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Current Opinion in Microbiology

The evolutionary success of regulated cell death in bacterial immunity

François Rousset and Rotem Sorek

Bacteria employ a complex arsenal of immune mechanisms to defend themselves against phages. Recent studies demonstrate that these immune mechanisms frequently involve regulated cell death in response to phage infection. By sacrificing infected cells, this strategy prevents the spread of phages within the surrounding population. In this review, we discuss the principles of regulated cell death in bacterial defense, and show that over 70% of sequenced prokaryotes employ this strategy as part of their defensive arsenals. We highlight the modularity of defense systems involving regulated cell death, explaining how shuffling between phage-sensing and cell-killing protein domains dominates their evolution. Some of these defense systems are the evolutionary ancestors of key components of eukaryotic immunity, highlighting their importance in shaping the evolutionary trajectory of immune systems across the tree of life.

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Current Opinion in Microbiology 2023, **74**:102312

This review comes from a themed issue on **Evolution of anti-viral defense**

Edited by **Philip Kranzusch**

Available online xxxx

https://doi.org/10.1016/j.mib.2023.102312

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Introduction

Phages have constituted a major evolutionary pressure on prokaryotes for billions of years, promoting a dynamic arms race that has yielded remarkable genetic innovations. As part of this arms race, bacteria have evolved successful strategies to defend against phages, among which are restriction-modification (RM) and CRISPR–Cas systems that directly target phage nucleic acids. However, early studies on phage–host interactions described another paradigm for bacterial defense, involving genetic systems that inflict cell death or growth arrest before completion of the phage replication cycle, a mechanism termed abortive infection (Abi) [1]. This phenomenon has been explored since the 1980s, mainly by studying the model organisms *Escherichia coli* and *Lactococcus lactis* and their phages [1], but its biological significance and general relevance to bacterial immunity were not fully appreciated.

In recent years, the prokaryotic immune repertoire was shown to be much more complex than previously envisioned [2–11]. To date, over 100 different antiphage systems have been described in bacteria and archaea, most of which were discovered since 2018. These discoveries surprisingly exposed numerous, highly abundant defense systems that mediate Abi, therefore bringing this defense strategy back in the spotlight. In this review, we show that defense by regulated cell death constitutes a major form of antiviral immunity among prokaryotes. We describe the evolutionary principles that make this strategy so successful in the microbial world and explain how it also contributed to the evolution of eukaryotic immune systems.

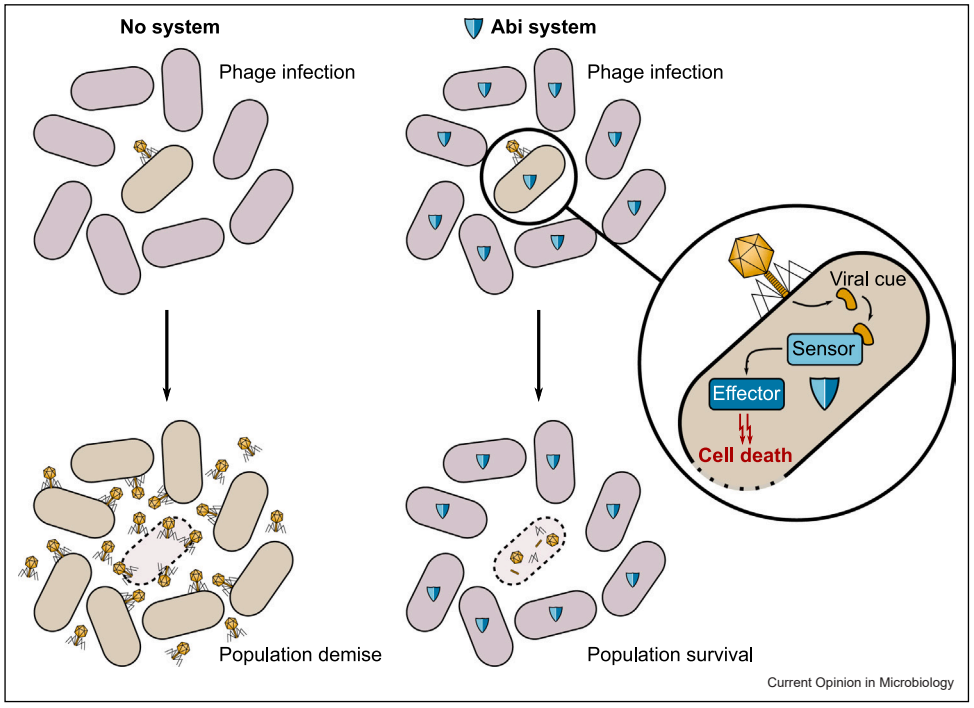
Principles of abortive infection

Abortive infection mechanisms inflict regulated death or dormancy of the infected cell before the phage can successfully complete its replication cycle. By preventing the release of new viral particles to the environment, Abi enables the survival of the surrounding microbial population that would otherwise be wiped out after a few infection cycles [12] (Figure 1). The general principle of regulated cell death in prokaryotes has long been disputed based on the widespread view of prokaryotes as planktonic unicellular organisms, questioning the benefit of this strategy in natural environments [13]. But in fact, microbial populations are frequently structured as communities of genetically identical or nearly identical cells, meaning that Abi would benefit kin cells and ensure the propagation of kin genes through evolution [13]. Since most phages have a narrow host range and usually only infect members of a single species, prevention of phage spread would protect only the kin but not unrelated cells [14].

Over 70% of prokaryotes encode abortive infection systems

Studies from recent years have significantly expanded our knowledge on the repertoire of defense systems encoded in the prokaryotic pan-genome [2–11], providing the opportunity to estimate the general importance of Abi mechanisms in bacterial defense. Examination of 131

Figure 1



General principle of abortive infection (Abi).During infection of an Abi-expressing cell, the sensor module detects a viral cue and transmits the signal to an effector module that executes cell death before completion of the phage replication cycle. As a result, no viable phages are released from infected cells and the surrounding population remains protected.

systems currently listed in DefenseFinder [15] shows that for 57 of them (43.5%), there is strong evidence in the literature supporting an Abi mechanism. Beyond some of the original Abi genes discovered in *L. lactis* and *E. coli* [1], this list includes many recently discovered antiphage systems, including Avs [11], cyclic oligonucleotide-based antiphage signaling systems (CBASS) [10], Lamassu [6], short prokaryotic argonautes (pAgo) [16–18], retrons [3,8,9], and many more.

We used DefenseFinder to analyze 3895 curated prokaryotic genomes [6], revealing a total of 26 740 defense systems (Figure 2a). This analysis shows that most sequenced prokaryotic genomes (72.5%) encode at least one Abi system, with an average of 1.71 systems per genome (Figure 2b and c). These numbers are certainly an underestimate, since the mechanisms of many recently discovered defense systems are still unknown. Interestingly, Abi systems are more prevalent in bacteria than in archaea (in 73.3% versus 45.6% of analyzed genomes), suggesting either differences in defense strategies or a gap in our knowledge of archaeal Abi systems (Figure 2d).

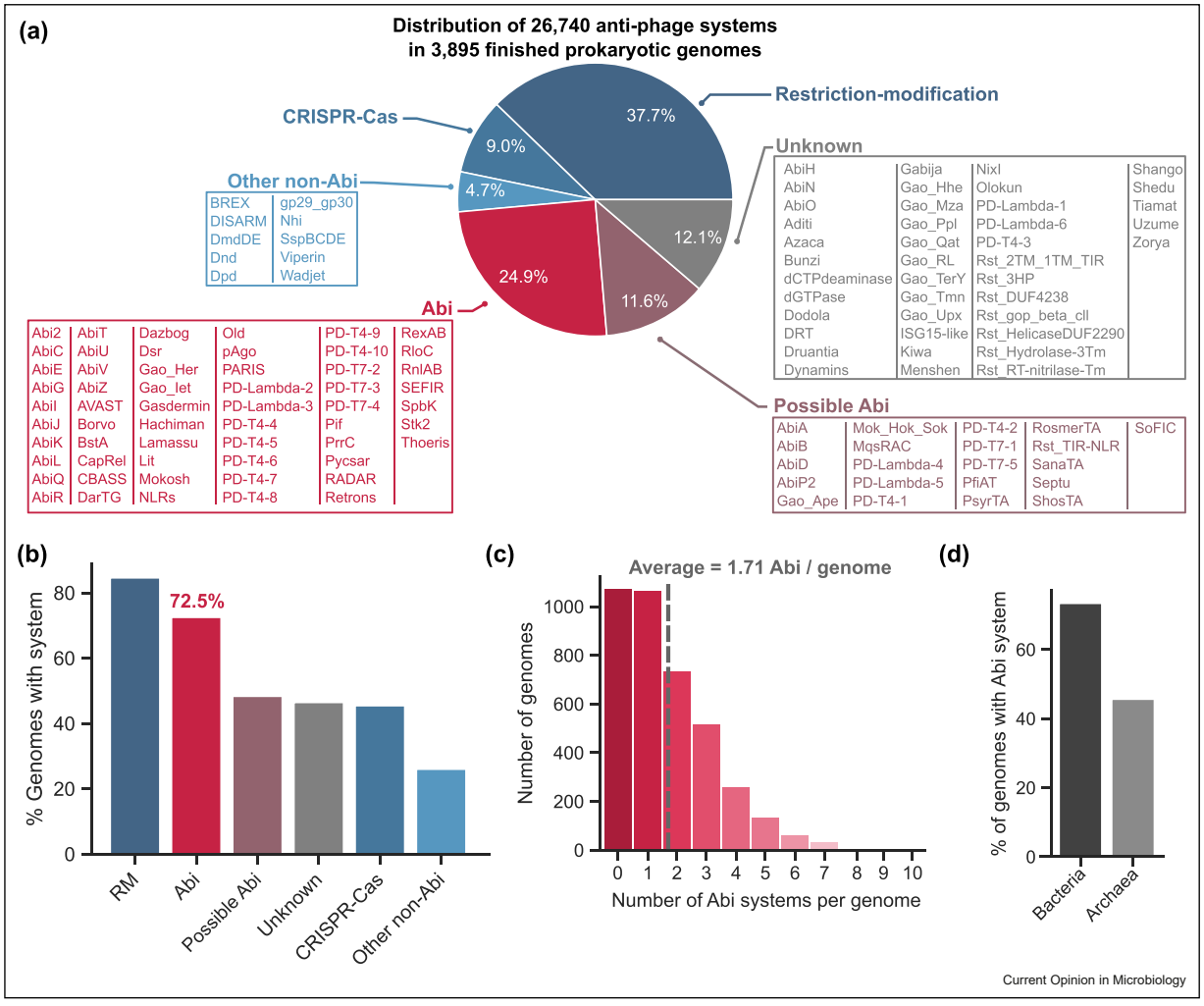
Genetic architecture of abortive infection systems

Most defense systems involving regulated cell death encode two key modules: the phage-sensing module, responsible for detecting that the cell has been infected; and the effector module, which executes cell death or growth arrest once activated by the phage-sensing module (Figure 1) [12]. These two modules are frequently found in two separate proteins. For example, in the defense systems CBASS, Pycsar, and Thoeris, the phage-sensing module is coupled with an enzymatic domain that produces a signaling molecule following phage detection [10,19,20], and the cell-killing module is encoded on a second protein that executes cell death upon binding the respective signaling molecule [19–27]. In other cases, including in the Avs, CapRel, and DSR proteins, the phage-sensing and cell-killing domains are present in the same protein, such that phage sensing results in a conformational change that activates the cell-killing domain [11,28,29].

Phage-sensing modules are sentinels for invasion

Defense systems that rely on regulated cell death pose a threat to the encoding cell, as inadvertent activation of the system would be toxic. As a result, such systems must be tightly blocked when the cell is not infected and should be activated only when phage infection is sensed. This gatekeeping function is enabled by the sensor module that detects a specific viral cue, and only then transmits the signal to the cell-killing module [12].

Figure 2



Abortive infection is a highly prevalent defense strategy in prokaryotes. **(a)** Classification of antiphage defense systems listed in DefenseFinder [15]. **(b)** Fraction of genomes encoding at least one system classified as RM, Abi, possible Abi, CRISPR–Cas, or unknown. Systems were classified as 'Possible Abi' if they encode an effector domain present in known Abi systems, or if they function as toxin–antitoxin systems. **(c)** Distribution of the number of known Abi systems per genome. **(d)** Prevalence of known Abi systems in bacteria and archaea (n = 3781 and 114, respectively).

In many cases, sensor modules detect infection by physically interacting with a conserved phage component. For example, proteins of the Avs family sense the phage terminase or portal proteins that are essential for packing DNA into the phage head [11]. These Avs proteins, which are evolutionarily related to eukaryotic nucleotide-binding domain leucine-rich repeat-containing proteins (NLRs) [11,30], have a C-terminal domain that directly binds the phage protein. This binding triggers a conformational change that induces oligomerization of the Avs protein and activation of its N-terminal cell-killing effector domain [11]. Similarly, the CapRel defense protein binds the phage major capsid protein [28], and the DSR2 defense protein recognizes the phage tail tube [29]. Defense systems can also sense a phage-specific nucleic acid structure, as suggested for short pAgo systems [16–18] as well as for Lamassu and CBASS systems that can detect single-stranded DNA or RNA secondary structures, respectively [31,32].

Sensor modules in Abi systems can also detect phage invasion by sensing its effect on host functions. For instance, the ToxIN system is activated by sensing that the phage has inhibited host transcription [33], while some retrons are activated when they sense that phage proteins inhibit the host RecBCD machinery [9,34].

There is an inherent conflict between Abi systems that defend via cell killing and non-Abi systems such as RM systems, which defend while preserving cell viability. If the cell encodes the latter, cell survival should be favored. To ensure this, there is frequently a temporal

separation in the activities of the two types of systems: RM and CRISPR–Cas systems target phage DNA at the very early steps of infection, while Abi systems frequently detect signals that appear later in the infection cycle, once it is clear that RM and CRISPR–Cas systems were not successful in clearing the phage DNA [34]. This is exemplified by the PARIS system [4] and the PrrC protein [35] that only trigger Abi in the presence of phage-encoded proteins that inhibit RM systems.

Cell-killing modules target essential cellular components

Once activated by their sensor modules, cell-killing effectors in Abi systems target essential host components, leading to rapid cell death or growth arrest. Targeted cell components are frequently nucleic acids, the translation machinery, small molecules essential for survival, or the cell membrane (Table 1).

A variety of effector nuclease domains in multiple defense systems degrade DNA or RNA nonspecifically, such that both phage and host nucleic acids are affected [11,22,23,33,36,37]. Effectors can block translation via tRNA modification or cleavage [28,35] or via proteolytic cleavage of the translation elongation factor Tu [38]. Multiple families of effector domains degrade the essential metabolite NAD^+, including Toll/interleukin-1 receptor (TIR) [18,19,24–26], SIR2 [6,16,20,29], and SEFIR domains [6]. Another type of effectors are nucleosidases that cleave ATP molecules into adenine and ribose-5′-triphosphate [39]. Depletion of NAD^+ and ATP not only deprives the cell from its main energy molecules, but was also suggested to activate the phage lysis machinery to induce premature cell lysis [39]. Another way to induce cell death is to directly breach the cell membrane. Multiple proteins have independently evolved as cell-killing defense effectors to execute this task: (i) patatin-like phospholipases degrade inner membrane phospholipids [10,40], (ii) a diversity of transmembrane effectors disrupt inner membrane integrity [19,27], and (iii) bacterial gasdermins form large pores in the inner membrane once activated by proteolysis, similar to their human homologs [41].

Shuffling through evolution: playing genetic Lego to evade resistance

Examining the repertoire of Abi systems present in microbial genomes reveals remarkable shuffling between sensor and effector modules (Figure 3). Avs proteins that sense the phage terminase and portal proteins can encode more than 15 different types of cell-killing domains, including those that target DNA, NAD^+, and the cell membrane [11]. Conversely, a given effector domain can be found associated with many types of sensor domains. For example, Cap4 nuclease effectors can be found in CBASS [21], Lamassu [6], and Avs systems [11], while SIR2 NAD^+-cleaving domains are found in many systems, including Thoeris [20], pAgo [16,29], and DSR [29] (Figure 3).

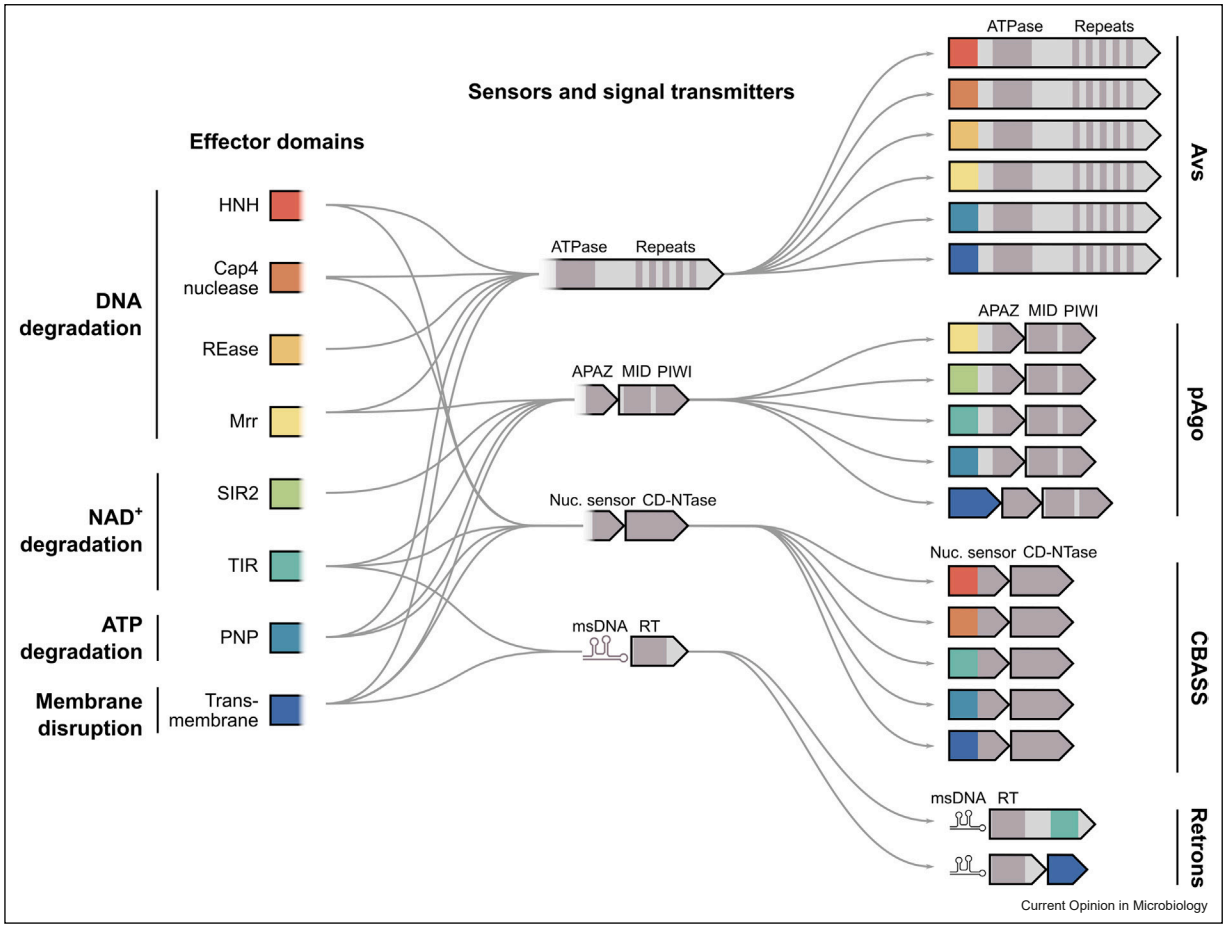
It is likely that evolutionary shuffling between sensor and effector modules in Abi systems serves to restore defense efficiency when phages evolve to escape bacterial defense. In some cases, phages can mutate their proteins to escape detection by the sensor module [9,11,29,34]. In other cases, phages may acquire anti-defense proteins that interfere with the cell-killing module [8,42,43] or disrupt signal transmission between the sensor and effector modules [44–47]. Such phage escape capabilities can be counteracted by shuffling between sensor and effector Abi modules. Indeed, it was experimentally shown that the sensor module of Thoeris

Table 1

| Major host-targeting effector functions in Abi systems.

Molecular function	Domain	Systems where it is found	References
DNA degradation	Cap4 nuclease	CBASS, Lamassu, and Avs	[6,11,21]
	NucC	CBASS, type-III CRISPR	[22,58,59]
	HNH	CBASS, Avs, and retrons	[3,9,11,23]
	Mrr	CBASS, Avs, and pAgo	[11,37,60]
	REase	Lamassu, Avs, and Detocs	[6,11,39]
	Toprim	Old, Retrons, PARIS, and Detocs	[3,4,9,39,61]
RNA degradation	ToxN	ToxIN	[33]
	RnlA	RnlAB	[36]
Translational arrest	RelA	CapRel	[28]
	Peptidase_U49	Lit	[38]
	P-loop NTPase	PrrC	[35]
NAD^+ depletion	TIR	CBASS, Pycsar, Avs, Retrons, pAgo, and SpbK	[9,11,18,19,24–26,62]
	SIR2	Thoeris, Dsr, Lamassu, pAgo, and Avs	[6,11,16,20,29]
	SEFIR	SEFIR	[6]
ATP degradation	PNP	CBASS, Detocs, Avs, and pAgo	[39]
Membrane disruption	Transmembrane	CBASS, Pycsar, Avs, Retrons, and Thoeris	[2,9,11,19,27]
	Patatin	CBASS	[10,40]
	Gasdermin	Gasdermin	[41]

Figure 3



Shuffling of sensor and effector modules promotes Abi diversity. A limited number of effector domains execute cell death and are repeatedly associated with different sensor modules, increasing the diversity of Abi systems. Examples of genetic shuffling between effector and sensor modules are shown. The C-terminal repeats in Avs proteins sense a phage component, inducing oligomerization of the protein through the central ATPase domain and activation of the effector domain. In short pAgos, the MID and Piwi domains are involved in the recruitment of short RNA guides to activate the effector domain upon binding to the target nucleic acid. In CBASS, a CD-NTase enzyme produces a cyclic oligonucleotide signal that is sensed by the nucleotide-binding domain of a second protein to activate its effector domain. In retrons, a reverse transcriptase produces a short msDNA, together forming a surveillance complex that detects phage invasion. Abbreviation: REase, restriction endonuclease; PNP, purine nucleoside phosphorylase; APAZ, analog of PAZ; MID, middle domain; PIWI, P-element-induced wimpy testis; Nuc binding, cyclic oligonucleotide-binding domain; CD-NTase, cGAS/DcnV-like nucleotidyltransferase; RT, reverse transcriptase; msDNA, multicopy single-stranded DNA.

can be exchanged between two Thoeris systems, leading to a corresponding swap in the phages recognized by the two systems [20]. Similarly, exchanging the phage-sensing domains between two CapRel proteins led to a switch in phage-sensing capacity [28]. On the other hand, the observed swapping of effector modules between Abi systems likely serves to restore defense when phages evolve to inhibit the effector function.

Gain and loss of abortive infection systems shape bacterial pan-immunity

Another level of evolutionary shuffling can be observed when examining the diversity of defense systems in the microbial pan-genome. Remarkably, strains of the same species can encode dramatically different sets of Abi systems, despite sharing a very similar core genome [15,48–50]. It was demonstrated that defense systems are dynamically lost and reacquired by bacteria via horizontal gene transfer on short evolutionary timescales, a process that leads to a 'pan-immune system' shared by strains of the same species [50–53]. Presumably, the residual toxicity of Abi systems inflicts a fitness cost that favors the loss of these systems in the absence of phage. Abi systems can then be regained by horizontal transfer, which is largely mediated by mobile genetic elements (MGEs) such as transposons, temperate phages, or integrative and conjugative elements [4,5,7,51–54]. Indeed, it was recently shown that ~90% of the defense

systems in strains of *E. coli* are carried on MGEs [48]. Abi systems are often compact and are therefore easily carried by temperate phages and phage satellites that are typically constrained by the size of cargo DNA they can package. Some temperate phages and their satellites encode Abi systems in dedicated hotspots in their genomes [4,5,7,54].

Conclusions and perspectives

Findings from recent years have brought Abi mechanisms in bacterial defense back into the spotlight. Beyond their abundance and function in protecting bacteria against phages, these systems have played an important evolutionary role in shaping the innate immune system of eukaryotes [55]. Indeed, the bacterial CBASS system was shown to be the evolutionary ancestor of the animal cGAS–STING antiviral pathway [10,25,56], and other components of the eukaryotic innate immune system, including gasdermins [41], NLRs [11,30], TIR signaling [20,45], and ATP nucleosidases [39], have all likely evolved from bacterial systems that exert cell death to defend against phages. Therefore, understanding bacterial Abi systems contributes to understanding the evolution of our own immune system. Accumulating evidence suggests that additional proteins involved in eukaryotic immunity evolved from bacterial Abi systems, highlighting the potential of studying these systems to generate novel insights on the innate immune mechanisms of eukaryotes [6,20,39,45,57].

Data Availability

Data will be made available on request.

Declaration of Competing Interest

R.S. is a scientific cofounder and advisor of BiomX and Ecophage

Acknowledgements

We thank members of the Sorek lab and the anonymous reviewers for critical comments. F.R was supported by the Clore Foundation postdoctoral fellowship and by the Dean of Faculty fellowship from the Weizmann Institute of Science. R.S. was supported, in part, by the European Research Council (Grant ERC-AdG GA 101018520), Israel Science Foundation (MAPATS Grant 2720/22), the Deutsche Forschungsgemeinschaft (SPP 2330, Grant 464312965), the Ernest and Bonnie Beutler Research Program of Excellence in Genomic Medicine, and the Knell Family Center for Microbiology.

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