Coupling immunity and programmed cell suicide in prokaryotes: Life-or-death choices

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Host-pathogen arms race is a universal, central aspect of the evolution of life. Most organisms evolved several distinct yet interacting strategies of anti-pathogen defense including resistance to parasite invasion, innate and adaptive immunity, and programmed cell death (PCD). The PCD is the means of last resort, a suicidal response to infection that is activated when resistance and immunity fail. An infected cell faces a decision between active defense and altruistic suicide or dormancy induction, depending on whether immunity is ''deemed'' capable of preventing parasite reproduction and consequent infection of other cells. In bacteria and archaea, immunity genes typically colocalize with PCD modules, such as toxins-antitoxins, suggestive of immunity-PCD coupling, likely mediated by shared proteins that sense damage and ''predict'' the outcome of infections. In type VI CRISPR-Cas systems, the same enzyme that inactivates the target RNA might execute cell suicide, in a case of ultimate integration of immunity and PCD.

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Abbreviations:
PCD, programmed cell death; **RM system,** restriction-modification system; **TA module,** toxin-antitoxin module.

Introduction

Parasites are intrinsic components of all replicator systems [1–3]. Virtually no cellular life form can eliminate parasitic genetic elements [4–6], and most organisms host diverse classes of such elements including viruses, transposons, and plasmids [7]. Thus, the entire history of life is a story of incessant arms races between parasites and hosts during which both sides evolve diverse offence, defense, and counter-defense strategies [1, 2, 8]. Nearly all cellular life forms, with the exception of some intracellular parasitic bacteria, combine multiple anti-parasite defense mechanisms [9]. The principal defense strategies include: (i) resistance whereby the receptor for a particular parasite, such as a virus, mutates to a form that is no longer conducive to the parasite entry into the host cell; (ii) innate immunity, i.e. diverse mechanisms that actively prevent the reproduction of different parasites; (iii) adaptive (acquired) immunity, i.e. mechanisms that involve collection of information on a specific parasite and utilization of that information for highly efficient and selective abrogation of its reproduction; and (iv) programmed cell death (PCD) (and possibly more broadly, programmed suicide of an organism) whereby an infected cell instigates a self-destruction program that prevents parasite reproduction from reaching completion, and thus, protects other cells from infection [9–11]. In bacteria, the functional systems that cause PCD, in many cases, can instead induce dormancy (stasis), i.e. a non-reproducing cellular state characterized by extremely low metabolic activity [12–14]. With the full realization of the importance of dormancy and addressing it where relevant, we hereinafter generically refer to PCD systems and mechanisms including dormancy induction. The PCD, in a sense, is a form of innate immunity inasmuch as the suicidal response is triggered indiscriminately by different pathogens. Nevertheless, given the

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fundamental biological difference between immunity responses, in which cellular organisms kill or inactivate pathogens, and PCD which entails cells (and possibly also multicellular organisms) killing themselves, we henceforth treat these strategies as distinct.

The recent discovery of adaptive immunity mediated by the CRISPR-Cas (Clustered Regularly Interspaced Palindromic Repeats and CRISPR-associated genes) systems in archaea and bacteria has attracted enormous attention and interest [15–18]. A big part of the furor is undoubtedly due to the utility of type II CRISPR-Cas (Cas9) as a new generation of tools for genome editing and regulation [19–23]. However, the CRISPR-Cas systems are also of major, fundamental biological interest, and notably, it is the unique mechanisms of these immune systems that make them such facile genome engineering tools. Arguably, the most striking aspect of the CRISPR-Cas function is that this is the only known case of adaptive immunity with heritable genomic memory, i.e. a mechanism of (quasi) Lamarckian inheritance of acquired characters [24]. Although some steps of the CRISPR-Cas response seem to involve selection, the major Lamarckian trend is obvious because the CRISPR-Cas systems modify a specific locus in the genome such that a unique phenotypic change (immunity to a specific virus or plasmid) is acquired and then transmitted across generations (in some cases, apparently millions of them) [25].

The discovery of CRISPR-Cas has stimulated extensive scrutiny of the principles and mechanisms of action of prokaryotic defense systems. In the process, multiple, intricate connections between immunity and PCD have become apparent leading to the concept of functional coupling between the two types of defense [26, 27]. Here we discuss different aspects of such connections, with an emphasis on recent discoveries showing that is some defense systems, immunity, and PCD effectively merge.

Immune systems possess an intrinsic suicidal potential

Even apart from PCD, which is dedicated machinery for altruistic self-destruction, immunity mechanisms are inherently suicidal. Simple considerations make this obvious. Immunity is a collection of mechanisms for abrogation of reproduction and destruction of parasites, above all, various mobile genetic elements including viruses. Given the fundamental unity of genetic systems across all life, cell, or virus, immunity is dangerous by design because it will inevitably attack the host itself unless kept in check. In the most general sense, this is a consequence of the laws of thermodynamics that prohibit error-less information transmission without commensurate energy expenditure [28, 29]. The numerous, often devastating autoimmune diseases are an obvious case in point [30, 31]. Additionally, autoimmunity has been demonstrated for the CRISPR-Cas systems [32–34], in accord with the conceptual notion that it is an inalienable property of immune systems. Thus, immunity can be maintained only when accompanied by efficient self/non-self discrimination mechanisms that evolve concomitantly with immunity itself. This happens when the benefits of protection from parasites are substantial, and/or when the immune systems themselves possess properties of

selfish elements and become "addictive" to the host as discussed later in this section.

The principles of self/non-self discrimination differ substantially between innate and adaptive immunity, and these distinctions reflect the major differences between these two types of immunity. Innate immunity systems recognize generic properties of the self, often modifications that these systems themselves introduce. The numerous, highly diverse and abundant restriction-modification (RM) systems present perhaps the most illuminating case in point [35–38]. The most common RM modules (known as type II) consist of two proteins one of which, a methyltransferase, is responsible for the modification (methylation) of the self and the other one, the nuclease, targets and destroys all unmodified DNA which, "from the point of view" of the RM systems is equivalent to non-self (there are many intricate variations on this theme among the RM systems that we do not have an opportunity to describe here).

The RM systems share key properties with typical toxin-antitoxin (TA) modules, the dedicated PCD inducers that are even more abundant in prokaryotes than RM [9, 39, 40]. In both the RM and TA modules, one part of the module acts as a poison and the other one as an antidote. The similarity between the two classes of systems is so pronounced that sometimes they are aggregately classified as toxins-antitoxins [41]. Yet, both the poison and the antidote function differently in immune systems compared to dedicated suicidal systems (Fig. 1). In the immune systems, the poison (such as a restriction endonuclease) directly attacks the foreign DNA, whereas the antidote (such as the corresponding methylase) protects the host genome. In contrast, in the dedicated PCD systems, the poison affects essential host molecules, such as mRNAs in the case of the TA systems, which include mRNA interferases as toxins. The antitoxin reversibly inactivates the toxin as long as the balance between the two components is maintained; again, there are many variants of TA systems in which the antitoxin functions differently, e.g. by inactivating the toxin mRNA [41–43].

The suicidal potential of the RM systems is obvious: the restriction endonuclease would kill the host whenever the methylation level of the host DNA perceptibly drops. In at least some RM systems, this potential is realized via post-segregational cell killing similar to that perpetrated by TA systems: once a RM system is lost from a cell after division, the dilution of the modification methylase leads to exposure of under-methylated DNA which is cleaved by the remaining restriction enzyme, thus, resulting in cell death [44–46]. Other RM modules attack the self DNA under specific stress conditions, in particular at arrested replication forks [47, 48]. Furthermore, type IV restriction systems (not RM because these lack the modification component) become suicidal when the bacteriophage carries its own methylase that methylates the host DNA at new sites resulting in its recognition as non-self by the type IV enzyme [49]. Both RM and TA systems are addictive to the host cells because when the genes encoding both components or the antitoxin only are lost, e.g. during cell division, the antitoxin rapidly loses activity (diluted in the case of RM and degraded in the case of TA), and typically, enough toxin remains to kill the microbial cell. Taken together, all these lines of evidence indicate that, although
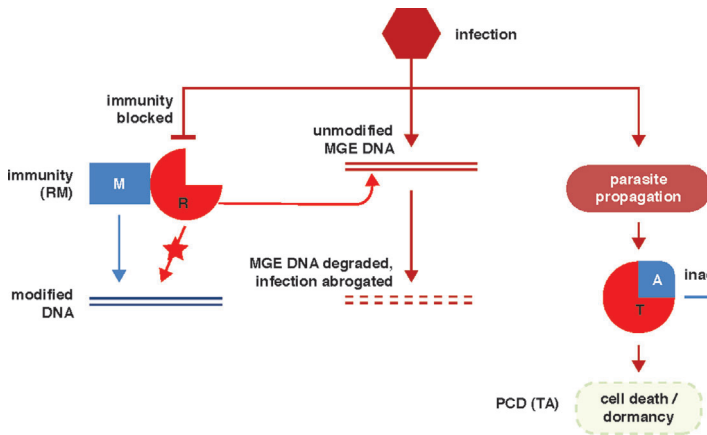
Figure 1. Immunity and programmed cell death: distinct but coupled defense strategies. The host DNA is protected from the action of the restriction enzyme by methylation whereas the invading DNA is sensitive. Innate immunity, in the form of RM systems, can inactivate the parasite DNA and block infection. However, if the innate immunity fails (e.g. due to the activity of the parasite-encoded antidefense system) and the parasite reproduces, the infection induces genotoxic stress which activates proteases cleaving antitoxins. The resulting activation of toxins leads to dormancy or PCD. M, modification enzyme; R, restriction enzyme; T, toxin; A, antitoxin; MGE, mobile genetic element.

the benefit of protecting a minority apparently outweighs the detrimental effects of the suicidal behavior [57].

Colocalization of genes and interaction of proteins of immune and PCD machineries in prokaryotes implies functional coupling

Apart from and beyond the suicidal properties of immune systems, the genomic loci encoding such systems often also include dedicated PCD modules, such as TA, and some proteins are shared by the two types of defense systems (Fig. 2). CRISPR-Cas, the most complex class of prokaryotic defense system, again presents the most remarkable cases in point. One of the key proteins in the first, adaptation phase of the CRISPR response, Cas2, is a derivative of the toxins of the VapD family of mRNA interferases [58, 59]. The primary role of Cas2 in CRISPR-Cas is that of a structural scaffold of the adaptation complex in which Cas1 is the active endonuclease component [60–62]. The interferase catalytic site is conserved in some but not all Cas2 proteins, and it has been shown that the catalytic residues of Cas2 are not required for adaptation [60]. Thus, at least in certain CRISPR-Cas systems, Cas2 might play a secondary role as a RNase, possibly a toxin [26], although catalytically active Cas2 proteins do not appear to be toxic when overexpressed in *Escherichia coli*. Indeed, non-sequence-specific nuclease activity of several Cas2 proteins against both DNA and RNA but typically, with a preference for RNA substrates, has been demonstrated [63–67]. The role of the nuclease activity of Cas2 in the CRISPR-Cas function remains obscure but evolutionary conservation of the catalytic site implies that it is functional in at least some microbes.

Many, if not most, CRISPR-Cas systems also contain additional nucleases, in particular (predicted) RNases of the HEPN (Hhigher Eukaryotes and Prokaryotes Nucleotide-binding domain) superfamily [68, 69] (Fig. 2). The RNase activity of two of these proteins, Csm6 and Csx1, has recently been experimentally demonstrated. Typically, the HEPN-containing Cas proteins additionally contain the CARF domain that adopts the Rossmann fold and is predicted to bind ligands, most likely nucleotides, and perform signaling functions [69]. Notably, the Csm6 protein that consists of a CARF and a HEPN domain is not required for the type III-B CRISPR-Cas interference [70] suggesting a different, accessory function for this protein. The HEPN superfamily consists of extremely diverse (predicted) RNases that are primarily involved in various defense functions. In particular, a highly abundant class of TA modules encompasses HEPN domain-containing proteins as the toxin moieties [68]. The HEPN domain-containing systems remain poorly functionally characterized but are common in many prokaryotes, and specifically, are the most abundant TA modules in

immunity appears to be the primary mode of action of the RM systems, they are not only accidentally suicidal and addictive via post-segregational killing, but also can be – and under various circumstances, actually are – turned into PCD devices. Given their addictiveness and frequent transfer on plasmids, both RM and TA systems can be considered a special kind of mobile genetic elements [50].

The adaptive immunity system, CRISPR-Cas, appears to be particularly precarious, in terms of the suicidal potential. Indeed, the CRISPR-Cas loci incorporate unique spacers that are employed as guides to recognize and cleave target nucleic acids [17, 18]. Obviously, self-recognition – i.e. targeting the spacer itself – by these guides would be damaging and potentially suicidal. The CRISPR-Cas systems have evolved specific safeguards against such direct self-recognition, typically in the form of a sequence motif (PAM, proto-spacer-adjacent motif) that is required for protospacer acquisition and subsequent recognition but is missing in the CRISPR, thus, preventing self-targeting [51–55].

However, direct self-recognition of the CRISPR arrays is only one form of suicidal autoimmunity to which CRISPR-Cas systems are prone. The second one involves the obvious possibility of acquisition of spacers from the host genome, followed by suicidal targeting [32–34]. It remains unclear exactly how, and strikingly, even whether CRISPR-Cas systems avoid this form of autoimmunity. In one model system – subtype I-E CRISPR-Cas – it has been demonstrated that substantial (although most likely, less than perfect) self/non-self discrimination is achieved via the recognition of actively replicating DNA that is undergoing RecBCD-mediated repair [56]. However, in another model, namely subtype II-A CRISPR-Cas, there seems to be no such discriminatory mechanism so that the CRISPR response is extremely wasteful: the majority of the bacterial cells are killed, yet,
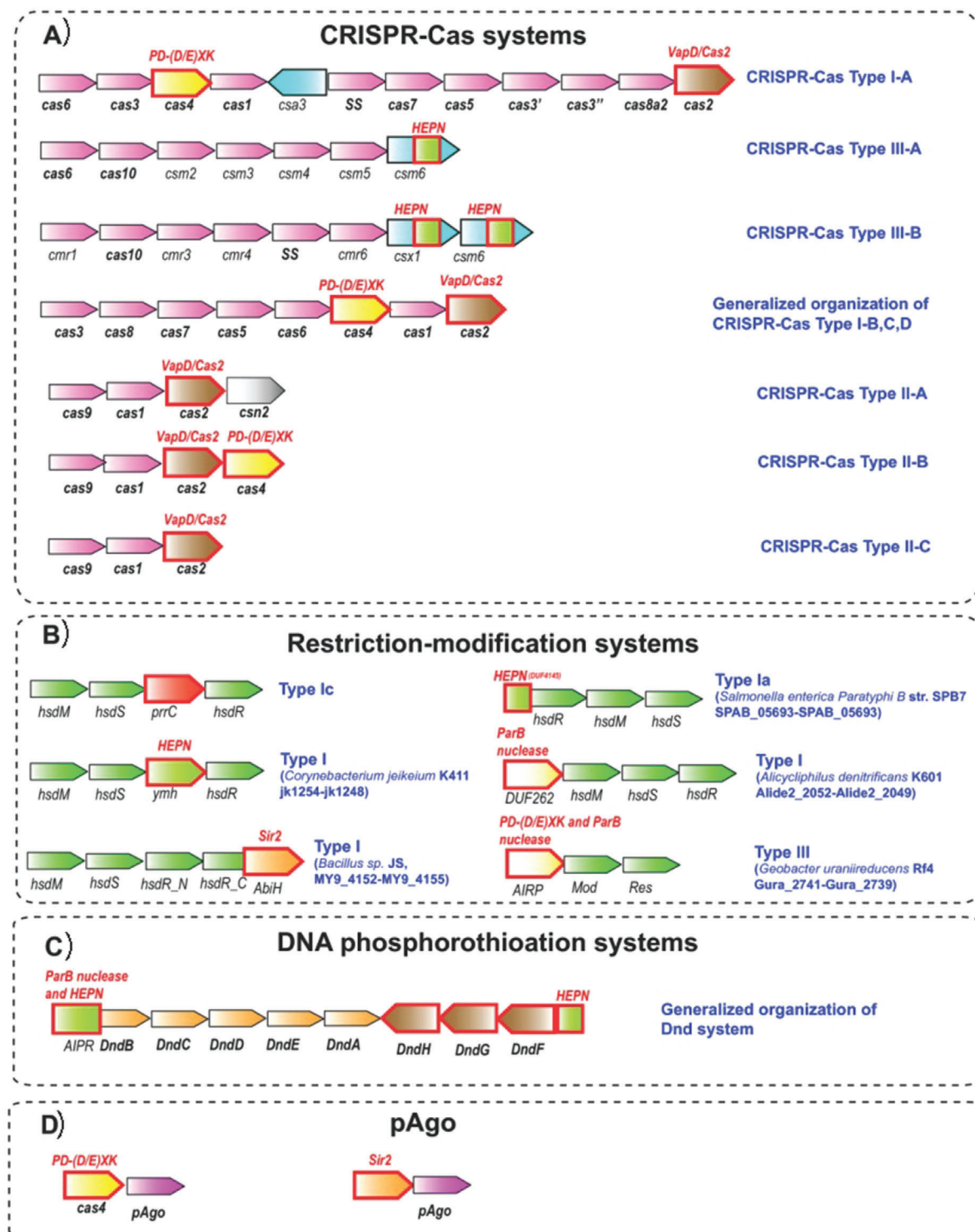
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Figure 2. Colocalization of genes encoding immune and PCD systems in bacterial and archaeal genomes. The core genes of CRISPR-Cas, RM, and DND systems in predicted operons are shown by pink arrows; genes with (predicted) toxin activity are shown by different colors, and the (predicted) toxin domains are indicated by red outline. The Csa3 protein in the Type IA system lacks the HEPN domain. HEPN, higher eukaryotes and prokaryotes nucleotide-binding domain; Sir2, ParB and REase, DEDD, nucleases from distinct superfamilies. **A**: CRISPR-Cas loci. Gene names follow the nomenclature and classification from [16]. **B**: Restriction-modification loci. Gene names follow the nomenclature and classification from [94]. **C**: Phosphorothioation loci. Gene names follow the nomenclature from [95]. **D**: Prokaryotic Argonaute genes, pAgo. Reproduced from [26] under Creative Commons License.

archaea [39, 68]. Accordingly, it appears likely that the HEPN domain-containing Cas proteins also possess toxin activity that could be masked by another domain of the same protein or by a distinct Cas protein. In some CRISPR-Cas systems, the CARF domain is fused to predicted nucleases that are unrelated to HEPN: in particular, Cas4 homologs which adopt the Restriction Endonuclease fold [69]. This apparent interchangeability of CARF-linked nucleases suggests the intriguing possibility that they are all toxins regulated through ligand-binding by the CARF domain.

A CRISPR-associated toxin activity has been directly demonstrated for the Csa5 protein of the type I-A CRISPR-Cas system of the archaeon *Sulofolobus solfataricus*. Infection of *S. solfataricus* with the SIRV2 virus induced the expression of Csa5 to the toxic level and resulted in cell death, hence suggesting that the toxicity of this protein indeed represents a PCD response to virus infection [71]. The Csa5 protein is the α-helical small subunit of the Cascade CRISPR RNA-processing complex of type I-A and does not appear to possess any nuclease activity [72], so the mechanism of toxicity remains obscure. These findings suggest that the CRISPR-associated toxicity is a broad phenomenon that goes beyond the known activities of toxic nucleases.

Apart from the CRISPR-Cas systems, comparative genomic analysis has revealed preferential association of dedicated PCD systems (TA) with innate immunity loci, such as RM [9, 26]. Taken together, these observations have prompted the hypothesis on functional coupling between immunity and PCD/dormancy [26]. Two versions of such coupling were considered. First, and most intuitively, PCD can be viewed as the strategy of last resort whereby the defense system senses the impending failure to stop virus reproduction in the given cell and accordingly switches to the suicidal mode, sacrificing the infected cell but saving other cells in the population. Alternatively, it has been speculated that faced with intense virus reproduction, the immune system would turn on the dormancy induction machinery, thus, not only protecting the surrounding cells but potentially, giving the infected cell a chance to recover once the virus clears. The two strategies might not be completely distinct given that there is never a guarantee that a cell re-emerges from dormancy. The presence, in numerous CRISPR-Cas loci, of genes encoding proteins, in which CARF domains are fused with diverse nucleases [69] (Fig. 2), implies the interesting possibility that the CARF domain functions as a sensor of defeat of the immune system in the battle with the virus, probably, in response to an alarmone that remains to be identified.

The immunity-suicide coupling hypothesis was construed on the basis of multiple but indirect lines of evidence. However, the experimental paradigm for this type of coupling is presented by an antiphage defense system that includes HEPN domain-containing RNases. These RNases, the bacterial RloC and PrrC proteins, are anticodon nucleases (ANCases) that both consist of an N-terminal NTPase domain and a C-terminal HEPN RNase domain [73, 74]. The PrrC ANCase is normally reversibly inactivated by components of a bacterial type I RM system but is activated by a phage RM inhibitor, resulting in an incision in the anticodon loop of tRNA^{Lys} which abrogates the synthesis of the phage late proteins [75]. This circuit shows the predicted coupling between immunity and PCD: inhibition of an innate immunity system (RM) triggers PCD through the activation of a toxin. The PrrC activity is additionally activated by GTP hydrolysis and stabilized by dTTP which accumulates in the phage-infected bacterial cells [76]. Thus, PrrC is effectively a toxin that is activated through sensing multiple signals emitted by the infected bacterium. Bacteriophages have evolved their ow, complex antidote, namely a pair of enzymes, polynucleotide kinase, and RNA ligase, that together repair the tRNA molecules cleaved by PrrC [77, 78].

Apparently, the activation of the RloC ACNase is the bacterial response to the phage tRNA repair system. RloC also cleaves tRNAs (in this case, tRNA^{Glu} and tRNA^{Gln}) but instead of simply incising the anticodon loop, this ACNase excises the wobble nucleotide, thus, precluding the repair by the phage kinase-ligase system [79]. Similar to PrrC, RloC is also stabilized by dTTP but does not seem to interact with RM. Instead, RloC contains a distinct domain that is a built-in sensor of double-stranded DNA breaks (DSB) [80, 81]. Once an elevated level of DSB is sensed, under genotoxic stress caused by phage infection or other factors, the sensor domain triggers a conformation change that turns the protein into an active toxin. Notably, activation of RloC coincides with activation of CRISPR so that the two systems are thought to provide complementary defenses [80]. Thus, the antiphage ACNases, especially PrrC, given its direct connection with RM, clearly demonstrate the link between dormancy induction (or at least, toxic effect), in this case through tRNA inactivation, and immunity mechanisms, such as RM and possibly CRISPR-Cas.

In the next section, we discuss a recent discovery that might directly link CRISPR-Cas to dormancy induction.

Type VI CRISPR-Cas systems: Dual immunity-suicide function

The recent discovery of new Class 2 CRISPR-Cas systems, driven by a comprehensive search for genomic loci that encode large proteins containing putative nuclease domains that could function as CRISPR-Cas effectors, has revealed what arguably is the most direct link between microbial immunity and PCD so far discovered [82–84]. Type VI effector proteins contain two HEPN domains that are predicted to possess RNase activity [82, 84]. Such an activity requiring both HEPN domains indeed has been demonstrated for the subtype VI-A effector (denoted C2c2, or provisionally, Cas13a) [83]. As expected of a RNA-targeting CRISPR effector, Cas13a provides efficient protection against the RNA bacteriophage MS2. In addition, Cas13a showed a distinct capacity that, though apparently highly unusual, in retrospect, could perhaps have been predicted. When primed with a cognate RNA, this protein becomes a promiscuous RNase that cleaves any RNA molecules present in the reaction mix with little sequence specificity (Fig. 3). Moreover, a decrease in bacterial viability was observed when Cas13a was coexpressed with the cognate RNA, suggesting dormancy induction [83]. Given the apparent minor contribution of RNA bacteriophages to the bacterial virosphere [85], it appears most likely that the principal functionality of subtype VI-A is defense against DNA phages that is realized through the toxic effect that is triggered by the recognition of a
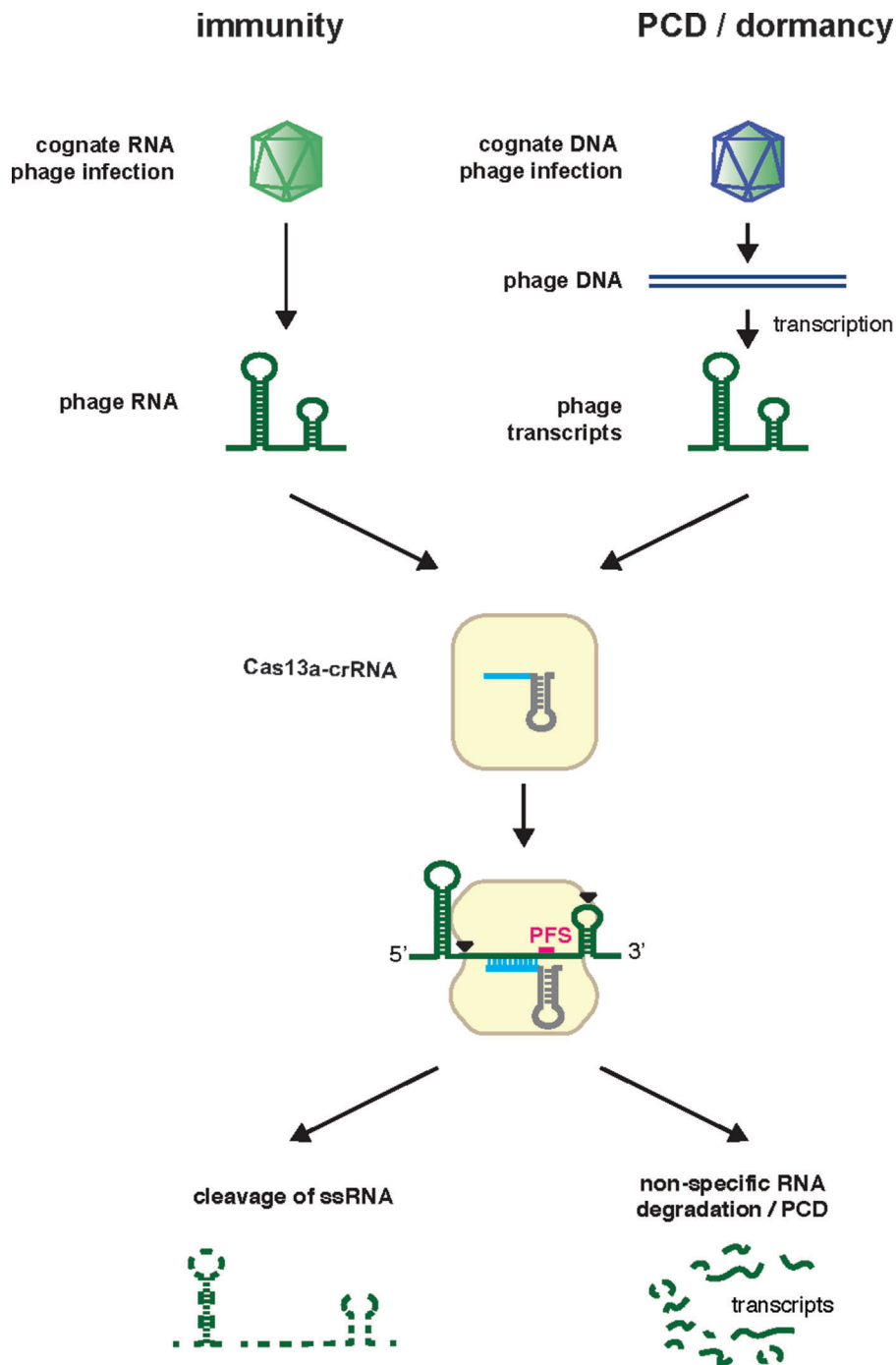
Figure 3. Type VI CRISPR-Cas systems: merging immunity with PCD. Upon infection with a RNA phage, for which a cognate spacer(s) is available, the Cas13a-crRNA complex recognizes and inactivates the target. However, the defense against DNA viruses is thought to proceed via the PCD route whereby recognition of the target (a virus transcript) triggers a conformation change in Cas13b, turning it into a promiscuous RNase. This activity then causes dormancy or PCD, hence preventing virus reproduction. Modified with permission from [83].

(Fig. 3). The mechanism of the transformation of Cas13a from an immune to a PCD/dormancy effector remains to be elucidated. A conformational change triggered by the formation of a complex with the cognate target RNA is a plausible, general explanation but the specifics that should become clear once structures of Cas13a complexed with different substrates are solved are of major interest. Regardless, however, type VI-A systems are a showcase for immunity-PCD coupling where the immune machinery itself appears to switch into suicidal mode.

Recently, two additional subtypes of type VI CRISPR-Cas systems have been discovered by computational screening of bacterial genomes [84]. The effector proteins of all type VI systems contain two HEPN domain, and by analogy to subtype VI-A, can be predicted to be able to switch to the PCD/dormancy mode. Moreover, the HEPN-containing proteins of Class 1 CRISPR-Cas systems might function in a similar fashion, especially given that some of these proteins combine a HEPN domain with a CARF (CRISPR-Associated Rossmann Fold) domain, a potential stress sensor [69]. Although the CARF domain-containing proteins have not been studied biochemically, several structures have been solved, and the presence of a conserved Rossmann fold strongly suggests that these proteins bind ligands, most likely nucleotide derivatives, and accordingly, could function as allosteric regulators of other Cas proteins [69].

What governs life-or-death decisions, and why bother with dedicated suicide machinery?

Regardless of whether the cell that turns on the self-afflicting program kills itself right away or goes into dormancy, with a chance of come-back, the factors that determine the decision are the same: the cell must "predict" the outcome of infection and act accordingly (Fig. 4). If, after the immune system recognizes foreign invasion, the sensor module "predicts" that the onslaught is likely to be manageable, the immune system is mobilized to its full capacity. If, on the contrary, the forecast is dire, the

cognate phage transcript and leads to dormancy or PCD. Clearly, this hypothesis remains to be tested directly.

Thus, the HEPN domain, an RNase that typically functions either as a toxin or as an immunity effector [68], appears to alternately act in each of these capacities in the case of Cas13a
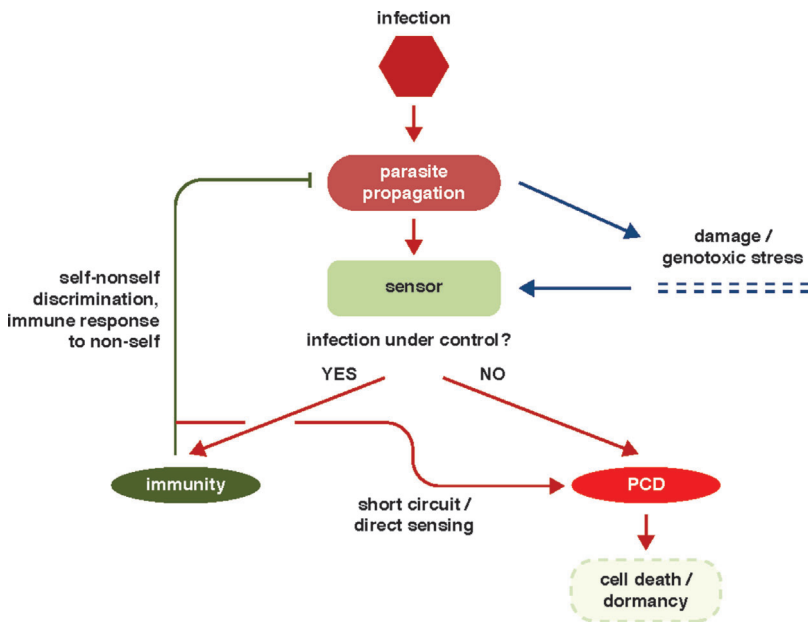
Figure 4. Switching from immunity to PCD through a sensor module. The sensors can be different for different immune and PCD systems, and could sense DNA damage directly, as in the case of RloC, or via an alarmone, possibly, a modified nucleotide, that remains to be identified, as in the case of the CARF domains associated with many CRISPR-Cas systems.

self-destruction program is turned on. The signals read by the sensor are likely to differ between defense systems. In some cases, the damage to the cell (genotoxic stress level) could be measured directly as exemplified by the DSB sensing by RloC [80]. The same ACNase as well as PrrC also senses the increased concentration of dTTP which accumulates during phage infection and effectively serves as an alarmone [76, 86]. The ligand(s) that serves as the signal for the CARF domain in the case of CRISPR-Cas system remain to be identified but the possibility that the CARF domain [69] is a toggle between the immune and self-afflicting responses appears imminently plausible. The nature of the switching signals, their threshold values and what determines these, and whether these features specifically depend on the character of virus-host interaction, are all intriguing directions for further study.

Type VI-A CRISPR-Cas systems (and conceivably, other variants of type VI) are a special case because they appear to short-circuit the typical defense relay by skipping or at least simplifying the damage-sensing step and employing the main immune effector as the suicide effector as well (Figs. 3 and 4). Indeed, Cas13a switches to the promiscuous mode in vitro where the only signal comes from the recognition of the target [83]. Type VI systems are rare among bacteria [84], and this might reflect the high cost of these systems to the host due to their "panic" response to invading DNA. Nevertheless, sensing of the target RNA concentration, which would reflect multiplicity of infection and/or the intensity of the expression of the virus genome, by the Cas13 proteins themselves, could occur even in this case. The more complex defense strategies that involve the dedicated "forecast module" (sensor) (Fig. 4), such as Class 1 CRISPR-Cas, are likely to outcompete the

simple ones where the self destruction program is activated at the first alarm signal.

Both immune systems with their suicidal proclivities, and especially, dedicated suicide devices are prone to misfiring and are thus, costly for the organism. What, then, are the factors that underlie the broad (although not universal) persistence of both these types of costly defense strategies? Mathematical modeling of the coevolution of different types of defense with pathogens as well as biological features of the defense systems seem to offer some clues [87, 88]. Detailed analysis of the coevolution models indicates that, assuming some basal level of innate immunity, adaptive immunity, and suicide can coexist within a relatively small region of the parameter space where the efficacies of both types of defense are limited [89]. Such a situation seems to correspond to the sensing toggle circuit outlined in Fig. 4, where the sensor "predicts" the outcome of infection and whether the immune system is likely to cope successfully. These considerations on coevolution of the immune and suicidal defense strategies apply to both adaptive immunity – which dominates when a cell encounters a familiar virus or plasmid – and innate immunity which acts against newcomers.

Immunity-suicide coupling is favored when the system includes dual function components that are involved both in immune and in suicidal activities [89]. This could be the case for the Cas proteins, such as Cas2, which is essential for adaptive immunity, but given its homology with interferases, might also display toxic properties. Furthermore, although the biological functions of the HEPN-containing Cas proteins, such as Csm6 and Csx1, are not well understood, it appears likely that they also contribute both to the interference stage of the adaptive immune response and, as toxins, to the self-destruction program [26, 68, 70, 90]. An intriguing question that remains to be addressed experimentally is whether or not CRISPR-Cas systems are capable of post-segregational cell killing.

From a complementary perspective, the persistence of the dedicated suicide systems as well as at least some immune systems, such as RM, has to do with the fact that TA and RM modules possess features of selfish genetic elements, or more specifically, make the host cells addicted to these modules by killing cells that purge them [91, 92].

Conclusions

Most organisms, even bacteria and archaea with small genomes, possess multiple layers of anti-parasite defense including both immune mechanisms that affect the invading agents and suicidal mechanisms. The coexistence of these fundamentally different defense strategies seems to be caused by the limited efficacy of immune systems that can be overcome by rapidly replicating parasites, under high multiplicity of infection and in other situations. The immune and suicidal strategies not only coexist and are often encoded

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in the same genomic loci but seem to be functionally coupled, in particular by virtue of sharing protein components. There are indications, although not yet solid evidence, that the switch from the immune mode to the suicidal mode of defense is governed by dedicated sensors that determine the level of damage inflicted on the cell and on that basis "predict" the outcome of the infection. In some cases, sensing the damage can be short-circuited as exemplified by the subtype VI-A CRISPR-Cas systems. However, such streamlined immunity-suicide systems are rare, hence suggesting that foregoing damage sensing could be costly. Although not the subject of this article, coupling of immunity with PCD and elaborate control of cell death are manifest also in eukaryotes and appear to be important in host-parasite interactions [93]. Thus, linkage between immunity and PCD, or in other terms, between parasite killing and suicide, appears to be a general attribute of cellular life forms. Understanding the coupling mechanisms as well as the specifics of damage sensing, which allows the cells to forecast the outcome of the infection and make informed life or death decisions, is an important and exciting direction of further research.

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