PROBLEMS & PARADIGMS
Prospects & Overviews

Phage lysis-lysogeny switches and programmed cell death: Danse macabre

Sean Benler | Eugene V. Koonin

National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health, Bethesda, Maryland, USA

Correspondence
Eugene V. Koonin, National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health, Bethesda, MD 20894, USA. Email: koonin@ncbi.nlm.nih.gov

Abstract
Exploration of immune systems in prokaryotes, such as restriction-modification or CRISPR-Cas, shows that both innate and adaptive systems possess programmed cell death (PCD) potential. The key outstanding question is how the immune systems sense and “predict” infection outcomes to “decide” whether to fight the pathogen or induce PCD. There is a striking parallel between this life-or-death decision faced by the cell and the decision by temperate viruses to protect or kill their hosts, epitomized by the lysis-lysogeny switch of bacteriophage Lambda. Immune systems and temperate phages sense the same molecular inputs, primarily, DNA damage, that determine whether the cell lives or dies. Because temperate (pro)phages are themselves components of prokaryotic genomes, their shared “interests” with the hosts result in coregulation of the lysis-lysogeny switch and immune systems that jointly provide the cell with the decision machinery to probe and predict infection outcomes, answering the life-or-death question.

KEYWORDS
abortive infection, CRISPR, kin selection, lambda switch, lysogeny, programmed cell death, toxin-antitoxin

INTRODUCTION

Viruses are ubiquitous and enormously abundant entities in the biosphere.[1,2] The unabating conflict between viruses and their hosts necessitates evolution of systems that distinguish self from nonself in all life forms.[3] The biological solution to the problem of self versus nonself recognition are immune systems, which are subdivided into innate and adaptive (acquired) ones, based on their mechanism of discrimination.[4] Innate immune systems recognize conserved nonself patterns, whereas variable nonself patterns are recognized and memorized by adaptive immune systems.[5] Almost all cellular organisms possess multiple, overlapping innate, and adaptive immunity mechanisms to restrict virus infection and invasion of mobile genetic elements. However, the multilayer organization of immune systems notwithstanding, their potential failure under overwhelming onslaught of parasites necessitates a cellular kill-switch, known as programmed cell death (PCD).[6]

Although originally PCD (also known as apoptosis) was discovered in animals and was considered a trait of multicellular life, more recently, it has become clear that most if not all unicellular eukaryotes as well as prokaryotes also possess PCD mechanisms.[7,8] Thus, PCD could be an intrinsic feature of all life, posing fundamental questions on the relevance of kin and group selection that are a hotly debated subject in evolutionary biology.[9–11] Arguably, PCD in unicellular organisms is an “altruistic” program that sacrifices a cell for the survival of multiple other cells in a population.[12–14] Such a mechanism can be maintained in evolution only through some form of kin or group selection.[15] Of further interest are the still incompletely understood sensing and signal processing mechanisms that underlie the “life-or-death decisions” made by cells.

Examination of immune systems in prokaryotes reveals multiple functional connections between immune systems and PCD.[16] For example, the adaptive immune system in prokaryotes, CRISPR-Cas (Clustered Regularly Interspaced Palindromic Repeats and
CRISPR-associated genes), frequently colocalizes with PCD modules in the form of toxin-antitoxin (TA) gene pairs and the two may be coregulated.[17,18] Type III CRISPR-Cas systems can induce cell dormancy or PCD when the promiscuous ribonuclease activity of one Cas protein is unleashed by cyclic oligoadenylates produced by another Cas protein in response to the specific target recognition.[19,20] Moreover, type VI CRISPR-Cas systems, while lacking a dedicated PCD machinery, instigate PCD themselves through indiscriminate degradation of cellular mRNAs by the Cas13 RNase that is induced by the recognition of the target virus genome.[21] Innate immune systems, such as restriction-modification, possess a similar intrinsic suicide potential as well as connections with TA modules.[22] Both the suicidal potential of immune systems themselves[23,24] and their frequent association with PCD modules increase the cost of their possession and necessitate strict regulatory control. The signals and “decision” process that drive the transition from sustained immunity to PCD are only beginning to be elucidated.[25]

All prokaryotes are parasitized by viruses (known as phages, in the case of bacteria) including those bacteria and archaea that are replete with innate and adaptive defense systems.[26–27] The life cycle of temperate phages, in contrast to obligate lytic phages, includes a stage when the virus integrates into the host cell genome. Each temperate phage harbors a genetic locus that governs the “decision” between lysing the host cell or integrating in the genome, known as the lysis-lysogeny switch. The switch locus is often accompanied by innate immunity and PCD-related genes that temperate phages deploy during lysogeny to defend their hosts from superinfection by other phages. There is a striking analogy between the phage lysis-lysogeny switch and the immunity versus PCD decision made by cellular defense systems. Indeed, (pro)phages also make a decision on whether the host cell should live (lysogeny) or die (lysis). Thus, the mechanisms of the lysis-lysogeny switches that have been studied in exquisite molecular detail could inform the understanding of the “life-or-death” decisions by immune systems that are at present barely known. Here, we outline the mechanisms employed by temperate phages to measure the immune status and physiological state of the host cells to decide between the lytic and lysogenic strategies, compare these mechanisms to those employed by defense systems, and discuss the accumulating examples of direct connections between prophages, defense systems and PCD. Although our discussion of the lysis-lysogeny switch focuses on phage Lambda, which is by far the best characterized phage model, fundamentally similar mechanisms operate in an enormous variety of prokaryotic viruses that interact with equally diverse defense systems.

**READING THE CUES: TEMPERATE PHAGES MEASURE HOST CELL STATE AND IMMUNE STATUS TO DECIDE BETWEEN LYSIS OR LYSOGENY**

The paradigm for virus “decision” between immediate replication (lytic pathway) or integration (lysogenic pathway) is the switch employed by *Escherichia coli* phage Lambda. More than a half century worth of genetic and biochemical dissection unveiled the intricacies of the Lambda “Epigenetic Switch” as discussed in detail in many extensive reviews.[28–31] Thus, below we outline only the general, essential features of the lysis-lysogeny switches.

Upon entering an *E. coli* host cell, one of the first genes transcribed from the Lambda genome directs the production of the transcription regulator cl that can function either as a repressor or an activator depending on the site in the phage genome where it binds.[32] Depending on the concentration of cl, either genes required for lysogeny (e.g., integrase) or genes involved in the lytic pathway (e.g., major capsid protein) are transcribed and translated.[33] High concentrations of cl inhibit the lytic pathway and promote integration (lysogeny), whereas low concentrations lead to the replication of Lambda DNA, production of virion structural proteins and host cell lysis.[29] At higher multiplicities of infection (more than one Lambda virion per cell), the outcome of infection is biased toward lysogeny,[34] potentially dictated by the collective “votes” of the infecting phage particles[35,36] and/or other physiological parameters of infection.[37] Thus, the phage assesses the immune status of *E. coli* by measuring the parameters of infection by itself and, as discussed below, by other viruses.[35] The physiological state of the host cell can also directly or indirectly inform the lysis-lysogeny decision through another protein, cII, that serves as a master regulator by promoting cl transcription.[33] The physiological inputs affecting cII include growth rate,[38] concentration of cyclic AMP,[39] ppGpp,[40] integration host factor protein,[41] and DNA damage.[42] Thus, the Lambda switch harmonizes multiple signals coming from the host cell state and infection status to commit to a lytic or lysogenic lifestyle.

Once dedicated to the lysogenic lifestyle and integrated into the *E. coli* chromosome, the lysis-lysogeny switch machinery poises Lambda to rapidly respond to changes in the cellular conditions. Such sensitivity is afforded by autoregulation that is hardcoded in the switch (Figure 1).[43] Recruitment of RNA polymerase by cl to its own promoter enhances transcription of cl and the downstream genes in a positive feedback loop. Such positive feedback ensures that the concentration of cl is sufficient to repress genes involved in lysis. However, excessive levels of cl hamper the transition to the lytic program.[44] As positive feedback increases the concentration of cl above a threshold, cl inhibits the recruitment of RNA polymerase in a negative feedback loop. The result is a finely balanced concentration of cl that provides for a highly robust control of phage development. For example, fewer than 1 cell per 10⁸ generations aberrantly switch to the lytic program,[45] but given an inducing signal, almost every prophage in a population of cells appropriately enters the lytic state.[46] The lambda switch optimally maintains the lysogenic state while affording the prophage the capacity to transition to the lytic program.

The best-characterized signal that initiates the lytic program in Lambda integrated as a prophage is DNA damage. Treatment with DNA-damaging agents or ultraviolet irradiation accumulates single-stranded DNA in the cell and activates the SOS-response.[46] As part of this response, single-stranded DNA is bound by the host RecA protein, and the RecA-DNA complex stimulates cl autoproteolysis.[42] Positive feedback compensates for the deficit of cl up to a certain threshold, but below that minimum, the feedback loop collapses and
lysis ensues. DNA damage is a common, but not universal, signal leading to phage induction of the lytic program. Less well-characterized signals sensed by the lysis-lysogeny switches in other temperate phages include various environmental triggers or proteins from related or unrelated infecting phages. Remarkably, despite the many idiosyncrasies of individual phage lysis-lysogeny decision switches, entire communities composed of diverse viruses switch from the lytic pathway to the temperate pathway as the host cell density increases. Indeed, lysogeny is the predominant observed behavior of phages in ecosystems with high microbial density, such as, for example, the human gut. As elucidated by genetic and biochemical dissection of Lambda, lysis-lysogeny switches function as 'sensors' that measure a signal (e.g., DNA damage) and calibrate the response of the (pro)phages to adopt the optimal reproductive strategy under the given conditions.

TEMPERATE PHAGES AND CRISPR-CAS: DANCING IN RHYTHM TO PREVENT A “CUT IN”?

When a phage switches from lysis to lysogeny or vice versa, it effectively makes a life-or-death decision for the host cell. The lytic pathway can be interpreted as a mechanism of PCD, but in this case, the death of the cell is not altruistic because the released phage particles infect and eventually kill other cells in the host population. By contrast, the lysogenic pathway immunizes the host upon integration of the phage into the cellular genome. Integrated phages (prophages) endow their hosts with immunity to other phages with identical or similar lysis-lysogeny circuits. For example, cl expressed from a Lambda prophage functions in trans to silence the lytic program of invading Lambda-like phages. In addition to excluding related phages, temperate phages also prevent infection by unrelated phages (i.e., superinfection, or using dance terminology, a “cut in”) by utilizing dedicated innate and adaptive immunity genes (see the section “Leading and following”). Thus, the cellular life-or-death outcome is a direct consequence of the phage lysis-lysogeny decision.

Cell death caused by phage reproduction during the lytic growth is, evidently, detrimental to the host, instigating an arms-race to evolve anti-phage defense systems. A major gap in our understanding is how such defense systems sense and ‘predict’ infection outcomes to make cellular life-or-death decisions. As temperate phages and defense systems coexist, we propose that the shared ‘interests’ of temperate phages and their hosts result in functional connections between the lysis-lysogeny switch and defense mechanisms, using CRISPR-Cas as a focal example. The coherence between prophage lysis-lysogeny switches and CRISPR-Cas could provide the circuitry to measure infection by exogenous phages and inform the cell if altruistic suicide is merited.

DNA damage connects CRISPR-Cas with the lysis-lysogeny decision of prophages

Cleavage of DNA by innate and adaptive immune systems is recognized by the cell as DNA damage and triggers a proportional SOS response. As discussed above, DNA damage also is a common signal that elicits the lytic program of prophages. Thus, nuclelease-based immune systems and lysis-lysogeny circuits could be connected based on the strength of the DNA damage signal and the accompanying SOS response. For example, a low-multiplicity infection of a cell with a parasitic element (e.g., phage or plasmid) that is susceptible to the immune system results in cleavage of the genome of the invading element and clearance, and emits a low level of the DNA damage signal (Figure 2A). By contrast, at high multiplicities of infection, the resulting DNA damage signal emitted by the immune system triggers destruction of cl-like proteins(s) of endogenous prophage(s) and induces their lytic program (Figure 2B). Indeed, it has been shown experimentally that DNA damage caused by CRISPR-Cas self-targeting drives the cleavage of phage cl-like proteins, activating the lytic pathways of defective prophages and ultimately killing the cell in a friendly fire type of event. Thus, destruction of foreign DNA by defense systems appears to be the signal that is sensed by prophages to estimate the parasite pressure and inform, and then, execute the life-or-death decision of the infected cell.

Phage integration and excision may function as a “CRISPR switch”

Conversely, phage integration and excision could serve as a regulatory mechanism for CRISPR-Cas activity. Notably, many viruses carry CRISPR spacers, typically, as parts of mini-arrays (but in some cases, within a full-fledged CRISPR-Cas system), and these
FIGURE 2  Potential connections between phage lysis-lysogeny switches and CRISPR-Cas. Nuclease-based immune systems generate DNA damage proportional to the multiplicity of infection, leaving cl intact under "controllable" infections (A), or destroyed by the SOS-response if the infection is "uncontrollable," instigating programmed cell death (B). Integration and excision of prophages from within CRISPR arrays functions as a molecular switch, where spacers are inactive (C) whereas the prophage is integrated or active after excision (D). Anti-CRISPR associated (Aca) proteins repress transcription of anti-CRISPR genes (Acrs) during lysogeny (E) but also promote lytic replication through an unknown mechanism. Aca's might localize to operator sites of lysis-related genes in the absence of bound cl, promoting their transcription while concomitantly derepressing the Acr locus, and thus, preventing nascent phages from destruction (F).

virus-encoded spacers can act as guides to direct site-specific integration of phages into a host CRISPR array carrying identical or closely similar spacers.[63,64] Such integration has been suggested to inactivate the target CRISPR-Cas system. However, some prophages have been shown to reversibly integrate and excise without executing the lytic program, acting as molecular switches by reconstituting the endogenous cellular locus.[65] For example, an A118-like prophage integrated in the comK gene of Listeria monocytogenes excises when the bacterium enters human macrophage cells.[66] Excision is not accompanied by the remainder of the lytic program; instead, excision restores comK functionality, expediting phagosomal escape and survival of the prophage that subsequently reintegrates into comK.[66]

Host cell processes that are similarly regulated by phage excision-integration include nitrogen fixation, sporulation, cold-shock, and DNA repair.[67] demonstrating how the lysis-lysogeny switch mediates the "shared interests" of phages and their hosts. We hypothesize that adaptive immunity can be regulated analogously through phage integration-excision into and from CRISPR arrays in response to specific environmental, cellular, or immune signals (Figure 2C,D).

Anti-CRISPR proteins couple CRISPR-Cas with the lysis-lysogeny decision of prophages

The best studied case of phage-mediated regulation of CRISPR-Cas immunity are the anti-CRISPR proteins (Acrs) that inactivate CRISPR-Cas systems.[68–70] Acrs are expressed from a strong promoter in the phage genome immediately upon infection of a cell to riposte surveillance by the host CRISPR-Cas system.[71] Acrs are also expressed at a low level during lysogeny, preventing costly "self" (prophage) targeting and enabling prophage persistence.[72,73] The dual transcriptional regime of the Acrs is arbitrated by regulatory proteins, known as Acr-associated (Aca) proteins, which tamper the expression from the Acr-Aca promoter that would otherwise disrupt the phage transcriptional program.[71,74] Aca proteins contain a Helix-Turn-Helix (HTH) DNA-binding domain that binds to the proximal Acr-Aca promoter in a dose-dependent fashion and autoregulates the operon, a mechanism that is shared by diverse phages and clearly resembles the lysis-lysogeny switch.[71,72,75] Gene fusions between the Aca HTH domain and Acrs have been identified, yielding hybrid Aca-Acrs.[71,76] The
The crystal structure of one such Aca-Acr hybrid, AcrIIA1 of the temperate *Listeria* phage A006, reveals an HTH domain that is, structurally, nearly identical to the HTH in the cI proteins of Lambda-like phages. Importantly, AcrIIA1 and its homologs promote induction through the HTH domain, independent of Cas9 inhibition, demonstrating that this bifunctional protein connects CRISPR-Cas regulation with the lysis-lysogeny switch.

The above data support the central role of the lysis-lysogeny decision of the prophage in governing the activity of CRISPR-Cas in the cell through the Aca protein. During lysogeny, Aca is principally located at operator sites within its cognate promoter in the prophage genome and inhibits Acr production (Figure 2E). In L. monocytogenes harboring A118-like prophages, Acr-Acr autoregulation maintains the concentration of the Cas9 protein at about 30% of that in prophage-free cells. The incomplete abrogation of Cas9 might afford a base level of adaptive immunity against heterologous phages. In response to a lysis-promoting signal, cI is destroyed, and operator sites in the lysis-related gene promoters are exposed. The Aca HTH domain might interact with the exposed lysis-related operator(s), thus, titrating Aca away from its cognate promoter (Figure 2F).

Although there is currently no experimental evidence of Aca interaction with additional promoters, binding to lytic promoters could explain how Aca promotes prophage induction. Moreover, relocation of Aca to lytic promoters would achieve a dual outcome, that is, increased lytic gene expression, on the one hand, and derepression of the Acr locus, on the other hand. Derepression of the Acr locus during lytic development results in the accumulation of Acrs and complete inhibition of the CRISPR-Cas system, which is required to prevent destruction of nascent progeny phages. Thus, the lysis-lysogeny decision of Acr-carrying prophages appears to govern the activity of CRISPR-Cas.

### WATCHING YOUR PARTNER’S STEPS: MANY IMMUNE SYSTEMS CHOOSE PROGRAMMED CELL DEATH IN RESPONSE TO LYTI, BUT NOT LYSOGENIC, DECISIONS OF VIRUSES

Not all immune systems immediately target invading parasites for destruction. From the perspective of a cell endowed with such defense mechanisms, the replicative strategy of the parasite is assessed as part of the cellular life-or-death decision. Specifically, certain prokaryotic immune systems recognize the lysis-lysogeny choice of incoming phages and “overrule” the lytic program of the phage by inducing PCD, whereas lysogeny is tolerated. The mechanisms involved in this phage-induced PCD are multiple, even if many of these are limited in their distribution to relatively narrow groups of prokaryotes. Thus, diverse abortive infection (Abi) modules, a type of TA systems encoded by many if not most bacteria and archaea, employ various molecular mechanisms to interrupt phage DNA replication and transcription during the lytic cycle of temperate phages, concomitantly, inducing host cell dormancy or death.

An entire class of such an abortive infection mechanism, the diverse cyclic-oligonucleotide-based anti-phage signaling systems (CBASS), was recently uncovered in a broad variety of bacteria. Comprons of CBASS sense phage peptides to elicit programmed cell death. Peptide-bound sensor proteins activate enzymes that synthesize a broad variety of cyclic oligonucleotides; in turn, the elevated concentration of the cyclic oligonucleotides that are recognized by sensor components of the CBASS system triggers deadly non-specific nucleases. Although it currently remains unknown which phage gene(s) encode the peptides that CBASS detects, this system has been recently demonstrated to trigger programmed cell death during the lytic cycle of phage Lambda. Systematic searches of bacterial genomes for anti-phage defense systems have revealed a staggering diversity of previously unknown ones of which most can be predicted to function via the Abi strategy, and many are likely to employ cyclic oligonucleotides as signals similar to CBASS. Furthermore, type III CRISPR-Cas systems contain a built-in CBASS-like mechanism that induces dormancy or PCD upon infection by a specific virus containing a cognate protospacer. Most likely, the diversity of Abi-like systems in bacteria and archaea is far greater than currently explored, and collectively, these systems comprise a major component of the network of microbial “pan-immunity.”

Many Abi modules include a transcription regulator that functions as a repressor of the toxin gene and is inactivated during lytic infection. These Abi systems resemble phage lysis-lysogeny switches not only conceptually, but even at the level of mechanistic details. In particular, as in the case of the phage lysis-lysogeny switches, expression of Abi systems is induced by DNA damage via the SOS response that cause degradation of the antitoxin that normally represses Abi transcription. Similarly, the *E. coli* MazEF TA system prevents lytic replication, but not lysogeny, of phage P1. In this case, the TA system interacts with the phage lysis-lysogeny switch by inhibiting the SOS response and thus preventing the degradation of cI and safeguarding lysogeny. Lysogeny is also safeguarded in *Staphylococci* spp., where a protein kinase is activated specifically during the lytic cycle of several phages, leading to global phosphorylation deregulation and cell death, whereas during the lysogenic pathway, the kinase is inactive. In *Mycobacteria*, PCD is elicited by several defense systems in response to superinfecting temperate phages entering the lytic, but not lysogenic, pathway. In bacteria that harbor type III CRISPR-Cas systems, transcription of the CRISPR targets is required to license DNA cleavage; as a consequence, lytic phage cycle is abrogated, in some cases, at least, via PCD, whereas quiescent prophages are tolerated. These are only examples: PCD induction in response to lytic phage growth seems to be a common strategy of altruistic defense in bacteria. From the perspective of temperate phages, lysogeny is an “escape” mechanism from these defense systems allowing the phage to co-reproduce with the host. From the cellular perspective, the tolerance of immune systems to lysogeny facilitates the acquisition of prophages that protect the cells from superinfection, as discussed below.
LEADING AND FOLLOWING: TEMPERATE PHAGES ENDOW CELLS WITH ADDITIONAL LAYERS OF IMMUNITY

Lambda again offers an informative model to understand how prophages exclude unrelated phages. A mutant of phage T4 (T4rl1) does not propagate on E. coli cells that harbor a Lambda prophage. The exclusion of T4rl1 is mediated by a pair of Lambda genes, rexAB,[98] immediately adjacent to cl (Figure 1). These genes are expressed during lysogeny and encode protein products that oligomerize, the RexB subunit being localized to the cell membrane and RexA to the cytosol.[98] Upon infection of E. coli by T4rl1, RexAB collapses the proton-motive force, killing the cell and preventing T4rl1 propagation.[98] The exact trigger that initiates RexAB-mediated PCD is not known, but disruption of cellular energetics by T4rl1 infection is a likely candidate.[99]

As in the case of TA systems, the proper ratio of RexA:B must be maintained to prevent anomalous PCD.[98] The balance of the two proteins during lysogeny is maintained via transcription regulation by cl. Lambda thus illustrates how transcriptional regulation by the lysis-lysogeny switch maintains a dedicated PCD device sensitive to cellular physiological state, immune status and infection by other phages. Notably, RexB also inhibits the toxicity of MazEF, preventing the inhibition of the SOS response and thus allowing the degradation of cl and induction of lysis under stress.[95] This multifunctional protein, therefore, connects prophage-mediate protection against superinfection with the lysis-lysogeny switch.

Temperate phages harbor a multifarious armament of innate immunity and PCD genes that are expressed during lysogeny. While Lambda has one switch locus with one set of PCD genes (RexAB), other phages harbor multiple loci that encode proteins mediating innate immunity and PCD (Figure 3). Apart from the RexAB-like modules,[100,101] examples include restriction-modification systems,[80] ppGpp (alarmone) synthetases,[80] cell division inhibitors, [102] and sRNAs.[103] A consistent theme among the protein effectors appears to be circumscription within and in the vicinity of the cell membrane. Preferential membrane localization is also observed among ancillary proteins encoded by genes associated with type III and VI CRISPR-Cas systems,[104-106] suggesting this subcellular localization enhances the efficacy of both innate and adaptive immunity. Collectively, these examples emphasize that temperate phages encode diverse innate immune and PCD repertoires that augment cellular defense systems and prevent superinfection by both related and unrelated phages during lysogeny.

Given that prophages and cellular immune systems coexist, what happens upon transition to the lytic cycle? As described above, certain defense systems sense products of phage genes that are expressed during the lytic cycle and elicit altruistic PCD, preventing spread of the phage to the surrounding population.[94] Another outcome is the irreversible loss of the ability of the phage to enter the lytic cycle, such that the immune response is never triggered. These immobilized phages are referred to as "defective," that is, remnants of complete prophages that are incapable of completing the lytic program autonomously due to the inactivation or deletion of some of the required genes.[26] Examination of defective prophages in prokaryotic genomes demonstrates that they are often vertically inherited and maintained by purifying selection,[107] which otherwise drives the loss of nonfunctional genes in prokaryotes.[108] Indeed, genes from defective prophages augment cellular physiology[109] and function in anti-phage defense.[110] Examples include the E. coli Lit protease, E. coli Prr tRNAse, Haemophilus influenzae HindIII restriction-modification enzymes and Lactococcus lactis AbiN.[111] Immobilization of mobile genetic elements, including plasmids, transposons, followed by recruitment of their components for defense functions, is a major route of evolution of prokaryotic defense systems, in particular, CRISPR-Cas adaptive immunity.[57,112,113] Immobilization might begin with a prophage acquiring an immune system that senses proteins produced during the lytic cycle of unrelated temperate phages and thus prevents their ability to complete induction while, at the same time, protecting the host from infection with related phages. Prophage maintenance can be further buttressed by acquisition of a second lysogeny-tolerant defense system, and so on, ultimately, resulting in the transformation of a prophage into a defense island, a constellation of multiple defense systems encoded within a single genomic locus, and a common phenomenon in prokaryotic genomes (Figure 4).[114-117] A remarkable case of phage domestication that is linked to bacterial PCD, although not directly to antivirus defense, is manifested by the Gene Transfer Agents (GTAs) that are present in many bacteria and archaea, but are most common and well-characterized in alphaproteobacteria.[118-120] The GTAs are deeply degraded prophages that retain only genes encoding structural and morphogenetic proteins and produce mini-phage particles that package random pieces of the host bacterial DNA, instead of the prophage genes. The expression of GTAs...
is induced under nutritional stress, resulting in the lysis of the host cell and infection of neighboring cells that, however, are not lysed but rather integrate some of the genes carried by the GTAs. Apparently, the GTAs are a case of prophages converted to altruistic PCD devices, with a double benefit to the bacterial population, in the form of nutrients supplied by the lysed cells and transfer of genes, some of which can confer selective advantage to the surviving cells.

**CONCLUSIONS AND OUTLOOK**

A fundamental realization in biology that is currently taking shape is that all cellular life forms possess some form of PCD and make life-or-death decisions when facing infection or other forms of stress. With respect to such decisions, there is a clear parallel between the immunity-PCD switch that appears to be built into most if not all defense systems, at least, in prokaryotes, and the lysis-lysogeny switch in temperate bacteriophages. Similarly to the defense systems, (pro)phages, effectively, make a life-or-death decision for the host cell when switching from lysis to lysogeny or vice versa. The defense systems make the decision to switch to PCD by sensing the state of the cell and, in particular, by measuring the level of DNA damage, and prophages rely on the same signals to activate the lytic program. The analogy aside, the major biological difference between defense systems and (pro)phages is that, in the former case, PCD is altruistic, that is protecting other cells in the microbial population from infection, whereas cell death by phage-induced lysis only serves the “interests” of the phage and is, in that sense, “selfish.” Beyond the formal parallel between their decision-making strategies, there are direct connections between (pro)phages and defense systems. Prophages can be considered a type of defense systems that protect the prophage-carrying cells from superinfection by both related and unrelated phages, in many cases, via PCD. In particular, prophages are activated and pushed into the lytic cycle by DNA damage caused by defense systems, such as CRISPR-Cas, resulting in cell death. Future experimental work could quantitate how much foreign DNA and how much damage is required
for defense systems to signal prophages that infection is "out of control" and that PCD is warranted. Moreover, defective, domesticated prophages can become dedicated PCD devices, the GTAs being an especially striking case in point. Ultimately, evolution of domesticated prophages can lead to their conversion into defense islands encoding multiple defense mechanisms. Long-term phage-bacteria co-evolution experiments could potentially yield direct observations of defense-related gene acquisition from temperate phages. Prophages also directly regulate the activity of immune systems, such as CRISPR-Cas, by various means, in particular, through the activity of the Acrs, which appears to be directly linked to the lysis-lysogeny switch. There is no doubt that additional, intricate forms of interaction between (pro)phages and immune mechanisms remain to be discovered, further broadening the general paradigm of evolutionary entanglement between mobile genetic elements and defense systems.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ORCID

Sean Benler https://orcid.org/0000-0001-7985-1294
Eugene V. Koonin https://orcid.org/0000-0003-3943-8299

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