Anti-CRISPR: discovery, mechanism and function

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Abstract | CRISPR–Cas adaptive immune systems are widespread among bacteria and archaea. Recent studies have shown that these systems have minimal long-term evolutionary effects in limiting horizontal gene transfer. This suggests that the ability to evade CRISPR–Cas immunity must also be widespread in phages and other mobile genetic elements. In this Progress article, we discuss recent discoveries that highlight how phages inactivate CRISPR–Cas systems by using anti-CRISPR proteins, and we outline evolutionary and biotechnological implications of their activity.

The Red Queen hypothesis proposes that organisms must continually evolve new mechanisms of resistance to parasites to avoid extinction[1]. The parasites, in turn, evolve countermeasures to circumvent these resistance mechanisms. This battle for survival can be tracked in the co-evolutionary dynamics of bacteria and viruses that prey on them, which are known as phages. Bacteria have evolved many diverse strategies to defend themselves against phage predation, including restriction–modification enzymes that inactivate target DNA by cleavage, toxin–antitoxin modules that lead to phage abortive infection and CRISPR–Cas systems that target and inactivate specific nucleic acid sequences by cleavage. In response, phages have evolved various mechanisms to overcome these defences, including expression of proteins that modify restriction sites[2–4] or degrade restriction–modification cofactors[5], antitoxin molecules that inhibit the activity of toxin-antitoxin abortive infection systems[6,7] and proteins that directly bind to and inactivate CRISPR–Cas machinery[8–14].

CRISPR–Cas systems provide bacteria with a sequence-specific defence mechanism against invasion by 'foreign' nucleic acids such as phages or plasmids[15–18]. Small CRISPR RNA (crRNA) molecules guide Cas proteins to recognize and destroy sequences that are complementary to the crRNA[17,19–22]. CRISPR–Cas systems are grouped into two broad classes (class 1 and class 2) encompassing six types, based on their phylogeny and mechanisms of action[23]. Class 1 systems, comprising types I, III and IV, use multisubunit Cas protein complexes for the recognition of targeted nucleic acids (for example, CRISPR-associated complex for antiviral defence (Cascade) in type I systems). By contrast, class 2 systems, comprising types II, V and VI, use a single effector protein (such as Cas9) that performs target recognition and nucleic acid cleavage functions[23,24]. Notably, the type II CRISPR–Cas9 system has been widely adapted for genome editing and other biotechnological applications[25].

The high potency and adaptive nature of CRISPR–Cas systems may be expected to provide a potent barrier to the propagation of mobile genetic elements (MGEs) in bacteria[15,16,26]. This includes not only phages and plasmids but also integrative and conjugative elements[27], as well as genomic islands[28,29]. When a bacterial cell with an active CRISPR–Cas system encounters a new MGE, it can 'immunize' itself through the incorporation of a small piece of the foreign DNA (known as a spacer) into the CRISPR array. If the same MGE is encountered again, it will be recognized as foreign and targeted for degradation[17]. MGEs can evade detection by CRISPR–Cas systems through the accumulation of mutations in their spacer-targeted sequence (known as the protospacer) or in the protospacer-adjacent motif (PAM). The PAM is a signature motif adjacent to the protospacer and is required by most CRISPR–Cas systems to distinguish the foreign protospacer from the identical 'self' spacer sequence that is part of the CRISPR array[30]. However, owing to the diversity of new spacers acquired randomly by different cells in a bacterial population, phages cannot completely escape CRISPR–Cas targeting in a continuous co-culture simply by random mutation[31]. Additionally, several type I CRISPR–Cas systems have been shown to rapidly acquire new spacers to re-immunize themselves against escape mutants through a process known as primed adaptation. This process relies on the protein complex Cascade to direct the adaptation machinery to a partially complementary protospacer sequence in a crRNA-dependent manner, which results in selective acquisition of new spacers from nearby sites on the MGE[32]. Despite the high potency of CRISPR–Cas immunity, several studies found no evidence that this type of bacterial defence reduces horizontal gene transfer over evolutionary timescales[33–36]. Indeed, a recent study showed that bacteria with CRISPR–Cas systems were even more likely than their CRISPR–Cas-deficient counterparts to possess integrated prophages[34]. However, a strong negative correlation between the presence of type III CRISPR–Cas and prophage number was reported, which indicates that type III systems may be more effective than other types in the limitation of the acquisition of prophages[34]. A comprehensive bioinformatic study showed that there was no relationship between the number of spacers in CRISPR arrays and the rate of horizontal gene transfer found in an organism[33]. Taken together, these data suggest that inhibitors of CRISPR–Cas systems are prevalent in MGEs across bacterial phylogeny and enable the horizontal gene transfer that occurs between bacterial lineages despite adaptive CRISPR–Cas immunity.

In this Progress article, we discuss the discovery of anti-CRISPR proteins and explore the recent insights into their functions and mechanisms of action. Moreover, we detail evolutionary and ecological implications and provide an outlook of the potential biotechnological applications of anti-CRISPR proteins.

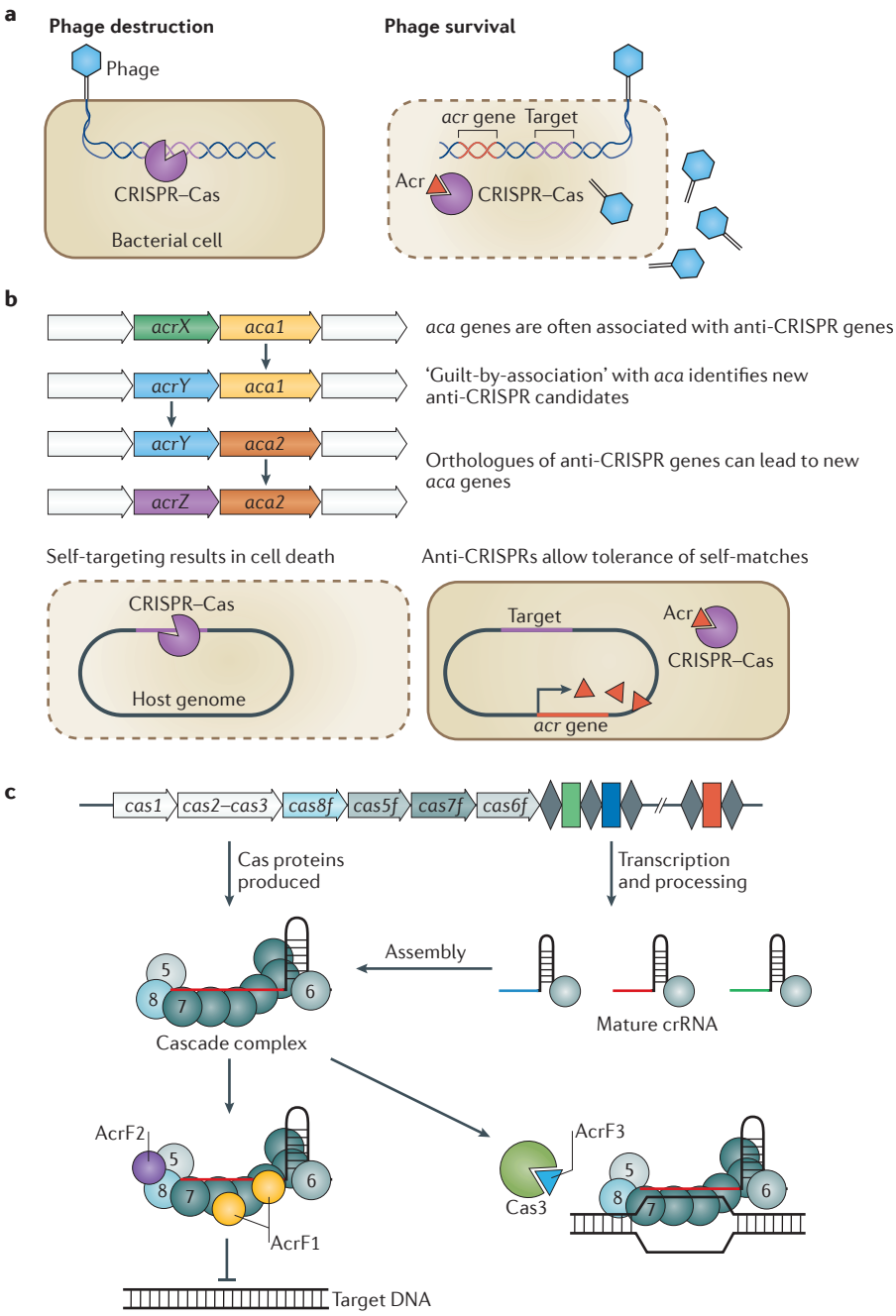

Figure 1 | **Anti-CRISPR gene discovery. a** | Carrying an anti-CRISPR gene (*acr*) can enable a phage to survive and propagate when it may otherwise have been targeted by the CRISPR–Cas system of the host. **b** | Upper panel: a visual representation of the 'guilt-by-association' method for identifying new candidate anti-CRISPR genes on the basis of their location immediately upstream of anti-CRISPR associated (*aca*) transcriptional regulator genes. Lower panel: a visual representation of the self-targeting method for identifying new candidate anti-CRISPR genes, which relies on the logical assumption that an active CRISPR–Cas system and a CRISPR RNA (crRNA, not shown) targeting the host genome cannot coexist without resulting in genome targeting and cell death. Therefore, anti-CRISPR proteins that are encoded in integrated mobile genetic elements and are present within the same cell could prevent cell death caused by self-targeting. **c** | The mechanism of action of three type I-F anti-CRISPR proteins. The CRISPR locus is transcribed and processed into mature crRNAs, which combine with Cas proteins to form a ribonucleoprotein complex known as Cascade. Cascade surveys the cell looking for sequences complementary to the crRNA. When a match is found, the Cas3 nuclease is recruited to the complex, and the target DNA is cleaved (not shown). AcrF1 (indicated as two yellow spheres) and AcrF2 (indicated as a purple sphere) interact with Cascade proteins Cas7f and Cas8f–Cas5f, respectively, and inhibit binding to the DNA target. AcrF3 (indicated as a yellow triangle) binds to the Cas3 nuclease and prevents it from cleaving the target DNA. Part c is adapted with permission from REF. 53, PLoS.

Discovery of CRISPR–Cas inhibitors

The first examples of active inhibitors of CRISPR–Cas systems were discovered in a closely related group of *Pseudomonas* spp. phages. These phages were able to infect and propagate in a *Pseudomonas aeruginosa* strain with an active type I-F CRISPR–Cas system even though they possessed protospacer sequences that should have been targeted by this system[8] (FIG. 1a). Sequence analyses pinpointed the genomic region responsible for this 'anti-CRISPR' phenotype and identified individual genes involved. In total, five distinct proteins (AcrF1, AcrF2, AcrF3, AcrF4 and AcrF5) were shown to inactivate the type I-F CRISPR–Cas system (TABLE 1). As none of these proteins perturbed the expression of *cas* genes or the production of mature crRNA molecules, it was hypothesized that they directly blocked CRISPR–Cas interference. In a follow-up study, four additional distinct families of small proteins (AcrE1, AcrE2, AcrE3 and AcrE4) were shown to inhibit the type I-E CRISPR–Cas system of *P. aeruginosa*[10] (TABLE 1). These proteins were encoded by genes in the same group of phages and positioned adjacent to the type I-F anti-CRISPR genes. Similar to the type I-F anti-CRISPR proteins, no homologues of the type I-E anti-CRISPR proteins were identified in MGEs of other bacterial genera.

The nine initially described anti-CRISPR protein families did not share any common sequence motifs that could lead to the discovery of new anti-CRISPR proteins that might be present in other species and/or active against other CRISPR–Cas types[8]. However, the genomic context of the genes encoding these proteins was very similar. Phages encoding anti-CRISPR proteins also encoded a putative transcriptional regulator known as anti-CRISPR associated 1 (Aca1; encoded by *aca1*) that contained a helix–turn–helix motif immediately downstream of the anti-CRISPR genes. By contrast, the phages that lacked anti-CRISPR genes also lacked *aca1* (REF. 8). Although investigations into the function of this regulator are still ongoing, the anti-CRISPR genes and *aca1* form a single operon, and the Aca regulatory protein seems to control the expression of the anti-CRISPR and *aca* genes for optimal activity during the phage infection cycle. Further studies used a 'guilt-by-association' bioinformatic approach to identify putative anti-CRISPR genes on the basis of their genomic location upstream of the putative regulator *aca1* in MGEs (FIG. 1b). Homologues of candidate

anti-CRISPR genes were observed upstream of a gene encoding a helix–turn–helix protein from a sequence family that was distinct from Aca1. In turn, this new family of Aca proteins (Aca2) led to the identification of additional candidate anti-CRISPR genes[11]. This work revealed and functionally validated five new families of type I-F anti-CRISPR proteins (AcrF6, AcrF7, AcrF8, AcrF9 and AcrF10), which are widespread in MGEs across the phylum Proteobacteria (TABLE 1).

Subsequently, by using this guilt-by-association method, the first inhibitors of a type II CRISPR–Cas system were discovered. Three families of small proteins (AcrIIC1, AcrIIC2 and AcrIIC3) were shown to block the type II-C CRISPR–Cas9 activity of *Neisseria meningitidis*, both in its native bacterial context and when it was used as a genome-editing tool in cultured human cells[9] (TABLE 1). An alternative bioinformatic approach was used to discover inhibitors of type II-A CRISPR–Cas9 systems (FIG. 1b). The study identified bacterial strains that encode type II-A CRISPR–Cas9 systems with crRNAs that match protospacers in their own genomes. Because self-targeting by the CRISPR–Cas system would be expected to cause cell death, it was postulated that any genome in which self-targeting occurred was likely to carry an anti-CRISPR gene in a MGE present within the same cell[12]. Expression of this endogenous anti-CRISPR protein from the MGE would inactivate the CRISPR system and allow the cell to tolerate self-targeting. By use of these criteria, four new families of anti-CRISPR proteins that inhibit the type II-A CRISPR–Cas system of *Listeria monocytogenes* were discovered[12] (AcrIIA1, AcrIIA2, AcrIIA3 and AcrIIA4) (TABLE 1). Two of these anti-CRISPR proteins, AcrIIA2 and AcrIIA4, were shown to be effective against the type II-A CRISPR–Cas9 protein of *Streptococcus pyogenes*.

Genes encoding anti-CRISPR proteins have been identified in myophages, siphophages, putative conjugative elements and pathogenicity islands. There are no common surrounding genetic features except the frequent presence of an *aca* gene immediately downstream of anti-CRISPR genes. Anti-CRISPR genes have been found both near the capsid and tail morphogenetic genes in phages and at the extreme end of their genomes. Some MGEs have two or three anti-CRISPR genes in a single operon, and the high variability in the arrangements and pairings of anti-CRISPR genes suggests that they may have been exchanged among MGEs[11]. Anti-CRISPR proteins are classified according to the type of CRISPR–Cas system that they inhibit. The naming convention that has been established includes the type of system inhibited, a numerical value referring to the protein family and the source of the specific anti-CRISPR protein. For example, $AcrIIC1_{Nme}$ is active against the type II-C CRISPR–Cas system, it was the first anti-CRISPR described for this system, and it is encoded in an integrated MGE in a *N. meningitidis* genome.

Anti-CRISPR mechanisms

To date, there have been 21 unique families of anti-CRISPR proteins described against type I and type II CRISPR–Cas systems (TABLE 1). Besides their small size, typically between 50 and 150 amino acids, there are no common features that are conserved among these protein sequences. None of the families has sequence similarity to any protein of known function. Over the past few years, mechanisms of action have been determined for six anti-CRISPR proteins by use of a combination of genetic, biochemical and structural studies.

The first mechanistic insight was provided by studies of the *P. aeruginosa* type I-F anti-CRISPR proteins AcrF1, AcrF2 and AcrF3 (REF. 37). AcrF1 and AcrF2 were both shown to interact directly with type I-F Cascade complex, inhibiting its ability to bind to DNA (FIG. 1c). However, although these anti-CRISPR proteins have the same functional outcome, they interact with different protein subunits. Indeed, two to three copies of monomeric AcrF1 bind to the Cas7f hexamer, which forms the extended backbone of the type I-F Cascade complex[37–39]. Critical interactions are formed between a cluster of three key residues on the surface of AcrF1 (REF. 40) and exposed lysine residues in the Cas7f protein backbone, blocking access of the DNA target[37,38,40]. By contrast, the small, acidic AcrF2 protein binds to two other proteins (Cas8f–Cas5f) in type I-F Cascade and sterically blocks DNA binding by competing with the target DNA for crucial interactions with two positively charged helices on the neighbouring Cas7f subunits, which are known as the lysine-rich vise[37,38,41]. In a mechanism similar to that of AcrF2, the AcrIIA4 anti-CRISPR protein

Table 1 | **Anti-CRISPR protein families**

Anti-CRISPR protein family	Characterized member	CRISPR system inhibited	Number of amino acids	Refs
AcrE1	JBD5-34 (*Pseudomonas aeruginosa*)	I-E	100	10
AcrE2	JBD88a-32 (*P. aeruginosa*)	I-E	84	10
AcrE3	DMS3-30 (*P. aeruginosa*)	I-E	68	10
AcrE4	D3112-31 (*P. aeruginosa*)	I-E	52	10
AcrF1	JBD30-35 (*P. aeruginosa*)	I-F	78	8
AcrF2	D3112-30 (*P. aeruginosa*)	I-F	90	8
AcrF3	JBD5-35 (*P. aeruginosa*)	I-F	139	8
AcrF4	JBD26-37 (*P. aeruginosa*)	I-F	100	8
AcrF5	JBD5-36 (*P. aeruginosa*)	I-F	79	8
AcrF6	$AcrF6_{Pae}$ (*P. aeruginosa*)	I-E and I-F	100	11
AcrF7	$AcrF7_{Pae}$ (*P. aeruginosa*)	I-F	67	11
AcrF8	$AcrF8_{ZF40}$ (*Pectobacterium* phage ZF40)	I-F	92	11
AcrF9	$AcrF9_{Vpa}$ (*Vibrio parahaemolyticus*)	I-F	68	11
AcrF10	$AcrF10_{Sxi}$ (*Shewanella xiamenensis*)	I-F	97	11
AcrIIA1	$AcrIIA1_{Lmo}$ (*Listeria monocytogenes*)	II-A	149	12
AcrIIA2	$AcrIIA2_{Lmo}$ (*L. monocytogenes*)	II-A	123	12
AcrIIA3	$AcrIIA3_{Lmo}$ (*L. monocytogenes*)	II-A	125	12
AcrIIA4	$AcrIIA4_{Lmo}$ (*L. monocytogenes*)	II-A	87	12
AcrIIC1	$AcrIIC1_{Nme}$ (*Neisseria meningitidis*)	II-C	85	9
AcrIIC2	$AcrIIC2_{Nme}$ (*N. meningitidis*)	II-C	123	9
AcrIIC3	$AcrIIC3_{Nme}$ (*N. meningitidis*)	II-C	116	9

targets type II-A CRISPR–Cas9 systems by mimicking double-stranded DNA (dsDNA) and occupying the PAM-binding site of *S. pyogenes* Cas9, thereby abrogating the ability of *S. pyogenes* Cas9 to bind target DNA[42–44]. By contrast, the anti-CRISPR protein AcrIIC3 blocks type II-C CRISPR–Cas9 from binding to target DNA but also induces the dimerization of Cas9 (REF. 45); thus, the mechanism by which AcrIIC3 interferes with target recognition is likely to be distinct from that of AcrIIA4, which does not lead to Cas9 dimerization. Each of these mechanisms enables foreign DNA to escape recognition and subsequent degradation by the CRISPR–Cas complexes.

In contrast to the anti-CRISPR proteins that bind to type I-F Cascade, AcrF3 was found to interact with the Cas3 helicase–nuclease effector, which is recruited after Cascade binds target DNA[37] (FIG. 1c). Structural studies that used X-ray crystallography[46] and cryo-electron microscopy[47] revealed that AcrF3 binds as a dimer to Cas3 and locks it in an ADP-bound form, thereby preventing its recruitment by Cascade. An analogous result is achieved by AcrIIC1, in which the anti-CRISPR protein AcrIIC1 was shown to block target cleavage in type II-C systems by binding the Cas9 HNH (His-Asn-His) endonuclease domain[45]. This impedes access to the active site and prevents the conformational changes that are required for cleavage activity. Although only a few anti-CRISPR proteins have been studied in detail, it is clear that there are many effective strategies used to inhibit CRISPR–Cas systems, and many additional novel structures and mechanisms are likely to be determined.

Evolutionary implications

As mentioned above, a recent study showed that phages are unable to evade a CRISPR–Cas system by mutation alone when targeted by multiple and diverse CRISPR spacers. The authors also demonstrated that a phage must carry at least one anti-CRISPR gene to avoid elimination when cultured with CRISPR–Cas-competent hosts[31]. This strong selection is likely to contribute to the broad diversity of anti-CRISPR sequences and mechanisms that have been observed in the currently identified collection of anti-CRISPR proteins.

The maintenance of a diverse repertoire of anti-CRISPR genes enables MGEs to overcome multiple CRISPR–Cas subtypes. In fact, the high diversity observed in CRISPR–Cas systems may be partly driven by the presence of equally diverse anti-CRISPR proteins and may explain the reason for having multiple CRISPR–Cas systems belonging to different types and/or subtypes in a single strain[24]. This would be in agreement with the Red Queen hypothesis, in which both MGEs and their hosts must continually evolve new defence and counter-defence strategies to persist[1].

CRISPR–Cas inhibitors encoded by phages and other MGEs could disseminate rapidly through bacterial communities. Many MGEs become integrated into the bacterial chromosome and thus could lead to permanent inactivation of the host CRISPR–Cas system through stable expression of the anti-CRISPR proteins. This process would render the host susceptible to subsequent gene transfer events, as a single anti-CRISPR gene could inhibit the ability of the CRISPR–Cas system to target all invading foreign sequences. Long-term CRISPR–Cas inactivation would be expected to result in loss of the *cas* genes or CRISPR loci or in the accumulation of deleterious mutations. In support of this hypothesis, 12% of bacterial CRISPR–Cas systems were computationally defined as incomplete owing to the presence of inactivating mutations or missing *cas* genes, and fewer than half of all sequenced bacteria and archaea encode CRISPR–Cas systems[23]. Some lineages of bacteria seem to lack CRISPR–Cas systems, which suggests that the advantage of having these systems may vary widely among species with different lifestyles[48]. Other studies have demonstrated that CRISPR–Cas systems can be readily lost from bacterial genomes if they prevent the acquisition of highly beneficial foreign DNA[49]. These and other studies highlight the fitness costs and benefits associated with CRISPR–Cas systems under different conditions[49,50]. Further investigations will be required to assess the impact of anti-CRISPR genes on horizontal gene transfer, including how they might influence the spread of antibiotic resistance and virulence genes.

Several studies have illustrated the broad range of activity of certain anti-CRISPR proteins. The majority of type I-F anti-CRISPR protein families have been shown to inhibit the type I-F CRISPR–Cas systems of both *P. aeruginosa* and *Pectobacterium atrosepticum*, which share only 39–63% pairwise sequence identity in their Cas proteins[11]. Similarly, two type II-A anti-CRISPR proteins were able to block Cas9 proteins that share 53% sequence identity[12], and a type II-C protein was shown to block multiple Cas9 proteins through its interactions with the highly conserved HNH endonuclease domain[45]. Assuming that homologues of confirmed anti-CRISPR proteins are active in the species in which they are found, this broad activity becomes even more pronounced. Predictions of the impact of anti-CRISPR activity on all type I-F and type II-C CRISPR–Cas systems show that the majority of species carrying these types of CRISPR–Cas systems are likely susceptible to one or more known anti-CRISPR proteins[9,11].

Examining a sequence similarity dendrogram of all CRISPR–Cas effectors[23], it is clear that the anti-CRISPR proteins discovered to date can affect a large proportion of CRISPR–Cas diversity (FIG. 2a). The discovery of anti-CRISPR proteins active against both type I and type II systems illustrates that these inhibitors are important when MGEs encounter both multisubunit (class 1) and single-effector (class 2) CRISPR–Cas systems. Taken together with the wide distribution of anti-CRISPR genes across both Gram-negative and Gram-positive bacteria (FIG. 2b), it is apparent that inhibition of CRISPR–Cas systems is a common strategy for MGEs that, with further investigation, is likely to be discovered for all types of CRISPR–Cas system and all prokaryotic organisms.

Biotechnological applications

Owing to the unique mechanisms of action of anti-CRISPR proteins, they can be used in creative ways to modulate CRISPR–Cas function[51]. For example, when AcrF3 and a promoter-targeting crRNA are expressed in cells with an active type I-F CRISPR–Cas system, Cascade binds to the promoter and, in the absence of Cas3 nuclease activity, results in a sequence-specific transcriptional repression system[37]. In this case, a Cas3-inhibiting anti-CRISPR protein (such as AcrF3) enables Cascade to stably bind, but not degrade, DNA, and to impede access to DNA by the RNA polymerase, thus preventing transcription. Other type I anti-CRISPR proteins that inhibit Cas3 and type II anti-CRISPR proteins that enable Cas9 DNA binding but not cleavage would be expected to have similar effects.

The potential biotechnological applications of type II anti-CRISPRs are more obvious, given the extensive use of CRISPR–Cas9-based genome editing technologies. Their use could be particularly important as a backup safety measure for gene drive and gene therapy technologies. Studies focused on type II-C[9] and type II-A[12] anti-CRISPR proteins provided proof of

principle that anti-CRISPR proteins can effectively block Cas9-based genome editing in cultured human cells. Thus, anti-CRISPR proteins that inhibit Cas9 can be harnessed to decrease off-target effects[44]. Anti-CRISPR proteins could also be used to restrict editing activity to particular tissues or developmental stages and to generally increase the safety and efficiency of genome editing technology based on Cas9. AcrIIC3 was shown to prevent catalytically inactive Cas9 from *N. meningitidis* from binding to DNA in human cells[9], and AcrIIA2 and AcrIIA4 were shown to inhibit transcriptional repression by catalytically inactive Cas9 from *S. pyogenes* in *Escherichia coli,* which indicates that anti-CRISPR proteins may also be used in CRISPR interference (CRISPRi), CRISPR activation (CRISPRa) and other applications that rely on Cas9 to localize fused effector domains to specific genomic loci[12,25,52]. These biological effects demonstrate the wide potential utility of anti-CRISPR-mediated control of CRISPR–Cas activity.

Conclusions and outlook

At this point, it remains unclear how anti-CRISPR genes evolved. An astounding amount of sequence and structural plasticity has been observed in these very small proteins, which is likely to be a result of extreme selective pressures from CRISPR–Cas immunity. As more anti-CRISPR proteins are discovered, patterns may start to emerge, but currently, the best hypothesis for anti-CRISPR evolution is *de novo* protein evolution: a process by which small ORFs can arise stochastically through mutation or insertions and exhibit a remarkable degree of evolutionary plasticity until they perform a function that increases the fitness of the 'selfish' genetic element on which they are encoded. This theory is compelling given the rapid generation time and high mutation rate of phages, coupled with the small size (<150 amino acids) of all known anti-CRISPR proteins.

Given the large number of anti-CRISPR proteins that have already been discovered in a short period of time, it is likely that there are anti-CRISPR proteins against most, if not all, CRISPR–Cas systems. A considerable proportion of CRISPR–Cas systems may be inhibited by anti-CRISPR proteins encoded by MGEs within the same genomes. However, the prevalence of CRISPR–Cas systems implies that they are still providing a widespread adaptive advantage. Based on the Red Queen hypothesis, it is possible

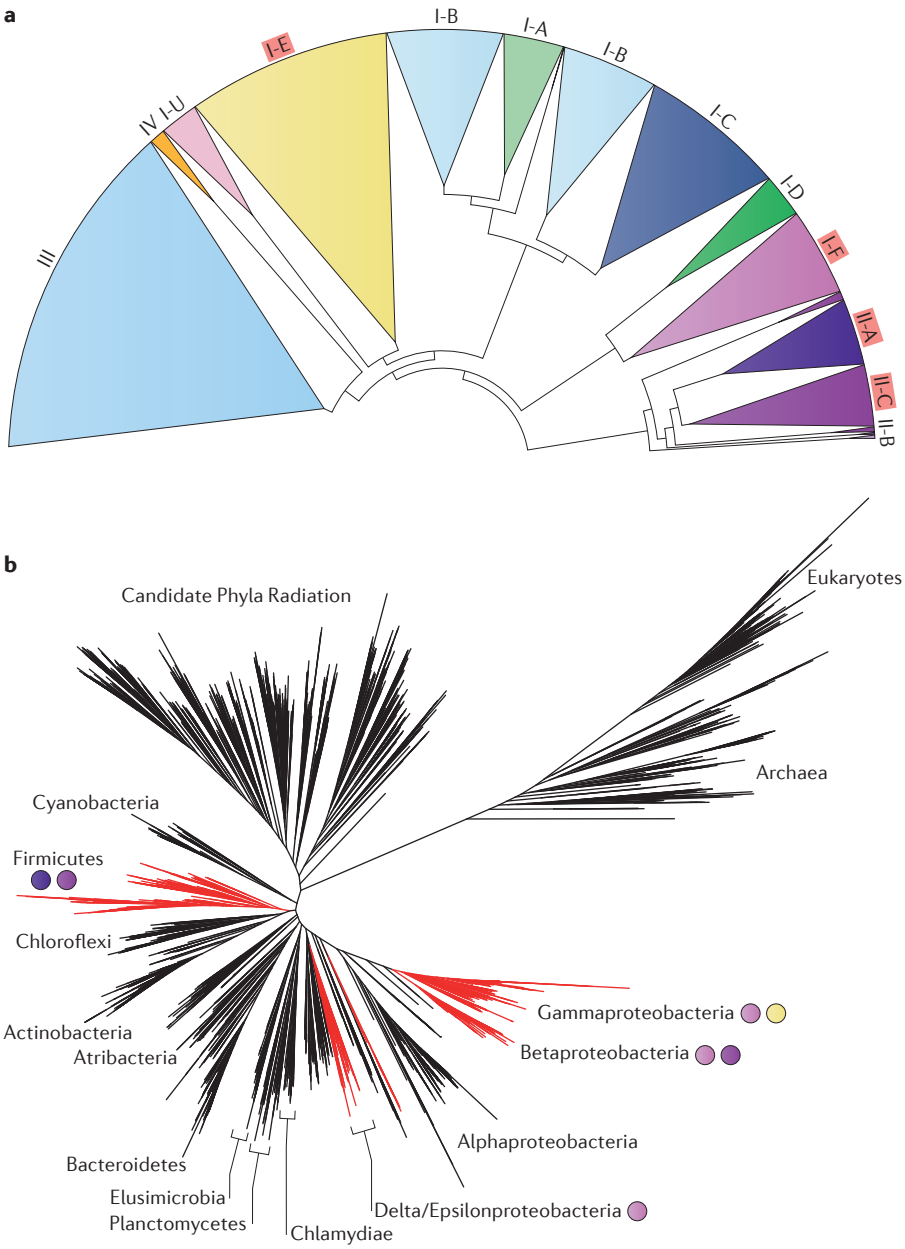

Figure 2 | **Inhibition of CRISPR–Cas systems by anti-CRISPR proteins is a common strategy across CRISPR–Cas system types and bacterial phylogeny. a** | A sequence-similarity dendrogram depicting the diversity of CRISPR–Cas interference proteins[23]. The CRISPR–Cas types for which anti-CRISPRs have been discovered are highlighted in red. The less abundant and more recently discovered types V and VI are not represented in this dendrogram but would not be expected to alter the main conclusions of this analysis. **b** | Anti-CRISPR genes are widespread across bacterial phylogeny. Major lineages in which anti-CRISPR genes have been discovered are highlighted in red on the phylogenetic tree[54]. Adjacent to each phylum name, coloured dots that match the CRISPR–Cas type colours of part **a** indicate which types of anti-CRISPR proteins have been found in at least ones in that phylum. Part a is from REF. 23, Macmillan Publishers Limited. Part b is from REF. 54, Macmillan Publishers Limited.

that bacteria and archaea could possess mechanisms to inactivate, overcome or co-opt anti-CRISPR activity, although it is still unclear what form this could take. We speculate that anti-CRISPR proteins like AcrF3, which do not inhibit DNA binding but prevent cleavage by CRISPR–Cas effectors, could potentially repurpose CRISPR–Cas systems into gene regulatory mechanisms in nature, using them to silence or activate gene expression.

Our current uncertainty concerning the functions and effects of anti-CRISPR proteins makes it impossible to predict

how they might ultimately influence aspects of bacterial physiology that are crucial to human health, such as the spread of antibiotic resistance and toxin genes. The identification and characterization of large numbers of anti-CRISPR genes, as well as investigations into their effects on horizontal gene transfer in co-evolving bacterial communities, will help answer these remaining questions. Clearly, we have only scratched the surface of the myriad anti-CRISPR sequences, structures and mechanisms of activity that exist.

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