells. *BMC Genomics*, **25**(1): 1169.
Zhang T, Tamman H, Coppieters 't Wallant K, et al. 2022. Direct activation of a bacterial innate immune system by a viral capsid protein. *Nature*, **612**(7938): 132–140.
Zhang XM, Qiu MY, Han B, et al. 2025b. Generation and propagation of high fecundity gene edited fine wool sheep by CRISPR/Cas9. *Scientific Reports*, **15**(1): 2557.
Zhang ZY, Todeschini TC, Wu Y, et al. 2025c. Kiwa is a membrane-embedded defense supercomplex activated at phage attachment sites. *Cell*, **188**(21): 5862–5877. e23.
Zhao AC, Li QY, Meng PF, et al. 2025. Reduced content of gamma-aminobutyric acid enhances resistance to bacterial wilt disease in tomato. *Plant Biotechnology Journal*, **23**(3): 792–806.
Zhou CY, Sun YD, Yan R, et al. 2019. Off-target RNA mutation induced by DNA base editing and its elimination by mutagenesis. *Nature*, **571**(7764): 275–278.
Zhou Y, Bravo JPK, Taylor HN, et al. 2021. Structure of a type IV CRISPR-Cas ribonucleoprotein complex. *iScience*, **24**(3): 102201.
Zhu GL, Zhou XZ, Wen MZ, et al. 2024. CRISPR-Cas13: pioneering RNA editing for nucleic acid therapeutics. *BioDesign Research*, **6**: 0041.
Zhu HC, Li C, Gao CX. 2020. Applications of CRISPR-Cas in agriculture and plant biotechnology. *Nature Reviews Molecular Cell Biology*, **21**(11): 661–677.
Zou X, Xiao XH, Mo ZR, et al. 2022. Systematic strategies for developing phage resistant *Escherichia coli* strains. *Nature Communications*, **13**(1): 4491.